



BIOHAZARDOUS WASTE HANDLING AND DISPOSAL GUIDE

I. Background & Regulatory Summary

Biohazardous waste includes any waste item that is contaminated with a biological material that is an infectious disease transmission risk or an environmental release risk (i.e., recombinant DNA). In the state of Tennessee, some forms of biohazardous waste are defined as “medical wastes” and are regulated for disposal purposes by the Tennessee Department of Environment and Conservation (TDEC). These regulated “medical wastes” include the following categories which may be applicable to work, research, and classes at TTU:

- Wastes generated by patients who are isolated to protect others from communicable diseases;
- Cultures and stocks of infectious agents, including specimen cultures from medical and pathological labs, cultures and stocks of infectious agents from research and industrial labs, wastes from the production of biological, discarded live and attenuated vaccines, and culture dishes and devices used to transfer, inoculate and mix cultures;
- Waste human blood and blood products such as serum, plasma and other blood components;
- Pathological wastes (i.e., tissues, organs, body parts, and body fluids) that are removed during surgery and autopsy;
- All discarded sharps (i.e., hypodermic needles, syringes, Pasteur pipettes, broken glass, scalpel blades) used in patient care or which have come into contact with infectious agents during use in medical, research or industrial labs;
- Contaminated carcasses, body parts, and bedding of animals that were intentionally exposed to pathogens in research in the production of biologicals, or in the in vivo testing of pharmaceuticals.

Unlike hazardous chemicals or radioactive waste, there is no one federal agency that clearly defines and regulates biohazardous waste. Several agencies associated with research funding have unique waste disposal requirements that may go above and beyond what TDEC regards as regulated “medical waste”. Therefore, it is the researcher’s responsibility to have a general knowledge of biosafety regulations & guidelines and how they apply to their work and the waste that is generated through the research and diagnostic service process.

Please review the regulatory/agency information in the following table. If your work will involve generating any of the wastes previously described, or any of the wastes in the table, then you will most likely need to segregate and manage some portion of your research waste as biohazardous waste. Additional training may also be required for handling these materials. If handle any of the following materials Contact Environmental Health and Safety (EHS) for information.

| Regulation | Activities covered by this standard | Biohazardous wastes |
|---|---|--|
| OSHA's Bloodborne Pathogens Standard | Work with human-derived materials including clinical and unfixed anatomical specimens, human cells and cell lines. | Those wastes that are contaminated to the extent where fluids can drip off or flake off of waste; liquid wastes; fresh (unfixed) tissues; sharps. |
| NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules | Work with molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from the replication of those previously described; regardless of whether work meets "exempt" criteria or not, all recombinant DNA work is to be carried out in accordance with biosafety level 1 containment practices at a minimum. | All contaminated solid and liquid wastes including sharps. |
| CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories" (BMBL) | Lab and animal studies involving work with microorganisms that can cause disease in humans; under certain circumstances, lab and animal studies involving microorganisms that are infectious to animals; diagnostic laboratory operations involving human or animal clinical specimens. | All cultures, stocks and items contaminated with these materials; in some cases, animal bedding and carcasses; biohazardous sharps. |
| USDA APHIS Permits | Work with any animal or plant-derived materials or pathogens that require an APHIS permit to receive or retain the material. | Permits will outline specific waste treatment requirements for the material in question. However, this usually involves segregation and biological inactivation of the material prior to disposal. |

II. Biohazardous Waste Categories

There are 4 general categories of biohazardous wastes based on the physical form of the waste. Each form must be segregated, identified, decontaminated and disposed of in an appropriate manner for the form in order to minimize occupational exposure and environmental release risks.

Biohazardous waste in any form should not be left untreated and unsecured in areas that are accessible to the public (i.e., left in hallways). Only lab personnel should remove treated biohazardous waste from the lab area and transport it to waste holding areas for final disposal.

A. Solid Biohazardous Waste (non-sharps)

This includes any non-sharp item that is contaminated with human or animal diagnostic specimen material (i.e., body fluids, tissue debris), any microbiological culture material (including recombinant DNA).

Examples include but are not limited to:

- Gloves and other disposable PPE contaminated with specimen or culture material.
- Plasticware such as pipettes or pipette tips, culture plates, specimen vials, etc. that are contaminated with biological specimens, bacterial and cell culture material, or nucleic acids.
- Towels and bench paper that are biologically contaminated (Note: Bench paper that is used in areas where samples or cultures are opened and manipulated must be regarded as biologically-contaminated and therefore removed and managed as solid biohazardous waste).
- All culture or sample containers that are contaminated with biological materials.
- Tubes of blood (note: glass blood vials that could break easily upon disposal should be segregated as sharps waste; see below).

Storage:

This type of waste must be collected for final treatment and disposal in a leak-proof container lined with a bag of moderate thickness to prevent punctures. An autoclavable red liner or a liner with the biohazard symbol may only be used if the material is infectious or potentially infectious. If the biological



material is not infectious to humans, an autoclavable clear liner should be used. The collection container must have a lid or other means of closure and the outer container must be labeled with the biohazard symbol regardless of the lab's operating biosafety level. Bench top containers should be used for collection of small quantities of contaminated dry goods (i.e., pipette tips, centrifuge tubes, etc.). Small plastic containers or wire bag racks lined with a biohazard bag are suitable for bench top collection. These containers do not need to have a lid (unless waste is contaminated with a pathogen) but daily disposal of the bag into a larger collection container such as the one shown to the right is strongly recommended.

B. Wastes Requiring Special Consideration

"Breakable" biohazardous wastes:



Tubes of blood and other "breakable" biohazardous waste can be troublesome to manage properly and safely for treatment and disposal. For small amounts of "breakable" biohazardous waste, these items may be placed in biohazardous sharps containers for disposal. However, if your lab generates a large amount of "breakable" biohazardous waste, please contact EHS for assistance with finding solutions for safer waste management.

Serological pipettes:

All plastic pipettes, regardless of contamination status should be segregated from other lab wastes because they readily puncture waste and trash bags which increases spill potential.



Treatment and disposal:

The purpose of solid biohazardous waste treatment is biological inactivation in a manner that reduces hazardous exposure risk for lab personnel and the environment. This is generally achieved by autoclave treatment of waste or treatment and disposal through a medical waste disposal contractor (i.e., licensed medical waste hauler) who will autoclave or incinerate the waste. Under the TDEC regulations, wastes are to be “rendered non-infectious by sterilization techniques prior to disposal”. This means that all items contaminated with a potentially infectious material must be autoclaved or managed through a medical waste disposal contractor for disposal.

Disinfection (i.e., bleach disinfection) of items such as serological pipettes contaminated with human cells does not preclude the need to manage these items as biohazardous waste for final treatment and disposal.

On-site Autoclave Treatment:

Autoclavable waste bags must be used in biohazardous waste collection containers. Bags must be placed in a secondary container (i.e., tray with raised sides), which is placed on a cart for movement to the autoclave facilities.

Practice notes on biohazard bags:

- Biohazard bags are a one-way means of disposal. Do not “dump” the contents from one biohazard bag into another as this action spreads contamination and increases your exposure to this waste.
- Biohazard bags need to be contained at all times during the collection, treatment, and disposal process. Some lab items may puncture bags and this can lead to leaks and spills. Bags awaiting autoclave treatment should be stored in trays, tubs, buckets, etc. (The only exception to this practice is when small quantities of biohazardous wastes that do not contain liquids are collected temporarily in bench top containers.)
 - Biohazardous waste bags awaiting treatment should always be stored in pans or secondary containment to prevent spills!

Ethidium bromide waste is to be collected in a leak-proof container with a lid lined with a sturdy, non-descript bag. The TTU Hazardous Waste label should be placed on the storage container as well. Biohazard bags must not be used for collection of other hazardous wastes. No biohazard labels!



If your lab works with human-derived materials or other materials that are an infectious disease risk for humans (i.e., RG2 / BSL-2 containment), you must use bags that bear the biohazard symbol. The bags should have a built-in heat indicator that allows for verification of autoclave treatment. Otherwise, autoclave indicator tape should be placed on the bag before autoclave treatment.

If your lab does not work with human-derived materials or other materials that are an infectious disease risk for humans (i.e., RG1), you may use bags that do not have the biohazard symbol. However, all biohazardous waste must be clearly identified as such from the point of generation through the point of treatment or biological inactivation. Therefore, if you chose to use autoclave bags without a biohazard symbol, you must employ some other means to identify the material as a biohazard. An example of how this can be achieved is placing the waste in a designated, biohazard-labeled secondary container for storage and transport. The waste bag can then be transferred from the biohazard-labeled secondary container to a regular autoclave pan for final placement in the autoclave.

Autoclave treatment of this waste must be performed in accordance with the biohazardous waste treatment parameters established for the autoclave. The treatment parameters can be established using a chemical integrator strip or a biological indicator with every load or at least quarterly. Note: Only personnel who have received training regarding the operation of the autoclave should use this device.

After the waste is treated according to established parameters, the autoclaved waste is cooled to room temperature and placed into a non-see-through black bag for final disposal.

Some departments regularly schedule pickup and disposal of waste through a medical waste disposal company contracted by EHS. In this case, the same procedures apply as above. However, bags do not have to be autoclavable, nor should they be placed in a non-see-through black bag.

Field Generation:

Waste should be collected and stored as previously outlined. Contact EHS for assistance with identifying disposal options. If you plan to transport waste for treatment and disposal, please refer to guidelines at the end of this document for this activity.

C. Liquid Biohazardous Waste

This includes bulk quantities of blood, blood products, body fluids from human and animal research origin and culture media. Note: Disposable primary containers or sample containers containing small quantities of liquids (less than 10 mls) should be managed as solid biohazardous waste.

Storage:



These liquids must be stored in closed, leakproof containers while awaiting treatment and disposal. Collection vessels should be secured so that they cannot be tipped over. Secondary containment is strongly recommended and can be achieved by placing the vessel in a bucket or deep tray.

Storage vessels or the secondary container must be labeled with the biohazard label if the liquids will not be treated and disposed of within the shift. If disinfectant is added to the vessel, provide labeling so that the chemical hazard is identified as well. For instance, if your collection flask contains waste cell media

and bleach, you should place biohazard label on the flask (or secondary container) as well as the words “bleach-treated cell culture materials” to properly identify both the chemical and biological hazard.

Treatment and disposal:

Liquid wastes may be treated and disposed of by either one or the other of the following methods:

- Chemical treatment of liquids with disinfectant; disposal via lab sink. Disinfectants may be used for “treatment” of liquid biological waste to prohibit growth of microorganisms. Here is an example for the use of household bleach.
 - Add household bleach to the collection vessel so that the bleach makes 10% to 15% of the final volume. Allow a contact time of at least 30 minutes. Carefully discharge the mixture to the sanitary sewer by way of the lab sink, and then thoroughly rinse down the sink with water. Remember to wear splash goggles, gloves, and a lab coat for handling of bleach and bleach-treated liquids.
 - NOTE: Diluted bleach solutions may go down the drain in most cases. However, many chemicals used for disinfection cannot be discarded down the drain. Contact EHS to determine if sink disposal of disinfectants other than diluted bleach solutions is acceptable.
- Autoclave treatment of liquids; disposal via lab sink. Place the closed collection vessel in a secondary container and transport by cart to the autoclave facilities. Treat by autoclave using the liquids cycle. (Remember to loosen or remove the closure on the vessel before placing in autoclave.) Discharge cooled, treated liquids to the sanitary sewer by way of the lab sink. Note: Only personnel who have received training regarding the operation of the autoclave should use this device.
 - Safety Note: PLEASE do not autoclave liquids containing chemical disinfectants!

D. Biohazardous Sharps

A biohazardous sharp is any device that is sharp enough to puncture the skin and that is contaminated with a biological material that is an infectious disease transmission risk, or an environmental release risk (i.e., recombinant DNA). Examples include but are not limited to:

- Needles, disposable syringes, capillary tubes & scalpels contaminated with human or animal blood.
- Microscope slides contaminated with unfixed human or animal specimen materials.
- Pasteur pipettes contaminated with cell culture waste media.
- Small glass/broken tubes of blood or microbiological cultures.

Storage:

Biohazardous sharps containers are those containers which are specifically designed for the collection and disposal of biohazardous or medical sharps. (Recycled food or reagent containers are NOT acceptable for collection and disposal of biohazardous sharps!)

A biohazardous sharps container is:

- constructed of puncture-resistant material,
- leak-proof on the sides and bottom,
- marked with the biohazard symbol, and



- has a restricted opening to prevent items from coming back out of the container, and to prevent someone from sticking their hand inside.

To protect yourself and others in your work area, place biohazardous sharps in a properly assembled (i.e., lid installed) biohazard sharps container immediately after use. This can be achieved by placing sharps containers within arm's reach of where biohazardous sharps are used.

Safety note: Do not recap needles. Do not bend or break sharp devices. Do not overfill sharps containers or use force to get an item into a sharps container. If your work involves handling sharps refer to the TTU "*Safe Sharps Handling SOP*" for additional information.

Treatment and disposal:

All sharps containers must be permanently closed and disposed of when $\frac{3}{4}$ full or whenever items do not freely fall into the container.

Disposal of biohazardous sharps containers will be accomplished through a medical waste disposal contractor coordinated through EHS. Please do not dispose of biohazardous sharps containers in the trash, regardless of treatment status.

Sharps containers must be permanently closed and wiped down with a disinfectant prior to removal from the lab and for disposal through EHS. If there are any liquids present in the biohazardous sharps container, it must be placed in a leak-proof secondary container with a secure lid (and a biohazard label) for transport to the waste collection site.

III. Procedure for Transporting Biohazardous Wastes

Whenever possible, biohazardous wastes should be treated and disposed of on-site. However, generation of biohazardous wastes in the field is often unavoidable and handling and transport will be necessary. Please follow the steps below in order to manage and dispose of these materials safely.

- Wastes that are generated in the field must be segregated and collected using the same principles as outlined for the lab environment.
- Bags of waste and sharps containers should be closed before removal from the site. (Bulk quantities of liquid waste should not be transported if at all possible. Contact EHS for assistance if the need arises to transport such material.)
- Bags of waste and sharps containers must be placed in a leak-proof secondary container with a secure lid (i.e., latchable, secured with tape, etc.) for transport to treatment facilities. The secondary container must be labeled with a biohazard symbol and an emergency contact name and phone number.
- Use a University-owned vehicle whenever possible for transport. Store and secure the transport container in a location in the vehicle whereby if an accident were to occur, the container or its contents will not be an exposure risk to the driver or the environment. For example, if transporting materials by car or van, store the container in the back seat or cargo bay. Secure the container with bungee cords or belts to keep the container upright and stable.
- When you arrive at your destination, transport the waste into the facility using the shortest available route, and move the materials with the aid of a cart. Do not use public elevators if at all possible and avoid traveling with the waste through common public areas. Do not touch door

handles, elevator buttons or other common contact surfaces with gloved hands. (Use the one-gloved hand technique, or get assistance from other staff for opening doors, etc.)

IV. References and Associated Standards

- Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard 29 CFR 1910. 1030
- Tennessee Department of Environmental Conservation (TDEC) Division of Solid Waste Management Rule 1200-1-7
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
- CDC/NIH “Biosafety in Microbiological and Biomedical Laboratories” (BMBL)
- University of Tennessee Biohazardous Waste Disposal Guide