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TISSUE CULTURE WASTE DISPOSAL GUIDELINES

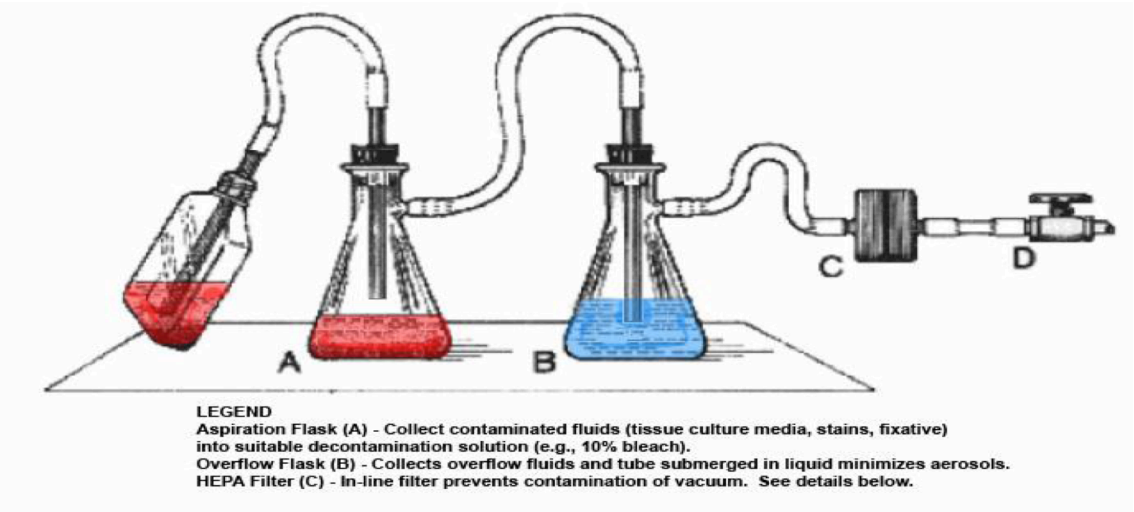
Overview

Potentially infectious cell or tissue culture waste media constitutes 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 are protected with High Efficiency Particulate Air (HEPA) filters and liquid disinfectant traps to prevent aerosolized microorganisms from being emitted into the laboratory or the system exhaust. The HEPA filter, installed inline, will isolate and confine infectious materials and prevent aerosol contamination of the vacuum pumps. These guidelines provide proper disinfection and disposal procedures for tissue culture wastes generated at GW.

Procedure and Set-up

Utilize appropriate personal protective equipment, engineering controls, and the following procedures to assemble a vacuum flask and ensure proper disinfection and disposal of tissue culture wastes. In the following illustration, the aerosol/fluid trap set-up consists of the following materials:

- Two (2) vacuum flasks (preferably plastic, to avoid breaking/spilling)
- Thick walled plastic tubing (to prevent tubing collapse)
- Plastic tubes
- Rubber stoppers
- High Efficiency Particulate Air (HEPA) filter



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.

1. 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) is connected to the overflow

flask (B), an in-line HEPA filter (C) is used to protect the vacuum system (D). The left aspiration flask (A) is used to collect the contaminated fluids (tissue culture media, stains, fixative) into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. Please note that the addition of a 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 chemical waste, or radiological waste, as appropriate. The OLS recommends the following filter options: Whatman (**HEPA-Vent** 6723-5000 and **Vacu-Guard** #6722-5000) or Millipore (SLFG05010)

2. Fill the Aspiration Vacuum Flask (A) with bleach to ~10% of the flask's volume. Do not use alcohol-based disinfectants. Note that when bleach and water are mixed together, the solution's disinfectant qualities only last 24 hours. Additional bleach may be required.

3. Label both flasks and indicate the constituents of the mixture; including the type of tissue culture media, disinfectant used, and other chemical constituents.

4. 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 the tissue culture waste into the flask containing disinfectant (A). Discontinue use when the vacuum flask is 75% full.

6. Add an additional volume of disinfectant required to achieve the manufacturer's recommended concentration (e.g., 10% bleach).

7. Stir to mix, and then leave at room temperature for 2 hours, or let sit overnight to ensure sufficient contact time with disinfectant.

8. If the tissue culture waste contains ONLY chemical constituents that are acceptable* for drain disposal then flush waste solution into a laboratory sink drain with copious amounts of water.

* **NOT ACCEPTABLE FOR DRAIN DISPOSAL:** Liquid waste with chemical constituents (e.g., heavy metals), other than the disinfectant, or radiological constituents, must be managed as a hazardous chemical waste:

1. 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.

2. Label utilizing a yellow self-adhesive hazardous chemical waste sticker, available from Health and Safety (safety@gwu.edu, or 202-994-4347). Identify all of the chemical constituents by listing full chemical names without abbreviations.

3. Store the waste bottle in a Chemical Waste Satellite Accumulation Area.

4. Once full or ready for pick-up, contact Health and Safety (safety@gwu.edu, or 202-994-4347).