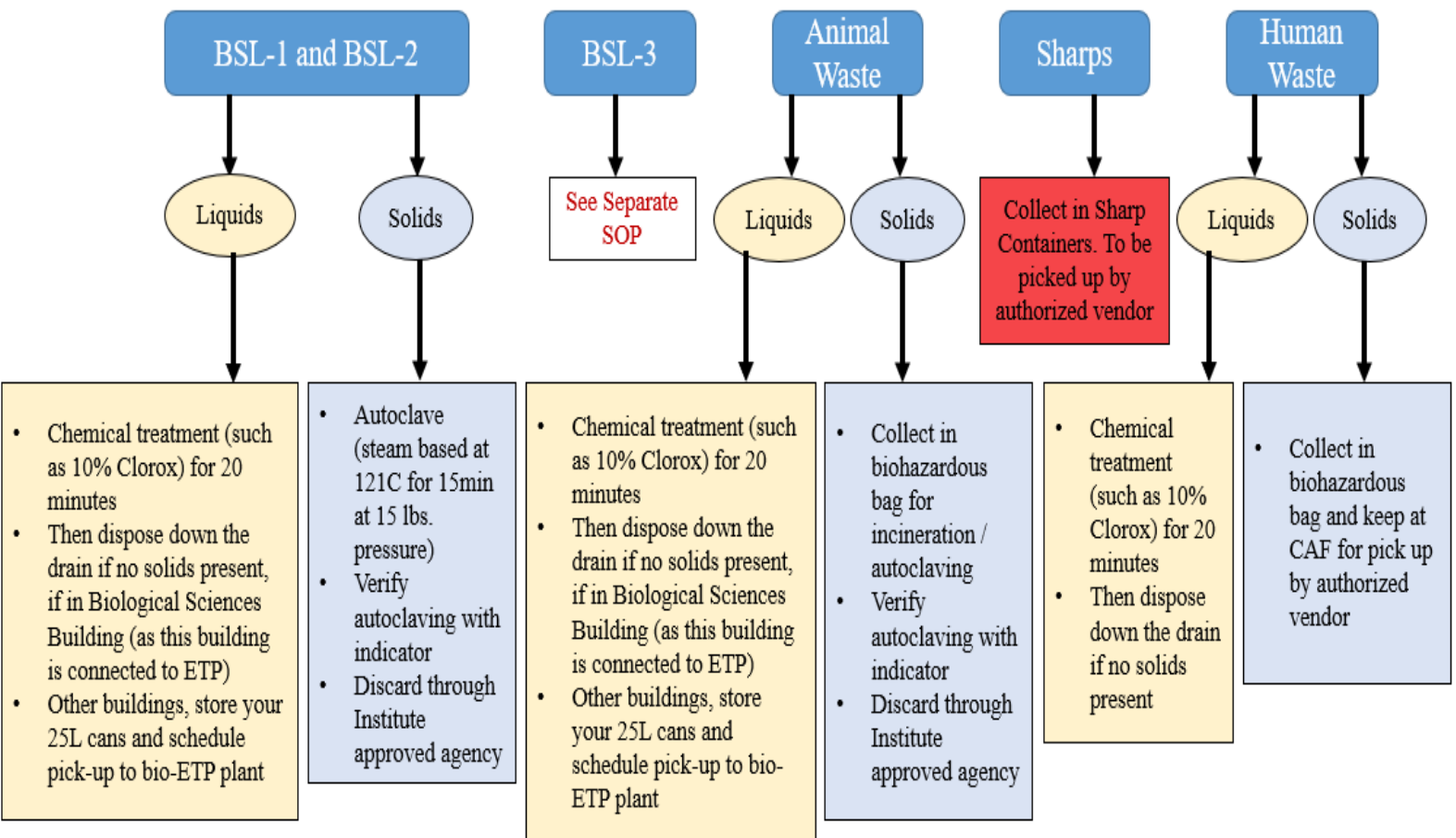




Basic Flow Chart Describing how to Discard Waste from Laboratories Working with Biological Specimens



## Standard Operating Procedure for the Safe Disposal of Hazardous Biological Waste

All experimental studies that include any life form (bacteria, virus, parasite, animal cells, plants, infected plants and plant tissues, soil used to grow plants and their pathogens, and experimental animals) or their products are considered biological waste (potentially hazardous and hazardous). Safe disposal practices of waste that is generated must be followed.

Contact the Biosafety Officer if you need assistance in determining whether your materials are biohazard waste, or have any other concerns.

**Note:** The researcher, laboratory, unit that is generating biological waste is responsible for safe disposal of that waste

**Waste is segregated into categories based on the classification of the laboratory that is generating it. This SOP is for BSL-1 and BSL-2 waste. For BSL-3 laboratories, please see separate SOP**

1. All "biological waste" generated by laboratories that handle any Biosafety Level-1 or Biosafety Level-2 organisms will be considered as biologically hazardous waste. That is, any items that came in contact with the organisms or contain these organisms, are considered as biohazardous waste
2. This waste may be divided into 2 broad categories
  - a. **Liquid waste:** It is preferable that the liquid waste be autoclaved prior to the next step.
    - i. Liquid waste has to be decontaminated with sodium hypochlorite solution for a minimum of 30 min.
    - ii. After such decontamination, the waste may be discarded as liquid waste down the drain, if no solids present, if in Biological Sciences Building (as this building is connected to ETP). Other buildings, store your 25L cans and schedule pick-up to bio-ETP plant or inform EHS Officer.
    - iii. Any solid particulates in this waste must be filtered out and discarded as biohazardous solid waste
  - b. **Solid waste:** Maybe further divided into non-sharp and sharp waste
    - i. Non-sharp waste must be sealed in autoclave bags and autoclaved for  $\geq 60$  min. at  $\geq 121^{\circ}\text{C}$  and 15 lbs. pressure.
      - The bags must be labeled with a clear indicator that shows when waste has been autoclaved and hazard inactivated (e.g., autoclave tape)
      - **The waste must then be handed over to an authorized waste collector.**
    - ii. Sharp waste must be placed in sealed puncture-proof plastic containers (preferably red in colour). Such puncture proof sharp waste containers may be purchased from many local vendors (contact OLSEH if you are not sure where to purchase these items). The containers must be labeled as biohazardous sharp waste and handed over to **authorized personnel** for disposal.
3. **Tissue and fluid from animals**
  - a. All animal tissue waste must be discarded in biohazardous bags and transfer the same to CAF for collection.
  - b. All animal fluid waste must be autoclaved, followed by decontamination with sodium hypochlorite solution. Drain the waste if no solids present, if in Biological Sciences Building (as this building is connected to ETP). Other buildings, store your 25L cans and schedule pick-up to bio-ETP plant or inform EHS Officer.
  - c. Histology waste (glass slides with tissue sections): any histology (or immunofluorescence or anything similar) slides must be autoclaved, and then discarded as sharp waste.

#### 4. Humans body fluids

- a. All human fluid waste is considered biohazardous waste , followed by decontamination with sodiumhypochlorite solution. Drain the waste if no solids present, if in Biological Sciences Building (as this building is connected to ETP). Other buildings, store your 25L cans and schedule pick-up to bio-ETP plant or inform EHS Officer.
- b. Histology waste (glass slides with tissue sections): any histology (or immunofluorescence or anything similar) slides must be autoclaved, and then discarded as sharp waste.

#### 5. Mixed Waste: defined as biologically hazardous waste mixed with chemicals and/or radioactive contents

- a. Ideally, mixed waste is not generated. If possible, researchers are highly encouraged to conduct experiments that will not generate mixed waste
- b. If mixed waste is generated out of necessity, Safety Officer from OLSEH must be contacted for handling the waste

**Cautionary point 1:** Procedure. This means that the selected procedure has been tested to be adequate for the inactivation of the waste. Documentation of this testing should be maintained with other survey and evaluation records. Direct testing for inactivation is frequently not possible. Where it is not possible, the methods used to measure the operational parameters must be verified initially and on a periodic interval thereafter.

A written Quality Assurance program must be in place. (For example, in the case of autoclaves using autoclave tapes to ensure proper sterilization, maintenance of the accuracy of thermometers, pressure gauges, and timing mechanisms). There must be documented evidence for the adequacy of the selected values of the adjustable parameters for the type, configuration, nature, and volume of waste to be processed.

**Cautionary point 2:** This includes procedures for the prevention of the dispersal of aerosols or liquids during the processing of the materials by the proper use of coverings, seals and ventilation as necessary

**Cautionary Point 3:** None of the autoclaved biohazard waste should be handled by any person other than the one identified for collecting biohazard waste

**Cautionary Point 4:** All laboratories must strive to maintain a logbook or electronic records of the type of biohazard waste generated, the person discarding the waste, date on which waste was autoclaved, and date on which the waste for collected by the identified waste-collector

#### Methods for sterilization by chemical treatment

1. Hypochlorite Solution (Bleach) - 500 ppm available chlorine (10%Clorox) **PREFERRED**
2. Phenolic Solution - 500 ppm active agent
3. Quaternary Ammonium Solution - 400 ppm active ammonium agent.

# Safety Guidelines for Biohazardous waste Disposal

## 1 Aim

To provide safety guidelines for biohazardous waste Disposal, management, and quality assurance.

## Definition

**Biohazardous waste:** waste that includes stocks or specimens, live or attenuated vaccines, cell lines, microbiological waste (bacteria, viruses, recombinant nucleic acids etc.) and material that has come into contact with any of these items, human blood/blood products and body fluids, items contaminated with blood/blood products and body fluids, biologically contaminated sharps including needles, needles attached to syringes, and blades etc.

## 2 How to handle the waste

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### 3.1 Evaluate your waste

To be able to handle the waste properly, first needs to determine whether the waste is hazardous or not, and whether handling of that waste is regulated by legislation. This step is often called classification or categorization of the waste.

#### 3.1.1 Categorization of the waste.

This means that hazardous chemical waste, radioactive waste, and infectious wastes are not to be mixed or placed in the same waste storage containers. Biohazardous waste collected in lab must be held in approved clear autoclave bags within clearly labeled “**Biohazardous Waste**” bins. Autoclave bags **MUST NOT** be filled more than half full (over filled bags will be returned for re-packaging).

General lab waste (i.e., paper towels, bench coverings, etc.) must be stored in separate bins and should be kept to a minimal.

## 3.2 Store your waste

Depending on the type of waste, there will be different requirements in terms of storage facilities. Waste can be in solid or liquid form, so it is important to store it according to its characteristics. Biohazardous waste must be stored in a sturdy, leak-proof container that is kept closed when not adding or removing waste. Different kinds of waste may require different types of storage containers. The container must be labelled with the words “**Biohazardous Waste**”, a clear description of the contents, and the date when the waste is first placed in the container. Containers must be stored on an impermeable surface with enough aisle space to allow for weekly container inspections.

### 3.2.1 Additional requirements for outdoor storage include:

- a) Controlling access to the containers
- b) Protecting the containers from the elements
- c) Storing containers of liquid waste on a curbed and impermeable surface to contain accidental leaks

## 3.3 Biohazardous Waste Treatment

Treatment depends on the nature of the biohazards. According to the Guidelines of the state pollution control board and WHO, there are number of options for the treatment of biohazardous waste prior to disposal. These include chemical decontamination, thermal decontamination (autoclaving) and Incineration. After proper treatment of biohazardous waste, hand it over to the agency that received biohazardous waste from IISc and record the weight of the waste handed over

### 3.3.1 Chemical Decontamination

Treat the waste with chemical decontaminants like hypochlorite solution or alcohols, each with their own advantages and disadvantages for use are available for the decontamination of waste containing bacteria, viruses, fungi and toxins.

### 3.3.2 Thermal Decontamination(Steam)

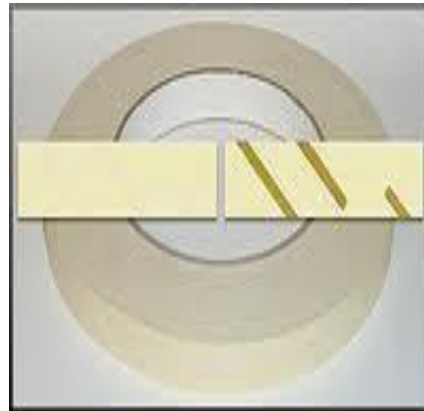
Infectious materials and toxins, together with associated waste can be effectively decontaminated by autoclaving. (Table) lists the types of biohazardous wastes produced laboratory and whether steam autoclaving is appropriate for decontamination. Please note that items requiring autoclave decontamination CANNOT contain chemical or radioactive contaminants.

S. No	Biohazard type	Decontamination by steam autoclave prior to landfill disposal	Alternative treatment method (required if “no” listed in previous column)	Categories
1.	Animal blood/body fluids	Yes		
2.	Microbiology Lab Waste: Animal cells, DNA/RNA ,Cultures of Bacteria Viruses, Fungi, Algae, Parasites etc.	Yes		
3.	Venoms/Toxins	Yes		
4.	Donned off PPE,PPE with spill Gloves, Shoe covers, Head covers from BSL3 laboratories	Yes	Handover it to the agency who is collecting Biohazardous Waste from IISc.	
5.	Sharps (needles, blades, etc.)	No	Handover it to the agency who is collecting Biohazardous Waste from IISc.	

## a) Procedure for Autoclaving Biohazardous Wastes

- Steam autoclaving is a method for decontaminating biohazardous materials is only permitted when autoclave efficiency/efficacy is verified by at least annual calibration of autoclaves and weekly cycle monitoring with the use of biological indicators. QC measures must be accurately documented in an autoclave log for verification.
- **Autoclave should be installed in a well-ventilated room away from the main laboratory and the corridors.**
- All biohazardous waste bags that are decontaminated via steam autoclave must be indicated as such by the presence of heat activated autoclave tape (presence of dark bars on tape indicates that the contents has reached high temperature).
- Appropriate personal protective equipment (PPE) must be worn at all times while autoclaving biological hazards .This includes closed toe/heel shoes, lab coats, gloves (heat resistant gloves for unloading autoclave) and eye/face protection. Autoclaves must be operated at **≥ 121°C for ≥ 60 minutes**. Biohazard bags should be loosely opened, and placed within a secondary containment vessel (i.e., tray) to allow maximum steam penetration during sterilization. Biohazardous waste bags **CANNOT** be filled more than half full, to maximize the efficiency of sterilization. Autoclave handler will return any bags that are over filled.
- DO NOT run samples previously treated with bleach (or any other strong oxidizer) or any other toxic chemicals/radioisotopes through the autoclave. After biological and chemical indicator assays have been successfully completed, biohazardous waste tags **must be removed**, and decontaminated bags should be placed in unmarked black garbage bags and disposed with regular garbage to the municipal landfill. Unsuccessful biological indicator assays require re- autoclaving of all waste processed since the last successful assay. As a result, frequent biological indicator assays are recommended.
- **Biological and Chemical Indicators** are sealed vessels containing a glass ampule of bacterial spores (usually *Geobacillus stearothermophilus*), a species of bacteria especially resistant to the steam sterilization process) within growth media and are used to ensure the efficacy of the autoclave run. To use, the biological indicator vial (attached to a string or other retrieval device) is placed in the center of a representative load within the autoclave (different load types should be tested separately) and the autoclave cycle is run as per procedures. A separate, negative control vial should be placed outside the autoclave for direct comparison following cycle completion.
- Following the cycle, the glass ampule is crushed, releasing the bacterial spores into the growth media. After incubation for 24-48 hours, growth of spores not killed during the sterilization cycle result in a diagnostic colour change of the media. A colour change, representing bacterial growth, indicates ineffective decontamination of the load. Maintenance of the vials original colour indicates successful decontamination. Maintenance of the negative control vials original colour indicates possible fault with the indicators and the cycle/test must be repeated with fresh biological indicators.

- Biological indicators must be used at least weekly to provide quality control for the decontamination of biohazardous waste (unless the autoclave has not been used for biohazardous waste decontamination in which case this must be recorded in the log).



- Chemical indicator tape is to be used on each item that is autoclaved to verify that the item has been autoclaved. Heat sensitive ink within the tape changes colour to indicate that the tape has been exposed to high heat. The presence of these lines does not indicate that the contents have been successfully decontaminated; only that it has reached high temperature Confirmation of decontamination with biological indicators (as previously described) is required prior to disposal

## b) Contingency plan

### □ Incident Response

- ✓ All incidents, including spills, must be reported to the supervisor and department.
- ✓ If any injury occurs, seek first aid and/or medical assistance as deemed necessary by the degree of the injury.
- ✓ Accident report must be filled out in the event of an injury.
- ✓ If clothing absorbs hot water/steam, remove clothing, and apply cool water/ice to the affected body part.
- ✓ A notice must be placed on the autoclave to indicate that the unit is out of service until the cause of the incident is identified, pro-active measures are taken to prevent such incidents in the future, and the autoclave is deemed safe for operation.

### □ Spill Clean-Up

- ✓ Spills may occur due to a boil-over or breakage of containers during the autoclave procedure. Use of secondary containers will make spill cleanup much easier – once cooled can be poured down the drain.
- ✓ No operation of the autoclave should be allowed until the spill is cleaned up. Spills not cleaned up will become harder to remove.
- ✓ The operator is responsible for the clean-up of the spill. Wait until the autoclave and materials have cooled down to room temperature before attempting to clean-up the spill.
- ✓ All spills to be reported to principal investigator/supervisor – record of spills to be kept



### 3.4 Trained personnel

The person who has any role in handling, storing, or otherwise managing hazardous waste is a necessary step for ensuring compliance with hazardous waste rules. Personnel must be familiar with each waste's hazards, appropriate safety procedures, and all aspects of compliance. For each of the personnel who will be engaged in any segment of the waste management system, it is necessary to provide adequate training and working conditions. The training should include an introduction to:

- Basic procedures for wasteManagement;
- Human and environmental risks
- Measures of precaution in waste management
- Responsibilities and authorities.

### 3.5 Keep records

The purpose of keeping records is to provide evidence that the waste is stored and disposed according to the procedures.