Management of Biohazardous Waste
Standard Operating Procedure

1.0 Introduction

This Biosafety Standard Operating Procedure (BSOP) outlines necessary procedures regarding the autoclave-based disinfection of biohazardous waste. These procedures will ensure that Memorial University of Newfoundland is in compliance with applicable biohazardous waste disposal guidelines and regulations. All autoclaves and autoclave users must also be in compliance with the general Autoclave Safe Use Standard Operating Procedure (BSOP-02).

2.0 Scope

This BSOP applies to all autoclaves owned by Memorial University of Newfoundland which are used to decontaminate biohazardous waste prior to disposal.

3.0 Responsibilities

This section outlines responsibilities within the university for the implementation of this BSOP.

a. Environmental Health and Safety (EHS)

- Review and amend this BSOP as necessary.
- Confirm that proper sterilization verification testing is being completed and records are being maintained.
- Ensure that autoclaves used for waste sterilization receive the proper sterilization verification testing and that records of this testing are maintained.

b. Department Heads

- Ensure that the autoclave settings required for proper waste sterilization are determined and utilized.
- Ensure that the requirements outlined in this BSOP are communicated to all applicable members of the unit/department.

c. Laboratory Supervisors/Principal Investigators

- Ensure that the proper packaging and labeling for waste awaiting autoclave treatment is available and utilized within the laboratory.
- Ensure that autoclaves used for waste sterilization receive the proper sterilization verification testing and that records of this testing are maintained.
- Ensure that the autoclave settings required for proper waste sterilization are determined.
- Ensure that validation and verification testing is completed and documented as outlined in this procedure.

d. Autoclave Users

- Package biohazardous waste appropriately, taking care not to compact the waste in the bag and not to overload the bag.

- Use the correct autoclave settings, as determined by the department, when treating biohazardous waste.
- Maintain the appropriate logs as outlined in this procedure as well as in BSOP-02: Autoclave Safe Use.

4.0 Definitions

Biohazardous waste: waste that includes human anatomical waste (not including teeth, hair and nails), , human and animal cultures, stocks or specimens, live or attenuated vaccines, cell lines, microbiological samples (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, and pathogenic/transgenic plants/plant parts including plant pests.

Biohazardous waste treatment: the processing of biohazardous waste which results in waste that is no longer considered hazardous and may be disposed of as municipal garbage.

5.0 In-lab segregation of Biohazardous Waste

Laboratory procedures involving biohazards at Memorial University of Newfoundland generate various types of waste (biological, radioactive, chemical, etc.) each with their own waste disposal requirements. As a result, in-lab segregation of wastes is vital for appropriate disposal.

Figure 1: In-lab biohazardous waste bins.

Biohazardous waste requiring disposal as per this protocol **MUST NOT CONTAIN** radioactive or chemical contaminants. For disposal of such wastes, please contact Environmental Health and Safety for guidance. Biohazardous waste collected in-lab must be held in approved clear autoclave bags within clearly labeled "Biohazardous Waste" bins (Figure 1). General lab waste (i.e. paper towels, bench coverings, etc.) should be kept to a minimal. Autoclave bags labeled with the biohazard symbol are **NOT** permitted for use. Autoclave bags **MUST NOT** be filled more than half full (over filled bags will be returned for re-packaging). Sharps are **NOT** permitted in autoclave bags. Please use CSA approved solid containers for sharps disposal. Half-filled autoclave bags should be tied closed with EHS approved biohazardous waste tags labeled with the principal investigators name and the date, as well as a description of the waste.

6.0 Biohazardous Waste Treatment

Materials classified as "biohazardous" according to Memorial University of Newfoundland's Biological Safety Manual can have different treatment requirements, depending on the nature of the biohazards. According to the Guidelines for the Management of Biomedical Waste in Canada, there are a number of options for the treatment of biohazardous waste prior to

disposal. These include chemical decontamination, thermal decontamination (autoclaving) and Incineration.

a. Chemical Decontamination

Many chemical decontaminants are available for the decontamination of solutions containing bacteria, viruses, fungi and toxins as well as for general surface decontamination. These include chlorine, iodine and alcohols, each with their own advantages and disadvantages for use. An appropriate method for decontamination must be approved by the Institutional Biosafety Committee prior to commencement of work with biohazards (contact EHS for advice on chemical treatment options for specific biohazards).

b. Thermal Decontamination (Steam)

Infectious materials and toxins, together with associated waste can be effectively decontaminated by autoclaving. Table 1 lists the types of biohazardous wastes produced at Memorial University and whether steam autoclaving is appropriate for decontamination. For items where steam autoclaving is inappropriate, alternative method(s) of disposal are listed. Please note that items requiring autoclave decontamination CANNOT contain chemical or radioactive contaminants.

c. Incineration

For items that cannot be appropriately thermally or chemically decontaminated (see table 1), incineration is the required treatment method. Items requiring incineration are transported off site by an externally contracted waste disposal vendor as hazardous waste. Hazardous waste disposal request forms can be found at:

https://www.mun.ca/health_safety/health-and-safety-management-system/laboratory-safety/

Table 1: Waste treatment options for biohazardous waste.

Biohazard type	Decontamination by steam autoclave prior to landfill disposal	Alternative treatment method (required if "no" listed in previous column)
Animal anatomical waste	No	Incineration
Animal blood/body fluids	**Yes**	
Human anatomical waste	No	Incineration
Human blood/body fluids	**Yes**	
Microbiology Lab Waste: Primary animal cell lines Established animal cell lines Primary human cell lines Established human cell lines Recombinant DNA/RNA Bacteria Viruses	**Yes**	
Fungi	**Yes**	
Venoms/Toxins	**Yes**	
Parasites	**Yes**	
Plants, plant parts and plant pests	No	Incineration
Plant cells and cell lines	**Yes**	
Prions	No	Incineration
Sharps (needles, blades, etc.)	No	Incineration

Data compiled from Table 3: Summary of treatment options for biomedical waste and Table 4: Summary of disposal options for untreated biomedical waste, Guidelines for the Management of Biomedical Waste in Canada.

7.0 Procedure for Autoclaving Biohazardous Wastes

The use of autoclaves to decontaminate the biohazardous wastes indicated in Table 1 must comply with **BSOP-02 Autoclave Safe Use**. Steam autoclaving as a method for decontaminating biohazardous materials is only permitted when autoclave efficiency/efficacy is verified by quality control (QC) measures including:

- Bi-annual certification of autoclaves (mechanical)
- Validation – validation of autoclave parameters with representative loads (at least annually) and weekly cycle monitoring with the use of biological indicators.
- Verification – verification that autoclave conditions were met by using chemical indicator (or acceptable alternative) as determined by LRA.
    - See detailed procedure for verification and validation in Appendix A.

QC measures must be accurately documented in an autoclave log (see BSOP-02) for review at all times. 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 have reached high temperature). This, in conjunction with QC measures previously described provides assurance that wastes are appropriately decontaminated prior to disposal.

Figure 2: PPE required for autoclaving.

Appropriate personal protective equipment (PPE) must be worn at all times while autoclaving biological hazards (Figure 2). 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 staff 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.

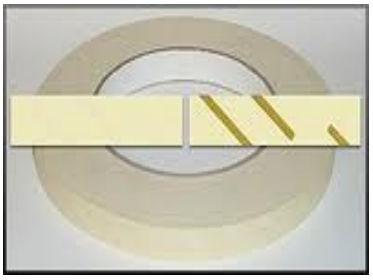
a. Using Biological and Chemical Indicators

Figure 3: Biological indicator vials.

Biological indicators (Figure 3) 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 must be tested separately) and the autoclave cycle is run as per procedures outlined in this BSOP. 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 color change of the media. A color change, representing bacterial growth, indicates ineffective decontamination of the load. Maintenance of the vials original color indicates successful decontamination. Maintenance of the negative control vials original color indicates possible fault with the indicators and the cycle/test must be repeated with fresh biological indicators.

Biological indicators must be used for cycle validation (at least annually for each load type) and **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). In addition to the required records mentioned in **BSOP-02: Autoclave Safe Use**, a log of all verification testing, including Biological Indicator testing, must be maintained. The Biological Indicator test log must include the date, the cycle time and settings, the indicator information (brand, expiry date, and lot #) and the test results. Validation records must be kept for a minimum of three years and be made available for inspection. The Biological Indicator Testing Log can be found in Appendix B.

Figure 4: Chemical indicator tape.



Chemical indicator tape can be used on items that are autoclaved to verify that the item has been autoclaved. Heat sensitive ink within the tape changes color 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.

Appendix A: Validation and verification of autoclave settings.

Validation: Validation is required in order to ensure that the autoclave cycle parameters used are adequate for effective decontamination of specific waste/load types. All autoclaves used for the decontamination of biohazardous waste must be validated:

- **at least annually,**
- following any repair/maintenance that may have affected the autoclaves' ability to meet cycle parameters.
- Prior to introducing new regulated materials/processes.

Validation tests must be completed and documented for **each load type processed** [e.g. solids (bench covering, gloves, paper towels, etc.), liquids (cultures, waste, etc.), glassware, etc.] using representative loads. The representative load would be the maximum quantity of material for that load type that could possibly be decontaminated per cycle. Results of autoclave validation must be documented in the Biological Indicator Testing Log (Appendix B) and be available for review during inspections.

Procedure:

1. Collect a representative waste load within a standard, clear autoclave bag (for solids) or within a standard beaker/flask ("vessel" for liquids).
2. Insert biological indicator (BI) vial into the centre of the load. Select a second BI for use as a positive control (i.e. non-autoclaved indicator).
3. Affix chemical indicator tape to the outside of the autoclave bag/vessel.
4. Load bag/vessel into autoclave, select cycle parameters and run cycle.
5. Upon run completion, check autoclave computer and/or print out to ensure that cycle parameters were met (document any issues in log).
6. Verify that chemical indicator tape has changed to a brown color (see Figure 4).
    - If the indicator did not change colour, but the test parameters (from the autoclave readout/printout) indicated it met the program parameters, re-run the load again (with new bio-indicators) with a longer run time.
    - If the chemical indicator on the bag/vessel changed colour to brown proceed to incubate the indicators.
    - If the chemical indicator does not change colour a second time, either reduce the load size, ensure it has been loaded into the autoclave properly or arrange for technical service of the autoclave.
7. Following the run, remove the BI from the load and incubate (with the positive control) as per the directions for the specific BI used.
8. Following 48 h of incubation assess the vials for color change:

- If the test vial (the one in the autoclave) is purple and the positive control is yellow following incubation, then the load was sterilized.
- Should both the test vial and positive control vial be yellow following incubation, then the load was not sterilized, and validation should be repeated with a longer run time and with new bio-indicators.
- Should the BI indicate a failure on the second run, either reduce the load size, ensure the autoclave was loaded properly or if necessary, arrange for technical service of the autoclave. Contact the BSO.
- Should both the test vial and the positive control vial be purple upon 48 hours of incubation then the spores were not viable. Re-test with a newer batch of bio-indicators.

9. Upon successful completion of the validation test (colour change on chemical indicator and test BI vial is purple with positive control vial yellow upon incubation) the autoclave parameters are approved for treatment of the type of biohazardous waste validated (all types of waste must be validated separately).

Verification: Once effective decontamination parameters have been established through validation, it is important that decontamination processes and procedures be monitored (verified) on a regular basis to confirm that established parameters continue to be met. The conditions of each autoclave cycle run must be verified to ensure that the parameters tested during validation are in place. Load verification must be completed when used for treatment of biohazardous waste as follows:

- Chemical indicator tape is used on each item in every autoclave run (which indicates that the appropriate temperature has been reached but not that contents have been decontaminated,
- BI are used to verify parameters are met prior to disposing of any treated waste (at least weekly).

Procedure:

1. Attach chemical indicator tape to each item that will be decontaminated. Attach tape to the exterior of autoclave bags.
2. Load autoclave with a single waste type and run based on the cycle parameters previously validated for that waste type (see validation section in the BSOP).
    a. Add BI within the chamber but not in contact with contaminated materials.
3. Upon completion of the autoclave run, ensure that chemical indicator tape has changed to a brown color (see Figure 4).
    - If the indicator did not change colour, but the test parameters (from the autoclave readout/printout) indicated it met the program parameters, re-run the load again (with new bio-indicators) with a longer run time.

- If the chemical indicator does not change colour a second time, either reduce the load size, ensure it has been loaded into the autoclave properly or arrange for technical service of the autoclave.

4. If chemical indicator tape has changed to brown on all items, items can be stored within the containment zone (CZ) pending successful completion of BI test.
    a. All waste treated since that last documented positive BI test must be stored until the next positive BI test – do not dispose until the next BI test confirms that the waste has been decontaminated.
    b. At least weekly (and at shorter intervals when processing large amounts of waste) perform BI testing as indicated in steps 2-9 in the validation instructions above.

Version History:

Version	Date	Author(s)	Notes
1.0	2015-02-23	Rod Hobbs	First writing.
1.1	2015-11-16	Rod Hobbs	Updating of hyperlinks.
1.2	2025-07-31	Rod Hobbs	Revision. IBC approval.

Appendix B: Biological Indicator Testing Log

Indicator Brand and Type: _____

Date	Tester initials	Test type (verification or validation)	Waste type	Cycle temp (°C)	Cycle time (min)	Indicator Expiry Date	Indicator Lot #	Test/control Result (+/-)	Notes/comments