Tissue Culture Waste Disposal Guide

Overview

The culturing and preparation of cells or tissues at Weill Cornell Medicine (WCM) generates liquid wastes that must be managed and assessed according to the biological, chemical, and radiological hazards contained within them.

Potentially infectious cell or tissue culture waste media constitute a biological waste that must be disinfected prior to disposal. The Centers for Disease Control & Prevention (CDC) and National Institutes of Health (NIH) recommend that the vacuum systems used to aspirate these wastes combine High-Efficiency Particulate Air (HEPA) filters and liquid disinfectant traps to prevent aerosolized microorganisms from being emitted into the laboratory or the system exhaust. When installed inline, the HEPA filter will isolate and confine infectious materials and prevent aerosol contamination of the vacuum pumps.

This EHS update provides proper disinfection and disposal procedures for tissue and cell culture wastes.

Applicability

This update applies to all generators of tissue culture wastes in laboratories at WCM.

Responsibilities

Environmental Health and Safety (EHS) ensures that the information provided to generators is concurrent with the laws and regulations governing the disposal of these wastes, providing assistance and training as needed.

Generators of tissue culture waste, including laboratory workers, principal investigators and laboratory supervisors, must verify that tissue culture wastes are properly managed and disposed of as established in this update; as well as in the EHS Drain and Trash Disposal of Chemicals Update, and the EHS Program Manuals 3.2 - Research Biosafety and 5.2 - Waste Disposal Procedures.

All laboratory staff must wear the appropriate personal protective equipment (e.g., gloves, lab coats, splash shields, etc.) when working in the lab.

Note: for guidance on biological waste disposal and clinical settings, refer to the applicable sections of the EHS Program Manual 5.2 - Waste Disposal Procedures.

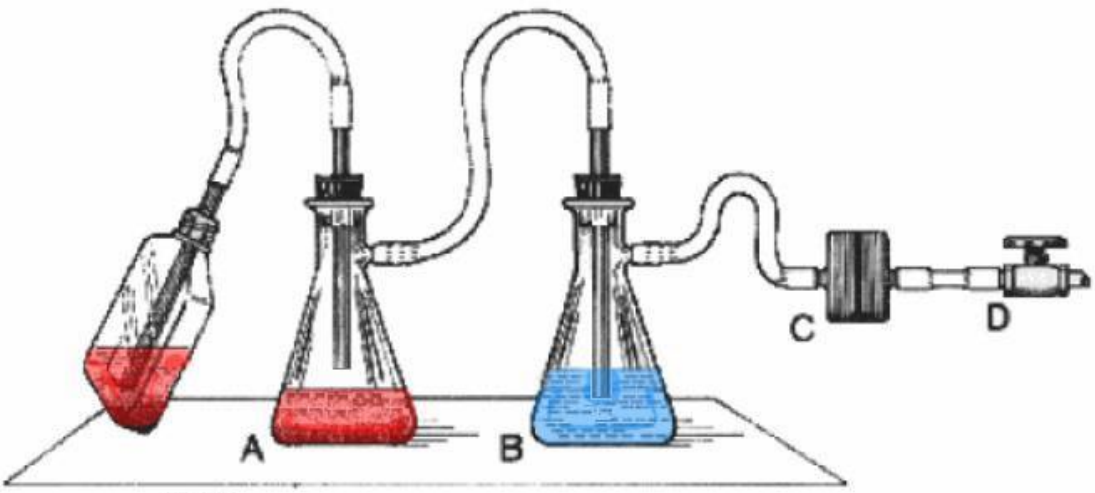
Procedure

Utilize the following procedures to assemble a vacuum flask and to ensure proper disinfection and disposal of tissue culture wastes.

The illustration on the next page shows an aerosol/fluid trap set-up consisting of the following materials:

- Two (2) vacuum flasks (preferably plastic, to avoid breaking/spilling)
- Thick walled plastic tubing (to prevent tubing collapse)
- Glass or plastic tubes
- Rubber stoppers
- HEPA filter

Weill Cornell Medicine
Environmental Health and Safety
TEL 646-962-7233 WEB weill.cornell.edu/ehs EMAIL ehs@med.cornell.edu
Weill Cornell Medicine | 402 East 67th Street, Room LA-0020 | New York, NY 10065



LEGEND
Aspiration Flask (A) - Collect contaminated fluids (tissue culture media, stains, fixative) into suitable decontamination solution (e.g., 10% bleach).
Overflow Flask (B) - Collects overflow fluids and tube submerged in liquid minimizes aerosols.
HEPA Filter (C) - In-line filter prevents contamination of vacuum. See details below.

This aspiration and disinfection mechanism follows the steps detailed below.

1. **SET-UP:** Utilizing thick-walled plastic tubing, aspirate the media from the sample flask (far left flask) to the aspiration vacuum flask (A). The aspiration vacuum flask (A) connects to the overflow flask (B), and the inline HEPA filter (C) protects the vacuum system (D).

   - The left aspiration flask (A) collects the contaminated fluids (tissue culture media, stains, fixative) into a suitable decontamination solution; while the right flask (B) serves as a fluid overflow collection vessel. Please note that the addition of a glass or plastic tube into the solution in flask B minimizes aerosols.
   - Filters must be replaced as needed—at a minimum, annually and when there is any evidence of deficiencies (e.g., filter blockage, failure, wetness). When changing out filters, dispose of them as regulated medical waste, hazardous waste, or radiological waste, as appropriate.

     - WCM Recommends Whatman (**HEPA-Vent** 6723-5000 and **Vacu-Guard** #6722-5000) or Millipore (SLFG05010) filters (shown on the right), available from VWR and Fischer Scientific.

2. **ADD DISINFECTANT**: Fill the Aspiration Vacuum Flask (A) with bleach to ~10% of the flask's volume. If using a different EPA-approved disinfectant, add the volume of disinfectant required to achieve the manufacturer's recommended concentration. **Do not use alcohol-based disinfectants**. Note that when bleach and water are mixed, the solution's disinfectant qualities only last 24 hours. Additional bleach may be required.

3. **LABEL:** Label both flasks with a Tissue Culture Waste Label, available from EHS, and indicate the constituents of the mixture; including the type of tissue culture media, the disinfectant used, and other chemical constituents.

4. **CONTAINMENT:** Place the vacuum flasks in secondary containment (e.g., bin or tray) if outside a biosafety cabinet to hold the liquid if it is spilled or released.

5. **ASPIRATE:** Aspirate the tissue culture waste into the flask containing disinfectant (A). Discontinue use when the vacuum flask is 75% full.

6. **ADD ADDITIONAL DISINFECTANT:** Add the volume of disinfectant required to achieve the manufacturer's recommended concentration (e.g., 10% bleach).
7. **STIR:** Stir to mix then leave at room temperature for 2 hours, or let sit overnight to ensure sufficient contact time with disinfectant.
8. **DETERMINE**: After disinfecting the biological characteristics of the tissue culture waste, the chemical constituents of the waste must be examined for proper disposal. Follow the WCM Drain and Trash Disposal of Chemicals procedure to determine if any of the chemical constituents, other than the disinfectant, are not acceptable for drain disposal (e.g., heavy metals).
   - **Not Acceptable for Drain Disposal = Hazardous Waste:** If there are any chemical constituents, other than the disinfectant, or radiological constituents that are not acceptable for drain disposal, the tissue culture waste must be managed as a hazardous waste as detailed in Manual 5.2 - Waste Disposal Procedures.
     - Collect: Collect the tissue culture waste into a sealable bottle. The bottle must remain sealed/closed at all times except when immediately adding or removing wastes from the bottle.
     - Label: Label utilizing a hazardous waste sticker, available from EHS or printable (seen on the right). Identify all of the chemical constituents by listing full chemical names without abbreviations.
     - Store: Store the waste bottle in a labeled Chemical Waste Satellite Accumulation Area.
     - Dispose: Once full or ready for collection, submit a chemical waste collection request via Salute.

   - **Acceptable = Flush:** If the tissue culture waste contains ONLY chemical constituents that are acceptable for drain disposal then:
     - pH ADJUST: Check and adjust the pH of the waste solution. If needed, either sodium hydroxide or potassium hydroxide can be utilized to produce a solution that has a pH higher than 5.0 and less than 11.0.
     - FLUSH: Flush waste solution into a laboratory sink drain with copious amounts of water.

Definitions

- **Disinfect:** to cleanse so as to destroy or prevent the growth of disease-carrying microorganisms.
- **HEPA Filter:** High Efficiency Particulate Air (HEPA) filters remove the most penetrating particle size of 0.3 µm with an efficiency of at least 99.97%.
- **Tissue Culture:** the growth of tissues and/or cells separate from the organism.

References

- EHS Program Manuals 3.2 - Research Biosafety and 5.2 - Waste Disposal Procedures
- Salute Safety - https://ehs.salutesafety.com/users/sign_in
- EHS Drain and Trash Disposal of Chemicals Update
- Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, HHS Publication No. (CDC) 21-1112 Revised December 2009