



Safe Operating Procedure

(Revised 1/26)

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 biological 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, contact EHS. The biowaste may be sent to an off-site

vendor and/or incinerated for treatment and disposal. On-site incineration is restricted to the conditions in UNL's permit and must be authorized by EHS.

Chemical Disinfection

Chemical disinfection is best reserved for nonporous, solid surfaces and items that cannot be autoclaved. Ensure that the entire surface is thoroughly soaked with the pathogen-specific disinfectant and that the recommended contact time is observed. Remove any organic material and dispose of it in a biowaste container for autoclaving, then proceed to wash the equipment, if applicable. 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. 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 (See 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. Label flask with the material to be collected, and disinfectant used. (e.g. *Tissue culture media % of disinfectant chemical compound*). Do not use bleach or ethanol as the disinfectant in vacuum traps. Bleach and alcohol cannot be autoclaved.
2. Include an in-line hydrophobic HEPA filter between the flask and the vacuum outlet. It is recommended to date the filter when installed.



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

3. Prior to aspirating media, add an approved concentrated disinfectant to the flask to achieve proper concentration when flask is full. Flush disinfectant through the tubing after each use.
4. When 2/3 full, waste liquid should be autoclaved. If hazardous chemicals are present in the media (e.g. methanol, selenium, heavy metals, etc.) the biohazardous liquid waste must be collected 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 tagged 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 solid and liquid 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 or liquid containers that are rated for autoclave use and the maximum temperature of the autoclave waste cycle used. If pathogenic, the bags must also be marked with the universal biohazard symbol. Autoclave indicator 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 solid biohazard waste bags must be placed in unmarked outer bags that are dark or opaque (i.e., not see-through) prior to disposal. **Autoclaved** liquid biohazardous waste can be disposed via the sanitary sewer/ lab sink if the contents are in conformance with the EHS SOP, **Sewer Disposal List**. If the autoclaved liquid waste is not compatible for sink disposal, tag the material for EHS pickup.



Do not use 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 60 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. 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 Biohazardous Waste Types

- **Human cell lines, 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 of via a vendor.
- **Laboratory waste from infectious agents** (i.e., culture plates, culture broths, media waste, used PPE, extraction and assay waste) must be autoclaved or validated inactivated prior to disposal.
- 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, plant, animal, arthropod) must be autoclaved or validated inactivated before disposal.
- **Research animal material, including carcasses, tissue, bedding, husbandry, food and water, excreta, etc.** that contain biohazardous material are generally disposed via incineration or through autoclaving prior to disposal. Autoclaving of small animals (arthropods, mice, small birds) is possible but should be done in consultation with EHS biosafety staff to ensure appropriate cycle parameters for complete decontamination. Animal caging and other non-porous surfaces can be treated by chemical disinfection or autoclaved and washed prior to reuse.



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

- **Soil and plant materials.** Soil and plant material (i.e., leaves, shoots, stalks, etc.) that contain r/sNA or is contaminated with human, animal or 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). Containers must be autoclaved prior to disposal or managed through EHS. Special marking, coloration, and other requirements apply to sharps contaminated with pathogens, including bloodborne pathogens. For more information refer to UNL's *Bloodborne Pathogen Exposure Control Plan*, which is available on the EHS web site. If a red biohazard plastic container is used for sharps contaminated with biohazardous material that is not pathogenic, the container must be clearly marked as "Non-Biohazardous Sharps." These containers should be autoclaved or disposed of through EHS when 75% full.



Figure 2 Contaminated Sharps Collection Containers

- **Pipettes and Pipette Tips** contaminated with biohazardous materials must be autoclaved prior to final disposal.
 - Contaminated pipettes and pipette tips should be collected in rigid, leak-proof, sealable, and labeled 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. 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 or Pasteur 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 pipettes and other pipette tips in a biosafety cabinet.
 - Pipettes and tips collected after autoclaving, should be placed in a rigid and sealable container for disposal (e.g. sturdy cardboard box).



Figure 3 Contaminated Pipettes and Pipette Tip collection containers