



Safe Operating Procedure

(Revised 4/24)

DISPOSING OF BIOHAZARDOUS MATERIALS, INCLUDING RECOMBINANT OR SYNTHETIC NUCLEIC ACIDS

Scope

This SOP applies to all work at UNL that is subject to the **UNL Biosafety Guidelines**. The content of this SOP is based on requirements established by the following standards:

- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*, National Institutes of Health
- *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, Centers for Disease Control and National Institutes of Health
- *Bloodborne Pathogens Standard, 29 CFR 1910.1030*, Occupational Safety and Health Administration

For the purposes of this SOP, the term “biohazard” applies to:

- Recombinant and Synthetic Nucleic Acids (r/sNA) in all forms, natural and synthetic (e.g., DNA, RNA, shRNA, etc.)
- Any waste material that is potentially infectious to humans, plants, or animals or contains human, animal or plant pathogens.
- Biological toxins
- Genetically-modified organisms, including animals, plants, microorganisms and arthropods
- Any material containing any of the above

General guidance as well as classes of biohazardous materials and their associated appropriate methods of disposal are described below.

General Guidance

Biohazardous waste must be treated prior to disposal. Most often this involves on-site autoclaving. In some cases, chemical disinfection may be appropriate. When neither of these options are feasible or appropriate, biowaste may be sent to an off-site vendor for treatment and disposal.

Chemical Disinfection

Waste items in a solid state that do not meet the definition of a hazardous or special waste and are chemically disinfected can be disposed of in the regular trash after sufficient contact time with the disinfectant. See the EHS SOP, **Chemical Disinfectants for Biohazardous Materials** for guidance on appropriate disinfectants and contact times. See the EHS SOP, **Items/Materials Prohibited from Trash Cans and Dumpsters** for more information on regulated waste management. Wastes in a liquid state that do not meet the definition of a hazardous or special waste and are chemically disinfected can be disposed via the sanitary sewer after sufficient contact time with the disinfectant. See the EHS SOP, **Sewer Disposal List** for more information on wastes suitable for sanitary sewer disposal.

Use of Vacuum Systems

Vacuum trap systems are commonly used in labs to remove supernatant and other waste liquid from cell and microbe culture plates and tubes. When setting up these systems it is essential to protect the vacuum line by utilizing a trap containing an effective disinfectant (see Figure 1). Vacuum aspiration systems in BSL-2 or higher labs must, at a minimum, have a trap containing an effective disinfectant **and** an in-line HEPA or equivalent filter (BSL-2/BSL-3 required components have an '*' next to them in Figure 1). Use of an overflow container after the trap and before the in-line HEPA filter is optional. Recommended filters are Cytiva Whatman™ HEPA-Vent Filter (Fisher #09-744-79) or MilliporeSigma™ Millex™ Filter (Fisher # SLFG85010). Filters should be checked regularly and replaced, as necessary.

Vacuum Flask Use Procedure:

1. Prior to adding media, fill the flask with bleach or other approved disinfectant to achieve proper concentration when flask is full.
2. Label flask with the biohazard symbol, material to be collected, and disinfectant concentration. (e.g. *Tissue culture media with 10% bleach*)
3. Include an in-line hydrophobic HEPA filter between the flask and the vacuum outlet. It is recommended to date the filter when installed.
4. When 2/3 full, waste liquid can be disposed of down the sanitary sewer if it has been ≥ 1 hour since liquid was last added to the flask. If hazardous chemicals are present in the media (e.g. methanol, selenium, heavy metals, etc.) the liquid waste must be collected



Figure 1 Vacuum aspiration system setup to protect a vacuum line.

by EHS. In this case, disconnect the flask from the vacuum line, and pour the liquid into a collection container that can be closed and tag it for pick up by EHS.

Off-site Treatment

Biohazardous waste that is managed through EHS for off-site treatment must be accumulated in specific containers. Contact EHS for additional information.

On-site Autoclaving

For a full discussion of autoclave use for disposal of biohazardous waste refer to the EHS SOP, **Autoclave Operation and Use**. Following is a summary of information contained in the referenced SOP.

Biohazardous waste that is autoclaved on-site to render it non-infectious or non-viable must be collected in bags that are rated for autoclave use and the maximum temperature of the autoclave waste cycle used. If pathogenic to humans, the bags must also be marked with the universal biohazard symbol. Autoclave tape must be affixed to the container prior to treatment (select a lead-free autoclave tape); the tape should be placed over the biohazard symbol in a "X" pattern. Any biohazard labels and markings not covered by tape must be obliterated following treatment and prior to disposal. Autoclaved waste bags must be placed in unmarked outer bags that are dark or opaque (i.e., not see-through) prior to disposal, and all liquids must be absorbed onto a solid media prior to placement in refuse containers or if allowed, they may be disposed via the sanitary sewer. Sewer disposal is limited to nutrient media and dilute disinfectants that are otherwise non-hazardous (do not contain chemical constituents regulated under hazardous waste laws, such as heavy metals, solvents, etc.). Contact EHS if you are unsure if a liquid can be sewer disposed.



Do not use red biohazard bags for any type of waste collection other than biohazardous waste (i.e., regular trash, radioactive materials not contaminated with biohazards).

Autoclaves used to decontaminate biohazardous waste must be regularly serviced, maintained and tested for performance using a biological indicator. See the EHS SOP, **Autoclave Performance Testing** for guidance. Biohazardous waste must not be disposed if the autoclave cycle did not achieve the desired performance; the waste cycle must be repeated until the required parameters for waste decontamination are achieved.

Autoclave waste cycles must meet certain requirements to ensure that the cycle is effective. Sterilization times for biohazardous waste can range from 30 minutes to 90 minutes, and temperatures can range from 121°C to 134°C. The selected parameters must be appropriate to the unique attributes of the specific biohazardous waste. It is not necessary to use a solid waste cycle for solid biohazardous waste decontamination. If a liquid cycle meets the required parameters, then that cycle (sterilization time of ≥ 30 min) will be appropriate as well. Keep in

mind that the time mentioned here is the sterilization time; the entire run will include additional time to reach sterilization temperature and then cooling/drying time.

Specific Waste Types

- **Human blood and other potentially infectious body fluids** and contaminated materials must be decontaminated prior to disposal as solid waste refuse or managed through EHS. Generally, this type of waste is decontaminated on-site by autoclaving or collected by EHS and disposed via a vendor. This material must not be incinerated on-site.
- **Laboratory waste from infectious agents** (i.e., culture plate and broths) must be autoclaved prior to disposal. Chemical disinfection is generally suitable for contaminated disposable and non-disposable laboratory equipment and consumables (i.e., pipette tips, glass, cuvettes, etc.). This material must not be incinerated on-site.
- Some, but not all **Biological Toxins** can be deactivated by autoclaving. Minimum cycle parameters for biological toxins are 121°C for ≥ 1 hour using a liquid cycle with slow exhaust. Contact EHS to confirm the specific toxin is compatible with autoclaving prior to proceeding.
- **Recombinant/synthetic nucleic acid-containing materials** (i.e., cultures, microbes, plasmids) must be decontaminated or inactivated before disposal. Chemical treatment with an appropriate disinfectant is an acceptable method of inactivation. Autoclaving of r/sNA-containing materials is also acceptable.
- **Research animal carcasses** and parts, contaminated bedding and feeds, excreta, etc. are generally disposed via incineration, autoclave or decontaminated by chemical treatment prior to disposal. If necessary, autoclaving of small animals (rodents, birds, rabbits, etc.) is possible, but should be done in consultation with EHS to ensure appropriate cycle parameters for complete decontamination. Cages and other non-removable, non-porous surfaces can be treated by chemical disinfection or autoclaved.



Note: For additional restrictions related to on-site incineration, refer to the EHS SOP, **Pathological Waste Incinerators - Operating Permit Requirements**.

- **Soils and other plant-related materials** Soil and plant material (i.e., leaves, shoots, stalks, etc.) that contains r/sNA or is contaminated with plant pathogens must be inactivated prior to disposal. Inactivation by autoclaving requires sufficient run time and specific temperatures and pressures. Soil is more difficult to inactivate, as it is very dense. See the EHS SOP, **Autoclave Operation and Use** for guidance on autoclave parameters for inactivating soil and plant material.

- **Animal diagnostic specimen shipping containers**, if contaminated, must be treated by autoclaving or chemical treatment prior to disposal, incinerated, or managed through EHS for disposal.
- **Sharps**, including glass, razor blades, needles, and other objects that can penetrate the skin and are potentially contaminated with biohazardous materials must be accumulated in rigid, leak-proof, labeled, containers (Figure 2). Full containers must be autoclaved or managed through EHS. Special marking, coloration, and other requirements apply to sharps contaminated with bloodborne pathogens. For more information refer to UNL's *Bloodborne Pathogen Exposure Control Plan*, which is available on the EHS web site.



Figure 2 Contaminated Sharps Collection Containers

If a red biohazard plastic container is used for sharps contaminated with biohazardous material that is not pathogenic to humans, the container must have the biohazard symbol thoroughly defaced and be clearly marked as “**Non-Human Biohazardous Sharps.**” (Figure 3). These containers should be autoclaved or disposed of through EHS when 75% full.

- **Pipettes and Pipette Tips** contaminated with biohazardous materials must be decontaminated prior to final disposal. Decontamination can be achieved by autoclaving or chemical treatment with an appropriate disinfectant.
 - Contaminated pipettes and pipette tips to be decontaminated by autoclaving should be collected in rigid, leak-proof, sealable, and labeled containers.



Figure 3 Non-Human Biohazardous Sharps Containers

- Containers can be the final collection container that will be autoclaved like a red sharps box or they can be temporary and emptied at the end of the working shift into a larger biohazardous waste collection container. **Temporary collection containers must be closed when not being actively filled or left unattended.**
- Collection containers **must** be closed when not actively being filled and labeled with the biohazard symbol if they are used to collect pipettes/pipette tips contaminated with human biohazardous materials. See example containers in Figure 4A.
 - A sturdy cardboard box lined with a biohazard bag is an acceptable alternative collection container for longer potential sharps like serological pipettes (Figure 4B).
 - A shallow pan with a lid (Figure 4C) can be filled with an approved chemical disinfectant and used to collect contaminated pipettes and tips. This is the recommended disposal method when working with serological and other pipettes in a biosafety cabinet.
 - Pipettes and tips collected after decontamination, should be placed in a rigid and sealable container for disposal (e.g. sturdy cardboard box).

Figure 4 Contaminated Pipettes and Pipette Tip collection containers

