

Biohazardous Waste Disposal Guide

I. DEFINITION OF BIOHAZARDOUS WASTE

Biohazardous waste is any waste generated from working in biological or biomedical laboratories that may contain infectious or potentially infectious substances or any agents or substances that are an environmental release risk (i.e., recombinant DNA). This includes materials that may present an actual or perceived biological risk to others on site. By using appropriate collection (autoclave bags in plastic pails) and decontamination methods (chemical disinfection, autoclaving) labs can render biohazardous waste to a non-hazardous state before collection and removal.

II. EXAMPLES OF BIOHAZARDOUS WASTE

- Microbiological cultures or stocks (including bacterial, viral, parasitic, fungal, etc.)
- Recombinant or synthetic nucleic acid molecules (including viral vectors)
- Organisms or cells that contain recombinant or synthetic nucleic acid molecules
- Cell/tissue cultures
- Anatomical or pathological waste (organs and tissue from humans or animals)
 - *NOTE: Collect ALL animal carcasses and animal anatomical waste for disposal by the Center for Comparative Research (CCMR). Collect all human anatomical waste for disposal by Environmental Health & Safety (EHS) or Clinical Pathology as applicable. These materials require special handling and disposal – see Sections VI.D and VI.E.*
- Human clinical specimens (feces, blood, urine or any other bodily fluid)
- Contaminated animal bedding
- Disposable personal protective equipment (PPE)
- Labware potentially contaminated with biohazardous agents (flasks, plates, pipets, tubing, etc.)
- Sharps (scalpels, razor blades, Pasteur pipettes, needles, syringes, etc.)

III. UNIVERSAL PRECAUTIONS

Applying a universal precautions approach in working with biohazardous materials is a prudent standard (i.e., managing all research biological materials as if they contain infectious or potentially infectious substances or are an environmental release risk). The Dartmouth Institutional Biosafety Committee (IBC) recommends that all biological wastes generated from research labs be managed as biohazardous waste. This action will assure that all biological research materials are inactivated or managed in a manner that minimizes the exposure risk for the general public and the environment.

IV. REGULATIONS

Unlike hazardous chemical or radioactive waste, there is no single federal agency that clearly defines and regulates biohazardous waste. Several agencies, some associated with research funding, have unique waste disposal requirements. Therefore, it is the researcher's responsibility to have a general knowledge



of biosafety regulations and guidelines and how they apply to their work and the waste that is generated through the research process. Please review the regulatory/agency information in the following table.

Summary of Federal Regulations/Guidelines for Biohazardous Waste

Regulation	Activities covered	Biohazardous waste
OSHA’s Bloodborne Pathogens Standard 29 CFR 1910.1030	Work with human-derived materials including clinical and unfixed anatomical specimens, human cells, and cell lines.	Liquid or solid waste from cultures or tissue prep; fresh (unfixed) tissues; sharps; labware; PPE.
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (<i>NIH Guidelines</i>)	Work with molecules that are constructed outside living cells by joining natural or synthetic DNA molecules that can replicate in a living cell, or molecules that result from the replication of those therefrom. All recombinant DNA work is to be carried out at BSL-1 containment practices and procedures 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 cause disease in humans and/or in animals; diagnostic lab operations involving human or animal clinical specimens.	All cultures, stocks, and items contaminated with these materials; in some cases, animal bedding and carcasses and 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. This usually involves segregation and inactivation of the material prior to disposal.

V. GENERAL INSTRUCTIONS

- i. Never place non-hazardous items in biohazard bags. Once combined they cannot be separated.
- ii. Hazardous chemicals and radioactive waste must never go into a biohazard bag. Creation of mixed waste should be avoided. Please contact EHS for assistance in ways to avoid the creation of such waste and for proper disposal if it cannot be avoided.

NOTE: Phenol waste associated with a DNA extraction is not a biohazard, it should be disposed of as hazardous chemical phenol waste.

- iii. Do not leave biohazardous waste in hallways, equipment rooms or areas with public access.
- iv. Liquids must be decontaminated with bleach (final 1:10 concentration) or 0.5% Wescodyne for 30 minutes before pouring the solution down the drain with a 10-fold excess of water.
- v. Do not autoclave liquid blood or other body fluids as they tend to congeal and make a mess; chemically decontaminate instead.

NOTE: Clotted blood must always be autoclaved (60 minutes at 121°C, slow exhaust) in lieu of chemical disinfection. Clotted blood is known to clog drains.



- vi. All aspiration traps must utilize an in-line HEPA filter to protect the building vacuum system. There should be fresh bleach or Wescodyne in the collection flask to decontaminate the aspirated waste as it is collected. Final concentration of bleach should be 1:10 and final concentration of Wescodyne should be 0.5% for proper decontamination.

VI. PROTOCOLS FOR SPECIFIC WASTE STREAMS

A. **SOLID, non-sharp biohazardous waste** (*gloves, flasks, test tubes, plastic pipettes etc.*)

- i. EHS provides 5 gallon plastic pails designed for the collection of biohazardous waste for autoclaving.
- ii. **Autoclave bags must always be orange** emblazoned with the word “biohazard” and the international biohazard emblem. NEVER USE ANY OTHER COLOR BAGS FOR AUTOCLAVED WASTE! The landfill may reject the shipment if they see non-orange biohazard bags or bags that have not obviously been autoclaved.
- iii. Do not fill autoclave bags beyond $\frac{3}{4}$ full.
- iv. Add ~250 milliliters of water to a standard-size orange biohazard bag before closing. This step is imperative to ensure the creation of steam within the bag. Use proportionately less water for smaller bags.
- v. Use a twist tie or piece of autoclave tape to loosely close the bag. Put an additional strip of autoclave tape on the side of the bag.
- vi. Never place autoclave bags directly on the floor. Bags must be placed in a secondary container or tray for transport and autoclaving. Use the metal trays provided by EHS. Avoid the use of Nalgene® since it is a poor conductor of heat and certain polymers are not autoclave-safe.
- vii. Autoclave tape only indicates exposure to heat – not adequate heat, time, or pressure. Therefore, darkened autoclave tape is only a marker that the bag has been in an autoclave, not that it has been sterilized. Failed cycles must be re-run even if the tape darkened. Use fresh tape for each cycle.
- viii. Autoclave operation must be validated quarterly with a biological indicator in conjunction with EHS. Autoclave tape that does not darken after a cycle indicates a problem with the autoclave. If you suspect your autoclave is not working properly, please contact EHS or Facilities.
- ix. If any liquid leaks from biohazard bags while in collection pails or while transferring to an autoclave tray, clean these spills immediately with a suitable disinfectant (fresh 1:10 dilution of bleach or 0.5% Wescodyne). Autoclave any leaking bags as soon as possible.
- x. Process for 60 minutes at 121°C (15 psi) if using a metal pan. If the use of a Nalgene plastic tray is unavoidable, you must process the load for 75 minutes.
- xi. After removal from the autoclave, allow the bag to cool completely. When cool, the autoclaved bag can be disposed of as general trash as directed in your building. Housekeeping will remove the autoclaved bags ONLY if they have been properly autoclaved and placed into the regular trash cans lined with clear bags as directed. They will not remove any bags left on the floor or in



the autoclave. NEVER dump any liquid that leaked from the bag down the drain until it has cooled completely. Molten agar will clog drains!

B. LIQUID biohazardous waste (*cell culture media, supernatant, blood and other bodily fluids*)

- i. For each 100 mL of biohazardous liquid, add 10 mL of sodium hypochlorite (household bleach) or 0.5 mL Wescodyne® (povidone iodine with detergent).
- ii. Mix and let stand 30 minutes.
- iii. Pour the mixture down a lab sink followed by a ten-fold excess of water.
- iv. If waste was disinfected directly in flasks or beakers, wash those vessels with soap and water after disinfection. If fluids were pooled for disinfection, the disposable plastic vessels the liquid was originally in must be autoclaved before disposal. Contaminated glassware must be soaked in 10% bleach or 0.5% Wescodyne before washing and reuse.

C. SHARPS (Needles, Syringes, Razor Blades, Glass pipettes)

- i. Place all sharps in biohazard puncture resistant containers obtained from the scientific stockroom or other suppliers. Use red bins on Hanover campus and pink bins at DHMC.
- ii. Never recap, bend, remove or clip needles. Never reach into a sharps container!
- iii. When $\frac{3}{4}$ full, snap lid closed and tape to securely shut.
 - In Borwell/Rubin/Williamson place the taped, closed containers next to the regular trash receptacles for disposal by DHMC housekeeping staff. Overfilled containers, open, or untaped containers will not be picked up.
 - In all other areas of the College, email ehs@dartmouth.edu for pick-up.

D. HUMAN Anatomical Tissue Waste

- i. Unfixed, unpreserved samples should be double bagged in black plastic, labeled and then frozen. Do not autoclave. Contact ehs@dartmouth.edu for disposal.
- ii. Fixed or preserved specimens should be double bagged in black plastic, labeled and have a Hazardous Waste Label attached indicating the preservative used. Do not autoclave. Contact ehs@dartmouth.edu for disposal.

E. ANIMAL Anatomical Tissue Waste and Bedding

- i. Animal carcasses, tissue and/or hair from animals must be double bagged in black plastic and stored in the refrigerator or freezer until collected for commercial incineration. CCMR disposal sites are listed below. For other areas of campus, contact CCMR staff.
 - In Borwell/Rubin/Williamson: Place the bag in the gray bin in the refrigerator located in the Borwell CCMR (room 382O02) or in the cooler in the Williamson CCMR (room 274F02).



- In Vail/Remsen: Place the bag in a plastic bin in the refrigerator in the Vail CCMR (001B).
- In Moore: Place the bag in the freezer in B52 (by the elevator).
- ii. Animal bedding contaminated with chemical hazards or biohazards must be disposed of according to the CCMR SOP “Hazards” (#510.20). These materials will be incinerated or autoclaved based on a risk assessment by the PI, IACUC, and EHS.
- iii. Animal carcasses/bedding containing radioactivity will be handled on a case-by-case basis by EHS. **Contact ehs@dartmouth.edu prior to beginning any experiment that may generate this material, even if Radiation Safety Committee approval has already been granted.** EHS assesses waste plans during initial approval but needs to prepare for the actual event.
- iv. Non-contaminated animal bedding is disposed of by the CCMR staff into the compactors located at the Borwell and Vail loading docks.

F. Biohazardous Material Combined with Radioisotopes

NOTE: Contact ehs@dartmouth.edu prior to beginning any experiment that may generate this material, even if Radiation Safety Committee approval has already been granted. EHS assesses waste plans during initial approval but needs to prepare for the actual event.

- i. Liquids must be disinfected by adding a suitable disinfectant to the container (10 mL bleach or 0.5 mL Wescodyne for each 10 mL of liquid to be disinfected). Note the infectious agent and disinfectant on a Hazardous Waste Label. Also, attach a completed Radioactive Waste Tag to the container. Dispose of all such liquid through EHS.
- ii. Solid material such as pipette tips and flasks must be soaked in a suitable disinfectant (10% bleach or 0.5% Wescodyne). Rinse and check for residual radioactivity in liquid. Dispose of all such liquids and labware through EHS.

VII. RESOURCES

- *Biosafety in Microbiological and Biomedical Laboratories*, 6th ed. Centers for Disease Control and Prevention and National Institutes of Health, 2020 June
<https://www.cdc.gov/labs/BMBL.html>
- *Bloodborne Pathogens*. Occupational Safety and Health Administration, 29 CFR 1910.1030
<https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030>
- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. National Institutes of Health Office of Science Policy, 84 FR 17858, 2019 April
<https://osp.od.nih.gov/biotechnology/nih-guidelines/>