V. PRACTICES AND PROCEDURES

A. Administrative Controls

1. Biohazard Warning Signs and Posting

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

All areas and laboratories that contain biohazardous agents must be posted with the biohazard warning sign (Figure 1). The background must be red/orange in color with a black universal biohazard symbol and black lettering.

![Figure 1](image)

All areas and laboratories that contain biohazardous or toxic agents must be posted with signs stating, "NO SMOKING, EATING, OR DRINKING PERMITTED"

All equipment (centrifuges, water baths, cryogenic freezers, incubators, etc.) that comes in contact with biohazardous materials must be labeled with the universal biohazard symbol.

2. Biosafety Levels

The levels are designated in ascending order, by degree of protection provided to personnel, the environment and the community. In general, Risk Group 1 agents are handled at Biosafety Level 1, Risk Group 2 agents at Biosafety Level 2 and so on.

**There are NO Biosafety Level 4 laboratories at Temple University.**

3. Vertebrate Animal Biosafety Levels

There are four animal Biosafety levels for experiments on animals infected with agents that produce or may produce human infection. As with Biosafety Levels, increasing levels of protection to personnel and the environment are provided as the order ascends.

**There are NO Animal Biosafety Level 4 facilities at Temple University.**

4. Medical Surveillance

A medical surveillance program will be provided through Occupational Employee Health at TUH for those personnel having substantial direct animal contact.
A medical surveillance program will be provided through Occupational Employee Health for those personnel who are occupationally at-risk of exposure to bloodborne pathogens. The program will include free hepatitis B vaccine, post-exposure evaluation and follow-up. For a more detailed explanation of this program, consult the University's Exposure Control Plan.

Designated personnel (Principal Investigators/Area Supervisors) are responsible to conduct a risk assessment for their area to determine the risk for nosocomial or occupational transmission of M. tuberculosis and implement an appropriate TB infection control plan.

Vaccines for which the benefits (levels of antibody considered to be protective) clearly exceed the risk (local or systemic reactions) will be offered free to all clearly identified at-risk personnel, because immunoprophylaxis may provide an additional level of protection. It is the Principal Investigator’s responsibility to ensure that laboratory personnel who work in Biosafety Level 2 or Biosafety Level 3 facilities receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g. hepatitis B vaccine, TB skin test, etc.) and periodic testing as recommended for the agent(s) being handled. Baseline serum samples may be collected as appropriate. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.

For more information, contact EHRS at (215) 707-2520.

B. Engineering Controls

1. Biological Safety Cabinets

BSC’s are designed to contain aerosols generated during work with infectious material through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. All personnel must develop proficient lab technique before working with infectious materials in a BSC. Three types of BSC’s (Class I, II and III) are used in microbiological laboratories.

The Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection. It provides protection to personnel and the environment from contaminants within the cabinet. The Class I BSC does not protect the product from "dirty" room air. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In many cases Class I BSC’s are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g. cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols that may flow back into the room.

The Class II BSC protects the material being manipulated inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination as well as meeting requirements to protect personnel and the environment. There are four types of Class II BSC’s: Type A1, Type A2, Type B1 and Type B2. The major differences between the four types may be found in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area.
### Comparison of Class II Biosafety Cabinet Characteristics

<table>
<thead>
<tr>
<th>BSC Class</th>
<th>Face Velocity</th>
<th>Airflow Pattern</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>II, A1</td>
<td>75</td>
<td>70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to the outside through a thimble unit</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>II, A2</td>
<td>100</td>
<td>Same as II, A1, but plenums are under negative pressure to room; exhaust air is thimble-ducted to the outside through a HEPA filter</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>YES [minute amounts (1)]</td>
</tr>
<tr>
<td>II, B1</td>
<td>100</td>
<td>30% recirculated to the cabinet work area through HEPA. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>YES [minute amounts (1)]</td>
</tr>
<tr>
<td>II, B2</td>
<td>100</td>
<td>No recirculation; total exhaust to the outside through hard-duct and a HEPA filter</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>YES (small amounts)</td>
</tr>
</tbody>
</table>

(1) In no circumstances should the chemical concentration approach the lower explosion limits of the compound.
The gas-tight **Class III BSC**, or glove box, provides the highest attainable level of protection to personnel, the environment and the product. It is the only cabinetry that provides a total physical barrier between the product and personnel. It is for use with high-risk biological agents and is used when absolute containment of highly infectious or hazardous material is required.

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

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**Selection and Placement of Biosafety Cabinets in the Laboratory**

Certain considerations must be met to ensure maximum effectiveness of these primary barriers. Contact EHRS prior to purchase of a biosafety cabinet to ensure you have selected an appropriate unit for the proposed usage. Contact your building administrator and EHRS to ensure proper installation and placement of a biosafety cabinet in your laboratory space.
Adequate clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance, and to ensure that the air return to the laboratory is not hindered. The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the protective laminar flow air curtain. The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1 mph. A BSC should be located away from open windows, air supply registers, or laboratory equipment (e.g., centrifuges, vacuum pumps) that creates turbulence. Similarly, a BSC should not be located close to a chemical fume hood.

2. Other Safety Equipment

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

Other safety equipment such as safety centrifuge cups and safety blenders are enclosed containers designed to prevent aerosols from being released during centrifugation or homogenization of infectious material. Containment controls such as BSC’s, safety centrifuge cups and blenders must be used for handling infectious agents that can be transmitted through the aerosol route of exposure. For assistance in the selection of a BSC or other safety equipment, contact EHRS by phone (215-707-2520).

For more information on PPE, a description of effective use of BSC’s and information on other safety equipment may be found in the Recommended Work Practices section below.
C. Recommended Work Practices

1. Pipettes and Pipetting Aids

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

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

2. Syringes and Needles

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

• Use disposable safety-engineered needle-locking syringe units whenever possible.
• When using syringes and needles with biohazardous or potentially infectious agents, work in a biological safety cabinet whenever possible.
• **Wear gloves.**
• Fill the syringe carefully to minimize air bubbles.
• Expel air, liquid and bubbles from the syringe vertically into a cotton pledget moistened with disinfectant.
• Do not use a syringe to mix infectious fluid forcefully.
• Do not contaminate the needle hub when filling the syringe in order to avoid transfer of infectious material to fingers.
• Wrap the needle and stopper in a cotton pledget moistened with disinfectant when removing a needle from a rubber-stoppered bottle.
• Bending, recapping, clipping or removal of needles from syringes is prohibited. If you must recap or remove a contaminated needle from a syringe, use a mechanical device (e.g. forceps) or the one-handed scoop method.
• Use a separate pan of disinfectant for reusable syringes and needles. Do not place them in pans containing pipettes or other glassware in order to eliminate sorting later.
• Used disposable needles and syringes must be placed in appropriate sharps disposal containers and discarded as infectious waste.

The Occupational Safety and Health Administration (OSHA) revised the Occupational Exposure to Bloodborne Pathogens Standard (29 CFR Part 1910.1030) in 2001 to include new efforts to help reduce needle stick injuries among healthcare workers and others who handle medical sharps. OSHA now requires the University to involve non-managerial employees in selecting safer medical sharps devices. Evaluative data will be made available in the Temple University Exposure Control Plan. If you use sharps and are interested in evaluating safer medical devices, refer to the Safety Device Evaluation Program.

3. Safe and Effective Use of Biological Safety Cabinets

In general:

Make sure your BSC is certified prior to use, when it is installed or after it is moved, and annually thereafter. (For information on cabinet certification contact EHRS by phone (215-707-2520).

• Understand how your cabinet works. The NIH/CDC document, Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, provides thorough information. Also consult the manufacturer’s operational manual.

• Monitor alarms, pressure gauges or flow indicators for any major fluctuation or changes possibly indicating a problem with the unit. Immediately notify EHRS of cabinets that are not operating properly. DO NOT attempt to adjust the speed control or alarm settings.

• Do not disrupt the protective airflow pattern of the BSC. Make sure lab doors are closed before starting work in the BSC.

• Plan your work and proceed conscientiously.

• Minimize the storage of materials in and around the BSC.

• Hard ducted (Type B2, Total Exhaust) cabinets should be left running at all times. Cabinets that are not vented to the outside may be turned off when not in use, however, be sure to allow the BSC to run for at least 10 minutes before starting work.

Operational directions
• Limit traffic in the area when the cabinet is in use.
• If there is an UV light incorporated within the cabinet, do not leave it on while working in the cabinet or when occupants are in the laboratory.
• Before using, wipe work surface with 70% alcohol. Wipe off each item you need for your procedures and place in cabinet.
• DO NOT place objects over the front air intake grille. Keep all materials at least 4 inches inside the sash. DO NOT place items on top of the unit or block the rear exhaust grille.
• Segregate contaminated and clean items. Work from "clean to dirty."
• Place a pan with disinfectant and/or a sharps container inside the BSC for pipette discards. DO NOT use vertical pipette discard canisters on the floor outside cabinet.
• It is not necessary to flame items. This creates turbulence in airflow and may compromise sterility; heat buildup may damage the filters.
• Move arms slowly when removing or introducing new items into the BSC.
• If you use a piece of equipment that creates air turbulence in the BSC (such as a centrifuge, blender) place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.
• Protect the building vacuum system from biohazards by placing an in-line HEPA cartridge filter between the vacuum trap system and the source valve in the cabinet.
• Clean up all spills in the cabinet immediately. Allow cabinet to run for 10 minutes before resuming work.
• When work is completed, remove all materials and wipe all interior surfaces with 70% alcohol.
• Remove lab coat and wash hands thoroughly before leaving laboratory.

4. Cryostats

Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent pose a risk because accidents can occur. Freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections.

When working with biohazardous material in a cryostat, the following is recommended:
• Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol.

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

• Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.

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

• Consider solutions used for staining potentially infected frozen tissue sections to be contaminated.

5. Centrifuge Equipment

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

Aerosols are created by practices such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, and resuspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, follow the procedures below:
• Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.

• Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
• Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.

• Always balance buckets, tubes and rotors properly before centrifugation.

• Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.

• Work in a BSC when re-suspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.

Small low-speed centrifuges may be placed in a BSC during use to contain aerosols.

High-speed centrifuges pose additional hazards. Take precautions to filter the exhaust air from vacuum lines; avoid metal fatigue resulting in disintegration of rotors; and use proper cleaning techniques and centrifuge components. Follow manufacturers' recommendations meticulously to avoid metal fatigue, distortion and corrosion.

Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them.

6. Personal Protective Equipment

OSHA Standard 29 CFR 1910.132 requires workplace assessment for potential hazards and mandates that employers provide appropriate PPE for employees. PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. PPE must be provided without cost to personnel. Supervisors are responsible to perform the assessments and to select and train employees in the use of routine items such as lab coats, protective gloves, safety glasses, face shields, etc. For assistance in selection of PPE contact EHRS. The following PPE is recommended for regular use:

Face Protection – Goggles or safety glasses with solid side shields in combination with masks, or chin length face shields or other splatter guards are required for anticipated splashes, sprays or splatters of infectious or other hazardous materials to the face. Information on safety eyewear is available at the EHRS website. EHRS can also provide information if prescription safety eyewear is required.

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

Gloves
Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxics and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice.
Delicate work requiring a high degree of precision dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required. In addition, for some workers, exposures to latex may result in allergic reactions. For assistance in glove selection, contact EHRS.

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

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

**Respirators**

In certain instances additional PPE may be required. Respirator selection is based on the hazard and the protection factor required. If your work requires the use of a respirator, you must participate in the [University's respiratory protection program](mailto:). Personnel who require respiratory protection must be medically evaluated by Occupational Health Services prior to respirator use. After evaluation, EHRS must assist the user in respirator selection and usage training. Contact EHRS to initiate the process.

7. **Aerosol Producing Devices**

The use of devices such as *ultrasonic disrupters, grinders and homogenizers* to disrupt biohazardous materials results in considerable aerosol production and should be performed in a BSC whenever possible. Special care and barrier protection (splash shields, goggles, bench napkins, gloves, etc.) are important not only during the agitation/disruption process but also when handling the finished product. Allow your vessel to sit for a short time to allow your product to settle before opening. Review the operations manual for the device you are using, paying special attention to those areas of the device that are susceptible to contamination by your product and decontaminate appropriately after use, especially when working with potentially infectious materials.

**Safety blenders** are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation and to withstand sterilization by autoclaving. If blender rotors are not leak proof, test them with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, cover the glass jar with a polypropylene jar to prevent spraying of glass and contents in the event it breaks. Use safety blenders in a BSC to prevent the accidental release of aerosol during the blending process. During use, place a towel moistened with disinfectant over the top of the blender. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. Decontaminate the device promptly after use.

**Lyophilizers** may be used to freeze-dry biohazardous material. Depending on each lyophilizer design, infectious aerosol production may occur when biohazardous material is loaded or removed from the lyophilizer unit. If possible, load sample material in a BSC. Be sure the vacuum pump exhaust is HEPA-filtered to remove any hazardous agents or, alternatively, vent the pump into a BSC. After lyophilization is completed, disinfect all surfaces of the unit that have been exposed to the agent. If the lyophilizer is equipped with a removable chamber, close it off and move it to a BSC for unloading and decontamination. Handle cultures as infrequently as possible and use vapor traps wherever possible.

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

Glass ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries. The cryogenic liquid storage unit must store in well ventilated rooms with a minimum of eight air changes per hour. All personnel must wear gloves, lab coat and safety glasses whenever handling cryogens. Gloves must be replaced frequently and immediately if they become contaminated or damaged in any way. In addition to the above items, personnel must wear any additional PPE (apron, boots, face shield, etc.) that is needed to prevent cryogenic material from contaminating their street clothes, skin, mouth, or other parts of the body under normal conditions. The use of polypropylene tubes eliminates this hazard. These tubes are available dust-free or pre-sterilized and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

8. Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols that may contain viable microorganisms. The use of a shielded electric incinerator minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available. The loops are semi-quantitative and can be used for counting bacteria.

The use of gas burners in BSC’s is not recommended. These burners can produce turbulence that disturbs the protective airflow patterns of the cabinet. In many biosafety cabinets, a portion of the total air volume is recirculated in the work area allowing flammable vapors or gases to accumulate thereby creating a fire hazard. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

If a gas burner must be used, select a touch-plate burner with a pilot light. In addition, appropriate hard piping from the house gas line must be used and an easily accessible emergency shut-off valve (specifically identified as such) must be placed on the outside of the biosafety cabinet.

9. Laundry

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

10. Housekeeping

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

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

- Secure biohazardous materials at the conclusion of work.
- Keep the laboratory neat and free of clutter - surfaces should be clean and free of infrequently used chemicals, biologicals, glassware and equipment. Access to sinks, eyewashes, emergency showers and fire extinguishers must not be blocked.
• Decontaminate and discard infectious waste – do not allow it to accumulate in the laboratory.
• Dispose of old and unused chemicals promptly and properly. To have your waste chemicals removed, complete an EHRS Chemical Waste Pickup form.
• Provide a workplace that is free of physical hazards - aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, avoidance of overloaded electrical circuits and avoidance of the creation of electrical hazards in wet areas.
• Remove unnecessary items on floors, under benches or in corners.
• Properly secure all compressed gas cylinders.
• Never use fume hoods or biosafety cabinets for storage.

Practical custodial concerns include:
• Dry sweeping and dusting that may lead to the formation of aerosols is not permitted.
• The use of a wet or dry industrial type vacuum cleaner is prohibited to protect personnel as well as the integrity of the experiment. They are potent aerosol generators and, unless equipped with high efficiency particulate air (HEPA) filters, must not be used in the biological research laboratory. Wet and dry units with HEPA filters on the exhaust are available from a number of manufacturers.

11. Biohazard Spill Cleanup Procedures

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

a. Biosafety Level 2 Spill Protocol

**NOTE:** If spill also involves radioactive materials, contact EHRS at 215-707-2520.

Instruct injured personnel to go to Occupational Employee Health (or to Student Health, if appropriate), immediately. Ask a co-worker to call ahead to alert Occupational Employee Health (or Student Health). If transportation assistance is required, call Campus Police at 1-1234 or 215-204-1234. After hours or on weekends, go to TUH Emergency Department.

**Small spills:**

Wipe up spill with a disinfectant-soaked paper towel & clean the surface with a suitable disinfectant.

**Larger spills:**

**Within a Biological Safety Cabinet (BSC)**

• BSC must run during cleanup to contain aerosols & HEPA-filter exhaust air.
• Don appropriate personal protective gear before initiating cleanup.
• Initiate clean up as soon as possible using a germicidal disinfectant (phenolic or iodophor). Alcohol is not recommended. Large quantities may create the risk of fire.
• If the spill is contained on a bench diaper, remove the contaminated bench diaper & discard as infectious waste.
• If the spill is on the work area surface, cover spilled material with disinfectant-soaked towels. Allow 20 minutes contact time then remove the contaminated towels & discard as infectious waste.
• Wipe down the interior of the cabinet & any splatter on items within the cabinet with a disinfectant-soaked towel.
• Wipe down non-autoclavable materials with disinfectant. Allow 20 minutes of contact time with disinfectant before any items are removed from cabinet.
• Place items designated as contaminated used sharps in an appropriate infectious waste sharps container using tongs/forceps. Place other contaminated disposable materials used in the cleanup process in an autoclave bag and process as infectious waste.
• Place contaminated re-usable items in biohazard bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and then clean for re-use.
• If the cabinet has a catch basin beneath the work surface & the spill resulted in liquids flowing into this area, more extensive decontamination is required.
  1) Ensure the drain valve under the cabinet is closed.
  2) Pour disinfectant onto the work surface & through the front and rear grilles into the drain pan. Allow 20-30 minutes contact time.
  3) Absorb spilled fluid-disinfectant from work surface with paper towels & discard in biohazard bag.
  4) Prepare to empty drain pan. Place disinfectant solution in a collection vessel. Attach flexible tubing to the drain valve. The tube should be of sufficient length to allow the open end to be submerged in the collection vessel to minimize aerosol generation.
  5) Open the drain valve & empty the drain pan into the collection vessel containing disinfectant. Flush the drain pan with water & remove the flexible tubing. Manage contaminated materials as if they are infectious.
  6) Remove protective clothing used during cleanup & place in a biohazard bag for autoclaving. Wash hands when gloves are removed.
  7) Notify Principal Investigator or supervisor and EHRS (215-707-2520). Consult with EHRS to determine whether formaldehyde decontamination of the cabinet and filters is necessary, especially if a high-risk agent or a major spill of a moderate-risk agent occurred.
  8) Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet.

Outside the Cabinet, Inside the Laboratory

• If a spill occurs in a Biosafety Level 2 facility, outside the BSC, notify other individuals in the laboratory to evacuate.
• Exit the laboratory to the hallway, closing the door behind you.
• Remove any contaminated clothing (turn contaminated portion inward) & place it in an autoclave bag.
• Wash all exposed skin.
• Place signs on door(s) to the laboratory warning individuals who may want to enter that a spill occurred & access is denied.
• Allow aerosols to settle for 30 minutes before re-entering the laboratory.
• Assemble supplies (disinfectant, sharps containers, towels, tongs, autoclave bags, etc.) before entering the laboratory.
• Don appropriate personal protective equipment (i.e. disposable gown, protective eyewear, gloves, shoe coverings & respiratory protection [if needed]).
• Clean up spill with a suitable disinfectant as follows:
  1) Surround spill area with disinfectant or diking material that is soaked in disinfectant.
  2) Place paper towels soaked in a disinfectant over the entire spill area.
  3) Allow 20-minute contact time with the disinfectant to ensure adequate germicidal action.
  4) Wipe down non-autoclavable materials with germicidal disinfectant.
  5) Place items designated as contaminated used sharps in an appropriate infectious waste sharps container. Place other disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
  6) Place contaminated re-usable items in biohazard bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and then clean for re-use. Remove protective clothing used during cleanup then place in a biohazard bag for autoclaving.
• Wash hands when gloves are removed.
• Notify Principal Investigator or supervisor & EHRS (215-707-2520)

Inside a Centrifuge
The potential for multiple infections from a single centrifuge accident is great. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. **All opening of centrifuges must be performed slowly.**

**Unsealed buckets:**
- If a centrifuge tube breaks while the centrifuge is running, turn off motor. Allow the machine to be at rest for 30 minutes before opening. If breakage is discovered after the machine has stopped, re-close the lid immediately & allow the unit to be at rest for 30 minutes.
- Unplug centrifuge before initiating clean up.
- Don strong, thick rubber gloves & other PPE before proceeding with clean up.
- Flood centrifuge bowl with a germicidal disinfectant. Place paper towels soaked in a disinfectant over the entire spill area. Allow 20 minutes contact time.
- Use mechanical means (such as forceps) to remove broken tubes & glass fragments. Place them in a sharps container for autoclaving & disposal as infectious waste.
- Remove buckets, trunnions & rotor then place in disinfectant for 24 hours or autoclave.
- Unbroken, capped tubes may be placed in disinfectant & recovered after 20 minutes contact time or autoclaved.
- Use mechanical means to remove remaining disinfectant soaked materials from centrifuge bowl & discard as infectious waste.
- Place paper towels soaked in a disinfectant in the centrifuge bowl & allow it to soak overnight, wipe down again with disinfectant, wash with water & dry. Discard disinfectant soaked materials as infectious waste.
- Remove protective clothing used during cleanup & place in a biohazard bag for autoclaving. Wash hands whenever gloves are removed.

**Sealed buckets (safety cups):**
- If breakage is suspected, remove the sealed bucket to a biological safety cabinet before opening.
- If breakage occurred, replace the cap on the safety cup loosely and autoclave.
- Notify Principal Investigator or supervisor & EHRS (215-707-2520).

**Outside the Laboratory; during Transport (on Temple’s Campuses)**
The major emphasis should be on preventing spills during transport. All transport of infectious materials must be in a rigid, securely sealed, watertight primary container, which is contained within a second rigid, leak proof sealed container. Sufficient absorbent should be added to the second container to take up contents in case of leakage from the primary container. The outer container must be labeled with the universal biohazard symbol.

If a spill occurs during transport, don gloves and initiate cleanup immediately as follows:
- Surround spill area with disinfectant or diking material that is soaked in disinfectant.
- Place paper towels soaked in a disinfectant over the entire spill area.
- Allow a minimum 20 minutes contact time with the disinfectant to ensure adequate germicidal action.
- Place **contaminated used sharps** in an appropriate infectious waste sharps container.
- Place other materials used in the cleanup process (including contaminated gloves) in an autoclave bag and process as infectious waste.
- Repeat decontamination of spill area after contaminated materials are removed.
- Wash hands as soon as possible.

Contact EHRS (215-707-2520) if assistance is needed.

**b. Biosafety Level 3 Spill Protocol**
NOTE: All laboratory personnel (faculty, staff, students) working with a Risk Group 3 agent in a Biosafety Level 3 facility must be trained in the use of respiratory equipment by the EHRS prior to beginning work.

If spill involves radioactive materials, contact EHRS immediately at 215-707-2520.

Instruct injured personnel to go to Occupational Employee Health immediately. Ask a co-worker to call ahead to alert Occupational Employee Health (215-707-4455). If transportation assistance is required, call Campus Police at 1-1234 or 215-204-1234. After hours, injured personnel should go directly to TUH Emergency Department.

Within a Biological Safety Cabinet (BSC)
- BSC must run during cleanup to contain aerosols & HEPA-filter exhaust air.
- Don appropriate personal protective gear before initiating cleanup (disposable back-closing gown, double gloves).
- Initiate clean up as soon as possible using a germicidal disinfectant (phenolic or iodophor). Alcohol is not recommended. Large quantities may create the risk of fire.
- If the spill is contained on a bench diaper, remove the contaminated bench diaper & discard as infectious waste.
- If the spill is on the work area surface, cover spilled material with disinfectant-soaked towels. Allow 20 minutes contact time then remove the contaminated towels & discard as infectious waste.
- Wipe down the interior of the cabinet & any splatter on items within the cabinet with a disinfectant-soaked towel.
- Wipe down non-autoclavable materials with disinfectant. Allow 20 minutes of contact time with disinfectant before any items are removed from cabinet.
- Place items designated as contaminated used sharps in an appropriate infectious waste sharps container using tongs/forceps. Place other contaminated disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
- Place contaminated re-usable items in biohazard bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and then clean for re-use.
- If the cabinet has a catch basin beneath the work surface & the spill resulted in liquids flowing into this area, more extensive decontamination is required.
  1) Ensure the drain valve under the cabinet is closed.
  2) Pour disinfectant onto the work surface & through the front and rear grilles into the drain pan. Allow 20-30 minutes contact time.
  3) Absorb spilled fluid-disinfectant from work surface with paper towels & discard in biohazard bag.
  4) Prepare to empty drain pan. Place disinfectant solution in a collection vessel. Attach flexible tubing to the drain valve. The tube should be of sufficient length to allow the open end to be submerged in the collection vessel to minimize aerosol generation.
  5) Open the drain valve & empty the drain pan into the collection vessel containing disinfectant. Flush the drain pan with water & remove the flexible tubing. Manage contaminated materials as if they are infectious.
- Remove protective clothing used during cleanup & place in a biohazard bag for autoclaving. Wash hands when gloves are removed.
- Notify Principal Investigator or supervisor and EHRS (215-707-2520). Consult with EHRS to determine whether formaldehyde decontamination of the cabinet and filters is necessary, especially if a high-risk agent or a major spill of a moderate-risk agent occurred.
- Run BSC at least 10 minutes after cleanup, before resuming activity in the cabinet.

Outside the Cabinet, Inside the Laboratory
- Notify other individuals in the laboratory to evacuate the laboratory immediately.
• Hold your breath and exit the laboratory to the anteroom.
• Remove contaminated clothing (turn contaminated portion inward; place into autoclave bag). Wash hands after gloves are removed.
• Wash all exposed skin with germicidal soap. If eyes were splashed, flush at eyewash station for 15 minutes then report to Occupational Medicine.
• Notify Principal Investigator or supervisor and EHRS (215-707-2520). EHRS will consult with the Principal Investigator to determine the appropriate method of decontamination and spill cleanup (personnel spill response or formaldehyde decontamination of the entire facility).
• Place a sign on the door to the BSL3 lab to warn individuals of the spill and advise them keep out of the lab.

If personnel spill response is required, do the following:
• Allow aerosols to settle for a minimum of 30 minutes before re-entering the laboratory.
• Assemble supplies (disinfectant, sharps containers, towels, tongs, autoclave bags and protective gear [disposable Tyvek suit/back-closing gown, protective eyewear, gloves, shoe coverings, respirator], etc.) before initiatiing spill cleanup.
• Don appropriate personal protective equipment (PPE). Double gloving is recommended.
• Clean up spill with a suitable disinfectant as follows:
  1) Surround spill area with disinfectant or diking material that is soaked in disinfectant.
  2) Place paper towels soaked in a disinfectant over the entire spill area.
  3) Allow a minimum 20 minutes contact time with the disinfectant to ensure adequate germicidal action.
  4) wipe down non-autoclavable materials with germicidal disinfectant, allowing 20-minute contact time.
  5) Place items designated as contaminated used sharps in an appropriate infectious waste sharps container using tongs/forceps. Place other contaminated disposable materials used in the cleanup process in an autoclave bag. Process as infectious waste.
  6) Place contaminated autoclavable re-usable items in biohazard bags, autoclavable pans with lids or wrap them in newspaper. Sterilize, preferably by autoclaving, and then clean for re-use.
  7) Repeat decontamination of spill area (floor and work surfaces) after contaminated materials are removed.
• Remove outer gloves before exiting laboratory to the anteroom.
• Remove protective clothing used during cleanup in the following order: shoe coverings, gown/suit, respirator, and gloves last. If reusable, wipe down respirator with disinfectant. Place disposable PPE in a biohazard bag for autoclaving.
• Wash hands with germicidal soap after gloves are removed; shower recommended.

**Inside a Centrifuge**
The potential for multiple infections from a single centrifuge accident is great. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. **All opening of centrifuges must be performed slowly.**

• If a centrifuge tube breaks while the centrifuge is running, turn off the motor, notify others in the lab, hold your breath, and evacuate. **Allow the centrifuge to be at rest for 30 minutes before attempting to open.**
• Notify Principal Investigator or supervisor and EHRS (215-707-2520). EHRS will consult with the Principal Investigator to determine the most appropriate method of decontamination and spill cleanup.
• Place a sign on the door to the BSL3 lab, to warn individuals of the spill and to keep out of the lab.

If personnel spill response is required, do the following:
Assemble supplies (disinfectant, sharps containers, towels, tongs, autoclave bags and protective gear [disposable Tyvek suit/back-closing gown, protective eyewear, gloves, shoe coverings, respirator], etc.) before initiating spill cleanup.

Don appropriate personal protective equipment (PPE). Double gloving is recommended. Re-enter BSL3 lab.

Unplug centrifuge and slowly open centrifuge.

If safety cup(s) is intact, remove unit to biological safety cabinet for further decontamination.

If integrity of safety cup(s) is breached, decontaminate all exposed surfaces before removing cup(s) to biological safety cabinet for further decontamination.

Outside the Laboratory; during Transport (within Temple campuses)
The major emphasis should be on preventing spills during transport. Please note that transportation of a large number of cultures at the same time is discouraged. If a cart is used to transport the material, it must have side rails. All transport of infectious materials must be in a rigid, securely sealed, watertight primary container, which is contained within a second rigid, sealed, leak-proof container. Sufficient absorbent should be added to the second container to take up contents in case of leakage. The outer container must be labeled with the universal biohazard symbol. The container should be placed on the bottom shelf of the cart.

If a spill occurs during transport, don gloves and initiate cleanup immediately as follows:

- Place absorbent towels, preferably soaked in a disinfectant, on the spilled material.
- Contact EHRS (215-707-2520). Remain nearby until EHRS arrives to assist in the spill cleanup.

D. Decontamination

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

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

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

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

**Methods**

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

**Heat**

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

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

Warranties and preventive maintenance plans for all autoclaves are strongly recommended by EHRS.

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

**Recommended procedures for autoclaving are:**

- All personnel using autoclaves must be adequately trained by their PI or lab manager. Never allow untrained personnel to operate an autoclave.
- Be sure all containment vessels can withstand the temperature and pressure of the autoclave. Be sure to use polypropylene / polyethylene autoclave bags.
- Review the operator’s manual for instructions prior to operating the unit. Different makes and models have unique characteristics.
- Never exceed the maximum operating temperature and pressure of the autoclave.
- Wear the appropriate personal protective equipment (safety glasses, lab coat and heat-resistant gloves) when loading and unloading an autoclave.
- Select the appropriate cycle: liquid cycle (slow exhaust) for fluids to prevent boiling over, dry cycle (fast exhaust) for glassware, fast and dry cycle for wrapped items.
- Never place autoclave bags directly on the autoclave chamber floor. Place autoclavable bags containing waste in a secondary containment vessel to retain any leakage that might occur. The secondary containment vessel must be constructed of material that will not melt or distort during the autoclave process. (Polypropylene is a plastic capable of withstanding autoclaving but is resistant to heat transfer. Materials contained in a polypropylene pan will take longer to autoclave than the same material in a stainless steel pan.)
- Never place sealed bags or containers in the autoclave. Polypropylene bags are impermeable to steam and should not be twisted and taped shut. Secure the top of containers and bags loosely to allow steam penetration.
- Position autoclave bags with the neck of the bag taped loosely and leave space between items in the autoclave bag to allow steam penetration.
- Fill liquid containers only half full, loosen caps or use vented closures.
- For materials with a high insulating capacity (animal bedding, saturated absorbent, etc.) increase the time needed for the load to reach sterilizing temperatures.
- Never autoclave items containing solvents, volatile or corrosive chemicals.
- Always make sure that the pressure of the autoclave chamber is at zero before opening the door. Stand behind the autoclave door and slowly open it to allow the steam to gradually escape from the autoclave chamber after cycle completion.
• Allow liquid materials inside the autoclave to cool down for 15-20 minutes prior to their removal.
• Dispose of all autoclaved waste through the infectious waste stream.
• Never leave the autoclaved biohazard materials unattended in the autoclave room.

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

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

**Liquid Disinfection**

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

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

Properties of common disinfectants may be found in 'Table 3: Properties of Common Decontamination Methods'.

**Vapors and Gases**

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

**Radiation**

Although ionizing radiation will destroy microorganisms, it is not a practical tool for laboratory use. Non-ionizing radiation in the form of ultraviolet radiation (UV) is used for inactivating viruses, bacteria and fungi. It will destroy airborne microorganisms and inactivate microorganisms on exposed surfaces or in the presence of products of unstable composition that cannot be treated by conventional means.
Because of the low penetrating power of UV, microorganisms inside dust or soil particles will be protected from its action, limiting its usefulness. UV is used in air locks, animal holding areas, ventilated cabinets and laboratory rooms to reduce levels of airborne microorganisms and maintain good air hygiene. Because UV can cause burns to the eyes and skin of people exposed for even a short period of time, proper shielding should be maintained when it is in use. UV lamps that are used for space decontamination should be interlocked with the general room or cabinet illumination, so that turning on the lights extinguishes the UV.

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

E. Infectious Waste Management

Infectious waste, as defined below, is regulated in Pennsylvania by the Department of Environmental Protection. It is the responsibility of generators to properly sort and dispose of all infectious waste following the policies and procedures established by EHRS. Infectious waste management varies across Schools within the University.

1. **Categories of infectious waste:**
   a. **Cultures and stocks** of infectious agents and associated biologicals, including the following:
      - Cultures from medical and pathological laboratories;
      - Cultures and stocks of infectious agents from research and industrial laboratories;
      - Wastes from the production of biologicals;
      - discarded live and attenuated vaccines except for residue in emptied containers;
      - Culture dishes, assemblies and devices used to conduct diagnostic tests or to transfer, inoculate and mix cultures.

   b. **Pathological wastes:** human pathological wastes, including: tissues, organs and body parts and body fluids that are removed during surgery, autopsy, other medical procedures, or laboratory procedures. Hair, nails and extracted teeth are excluded.

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

   d. **Animal wastes:** contaminated animal carcasses, body parts, blood, blood products, secretions, excretions and bedding of animals that were known to have been exposed to zoonotic infectious agents or non-zoonotic human pathogens during research (including research in veterinary schools and hospitals), production of biologicals or testing of pharmaceuticals.

   e. **Isolation wastes:** biological wastes and waste contaminated with blood, excretion, exudates or secretions from:
• Humans who are isolated to protect others from highly virulent diseases,
• Isolated animals known or suspected to be infected with highly virulent diseases.

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

2. **Handling**
All infectious waste from University laboratories must be autoclaved by the generator prior to disposal municipal trash, or must be placed in red biohazard bags in appropriate infectious waste containers for eventual pickup by a certified infectious waste hauler for eventual incineration. Treatment of infectious waste, other than by autoclaving, must be reviewed by EHRS.

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

Potentially infectious and biohazardous waste must be separated from general waste at the point of generation (i.e., the point at which the material becomes a waste) by the generator into the following three classes as follows:
- **Used Sharps**
- **Fluids (volumes greater than 20 cc)**
- **Other**

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

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

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

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

3. **Mixed Waste**
Provisions must be made for potentially infectious waste with multiple hazards, e.g., radioactive material contaminated wastes, or wastes substantially contaminated with
toxic/carcinogenic compounds. Contact EHRS (215-707-2520) regarding the disposal of these wastes.

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

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

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

5. **Monitoring Treatment of Infectious Waste**
   If infectious waste is to be autoclaved (treated) on-site, rather than being removed by a licensed biomedical waste hauler, then the efficacy of the autoclave process has to be tested and documented. The principal investigators or supervisors of steam sterilizers are required to ensure that steam sterilizers are inspected at least annually. The inspection should consist of a calibration for temperature and pressure, using an instrument approved by the National Institute of Standards and Technology. The steam sterilization process must be monitored on a weekly basis with a biological indicator (using *Bacillus stearothermophilus* or a suitable substitute). The principal investigators or supervisors are required to maintain records on the annual inspection and weekly efficacy validation. These records must be produced upon demand.

6. **Animals**
   Disposal of research animals and animal parts that are considered to be infectious waste is coordinated through University Central Animal Facility (CAF). Disposal of animal carcasses in the general trash is prohibited.

F. Transport of Biological Materials

1. **Intramural Transport**

   When transporting biohazardous materials on campus, take precautions to communicate the hazard to those around you as well as to prevent an accidental spill. Transport all biohazardous materials (tissues, blood samples, contaminated supplies, etc.) in a rigid, securely sealed, watertight primary container, contained within a second rigid, sealed, watertight container. Add sufficient absorbent to the second container to take up contents of the first container in case of leakage. Label the outer container with the universal biohazard symbol.

   When transporting infected animals between the animal facility and the laboratory, place them in cages fitted with filter bonnets and transport them on carts with sides. Outer containers and/or animal cages must be labeled with the universal biohazard symbol.

2. **Extramural Transport and Permitting**

   The packaging and shipping of biological materials for extramural transport must comply with federal and international shipping requirements. It is the intent of the regulations that biological material which may contain infectious agents will be packaged and shipped in such
a way that the contents will not leak and will arrive in good condition. The shipper (i.e., person with direct knowledge of what is being shipped) must be trained every 2 years and be familiar with the most current packaging and shipping requirements. Consult EHRS for guidance in the packaging and shipping of diagnostic specimens, biological and infectious substances, import and export of biological materials and live organisms, and resources for appropriate forms and supplies.

G. Permits

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

The following vectors require import permits:
1. **Animals** known or suspected of being infected with any disease transmissible to man. Importation of turtles less than 4 inches in shell length and all nonhuman primates requires an importation permit issued by the CDC, Division of Global Migration and Quarantine.

2. **Biological materials**: Unsterilized specimens of human and animal tissue (including blood), body discharges, fluids, excretions or similar material, when known or suspected to be infected with disease transmissible to man.

3. **Insects**: Any living insect or other living arthropod, known or suspected of being infected with any disease transmissible to man. Also, if alive, any fleas, flies, lice, mites, mosquitoes or ticks, even if uninfected. This includes eggs, larvae, pupae, and nymphs as well as adult forms.

4. **Snails**: Any snails capable of transmitting schistosomiasis. No mollusks are to be admitted without a permit from either CDC or the Department of Agriculture. Any shipment of mollusks with a permit from either agency will be cleared immediately.

5. **Bats**: All live bats require an import permit from the CDC and the U.S. Department of Interior, Fish and Wildlife Services (202) 358-2095

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

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

Application forms are available online or may be obtained directly from EHRS (215) 898-4453 or by calling CDC at (404) 498-1600. Completed forms may be returned to CDC by mail (1600 Clifton Road NE, Mailstop E-79 Atlanta, GA 30333) or FAX (404) 498-2275. Application to CDC for the importation permit should be made 10 working days in advance of the shipment date to allow time for processing, issuance and delivery of the permit and shipping labels to the permittee.
Other Permits:

U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits are required to import or transport infectious agents of livestock and biological materials containing animal, particularly livestock, material. Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials, and suspensions of cell culture grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA due to the potential risk of introduction of exotic animal disease into the U.S. Applications for USDA/APHIS permits may be obtained online or from EHRS (215) 898-4453. Further information may be obtained by calling the USDA/APHIS at (301) 734-3277.

Export of infectious materials may require a license from the Department of Commerce. Call (202) 512-1530 for further information.