



## Bio Basics Fact Sheet: Autoclaving Biological Waste

### What is Covered:

- All disposable lab ware, except Pasteur pipettes, contaminated with potentially biohazardous materials (blood, body fluids, human cell culture media, bacteria culture media, viruses, recombinant DNA, etc). **Note:** gloves, regardless of contaminated or not, and paper towels used for work surface or equipment decontamination must be disposed of in a clear autoclave bag or red biohazard bag.
- Pasteur pipettes should be disposed of in sharps container.
- Culture plates
- Culture media
- Animal cages and bedding from infected animals

### Background:

Studies have shown that the processing time necessary to achieve decontamination of biological material depends on several loading factors. Load size, type of container, and moisture content all impact decontamination time. A **60 minute** autoclave cycle (excluding exhaust time) is recommended to allow enough time for the center of the load to come up to temperature and have sufficient steam penetration. This will insure that the temperature in ALL parts of the load has reached 121°C for at least 20 minutes. If you wish to autoclave waste for less than 60 minutes you must verify effectiveness, see Autoclave Testing below.

In order to standardize the autoclaving of wastes and to assure that all loads, regardless of size or content, are properly decontaminated, the following procedure must be followed.

### Procedure:

- Place materials in a clear autoclave bag (available from U stores).
  - No Biohazard symbol should be visible. Don't use red or orange biohazard bags to autoclave biohazardous waste since these bags cannot be disposed of as regular trash. The University needs to pay a premium to dispose biohazard waste.
  - Bag should be loosely packed and not more than 3/4 full.
  - Do not seal bag shut
    - if using the supplied rubber bands place on loosely, do not twist.
    - opening should be at least one inch in diameter.
- Use autoclave indicator tape on outside of bag to show that waste has been processed. Tape does not prove decontamination effectiveness, see **Autoclave Testing** below.
- Place waste material on a large, metal, leak-proof tray.
  - Metal containers transfer heat more efficiently than plastic containers.
  - Container should be large enough and shallow enough to allow for ample steam circulation.
  - If autoclaving more than one bag at a time, be sure there is ample room between the bags so steam circulation is not impaired.
- Autoclave at 121°C for **60 minutes**.
- After autoclaving is complete, tape bag shut or tighten rubber band and place in regular trash receptacle.
- To autoclave liquid waste, place liquid in beaker or flask, not in autoclave bag.
- Autoclaved liquid culture waste can be sewered unless hazardous chemical waste is present. If hazardous chemical waste is present follow procedures in the Hazardous Chemical Waste Management

Guidebook at [http://www.dehs.umn.edu/hazwaste\\_chemwaste\\_umn\\_cwmgbk\\_sec2.htm](http://www.dehs.umn.edu/hazwaste_chemwaste_umn_cwmgbk_sec2.htm). **Do not sewer** melted agar as it will congeal and clog the plumbing.

## Autoclave Testing:

- All autoclaves used for waste decontamination should be regularly tested for effectiveness.
- Autoclave indicator tape **does not** prove decontamination effectiveness. Tape only indicates that the outside of the container came to temperature, it does not reflect time or conditions inside the load.
- For highest confidence in decontamination effectiveness, each load should be tested. If autoclaving for less than 60 minutes, each load must be tested. 3M Comply (Thermalog) chemical integrator strips provide immediate test results.
  - Can be purchased from U Stores - CX12607.
  - Attach the indicator strip to a stick or string and put in the **center** of the load.
  - Retrieve after autoclaving for confirmation that the entire load has been exposed to the conditions necessary for decontamination.
  - If the indicator reads unsafe, autoclave again.
- It is also recommended that autoclaves be tested monthly with a spore indicator.
- Autoclave testing log must be kept for two years. The log is available at <http://www.dehs.umn.edu/Docs/AutoclaveLog.doc>

## Personal Safety Precautions:

- When unloading an autoclave, wear heat resistant gloves, eye protection, and lab coat.
- To prevent steam burns, make sure that the autoclave pressure is near zero before opening the door.
- Allow steam to escape gradually by slowly cracking open the autoclave door. Allow load to cool for 10 minutes before removing.
- Do not autoclave sealed containers or full bottles with narrow necks as they may explode.
- Do not autoclave materials containing solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), or radioactive materials.

## Red Bag Use:

Red bags use vs. autoclaving waste. Please use University resources responsibly. Red bag disposal costs the University 10X more than disposal of autoclaved waste. Red bags are intended for use where an autoclave is not available.

## Additional Notes:

- All Pasteur pipettes, needles and syringes, broken glass, slides and cover slips, should be placed in sharps containers for pick up. Do not autoclave.
- Liquid wastes can be decontaminated with one part bleach to ten parts liquid for 30 minutes and sewer. **Do not add bleach to materials that may contain ammonia or strong acids as dangerous chlorine gas may be produced.**
- As an alternative to autoclaving, contaminated non-liquid materials can be placed in a red Biohazard bag and be collected as Biohazard waste. Check with your Research Safety Officer (RSO) to determine the proper procedure to follow for your area.
- See the Infectious Waste Disposal Chart at [http://www.dehs.umn.edu/bio\\_wastedisptble.htm](http://www.dehs.umn.edu/bio_wastedisptble.htm) for additional waste disposal information.

## Reference:

*Biological Safety Principles and Practices* edited by Diane Fleming & Debra Hunt, ASM Press, Washington D.C., page 393-395, 2000