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1.0 Introduction

This Standard Operating Procedure outlines necessary procedures regarding the autoclave-based disinfection of biohazardous waste, including validation and verification controls. The procedure will ensure that Queen's autoclaves are in compliance with applicable guidelines and regulations. All autoclaves and autoclave users must also be in compliance with the general Autoclave Standard Operating procedure (SOP-LAB-02).

2.0 Scope

This SOP applies to all autoclaves owned by the University which are used to treat biohazardous waste.

3.0 Applicable Legislation and Guidelines

- Environmental Protection Act –R.R.O. Regulation 347, 1990
- Ontario Regulation 558/00, Amending Reg. 347
- Guideline C-4, The Management of Biomedical Waste
- Guideline C-17, Non-Incineration Technologies for Treatment of Biomedical Waste
- Laboratory Biosafety Guidelines 3rd Edition, 2004, Public Health Agency of Canada
- Containment Standards for Veterinary Facilities, Canadian Food Inspection Agency

4.0 Responsibilities

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

4.1 Department of Environmental Health and Safety

- Provide any necessary Biohazardous Material labels.
- Review and amend this Standard Operating Procedure as necessary.
- Confirm that proper sterilization verification testing is being done, and records are being maintained.

4.2 Department Heads and Safety Officers

- Ensure that waste-treating autoclaves receive the proper sterilization verification testing and maintain records of this testing.

- Ensure that the autoclave settings required for proper waste sterilization are determined.

4.3 Laboratory Supervisors/Principal Investigators

- Ensure that the proper packaging and labeling for waste awaiting autoclave treatment is available.
- Ensure that waste-treating autoclaves receive the proper sterilization verification testing and maintain records of this testing.
- Ensure that the autoclave settings required for proper waste sterilization are determined.

4.5 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.

5.0 Definitions

Decontamination: The process by which materials and surfaces are rendered safe to handle and reasonably free of microorganisms, toxins, or prions; this may be accomplished through disinfection, inactivation, or sterilization.

Decontamination Technology: Equipment proven by validation to render materials safe to handle and reasonably free of microorganisms, toxins, or prions. Examples include autoclaves, incinerators, tissue digesters, and effluent decontamination systems

Sterilization: A process that completely eliminates all living microorganisms, including bacterial spores.

Biohazardous waste: waste that includes human anatomical waste (not including teeth, hair and nails), animal waste, human and animal cultures, stocks or specimens, live or attenuated vaccines, cell lines, and material that has come into contact with any of these items, human liquid blood or semi-liquid blood and blood products, items contaminated with blood or blood products that would release liquid or semi-liquid blood if compressed, body fluids visibly contaminated with blood, and body fluids removed in the course of surgery, treatment, autopsy,

or for diagnosis, sharps including needles, needles attached to syringes, and blades, or is cytotoxic waste.

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.

Pathological waste: the following materials are considered pathological waste:

- Any part of the human body, including tissues and bodily fluids but excluding non-infectious fluids, extracted teeth, hair, nail clippings and the like.
- Any part of the carcass of an animal infected with a communicable disease or suspected by a licensed veterinary practitioner to be infected with a communicable disease.
- Non-anatomical waste infected with a communicable disease.
- Waste derived from any of the waste listed above, including autoclaved pathological waste.

Validation: Autoclave validation is more stringent than verification and is a requirement under regulations enforced by the CFIA and PHAC. It tests that cycle parameters are effective at inactivating materials under the most challenging load conditions for a particular cycle used. It is to be conducted annually, after repairs or modifications to the equipment, and/or introduction of new regulated materials or processes. Validation is required for each load type, e.g. solid waste, liquid waste, glassware, using representative loads.

Representative Load: A simulation batch of materials of a particular load type (e.g., plastics, waste, liquids, carcass), including mixed load types (e.g., containing pipette tips, agar plates and gloves), used to validate a decontamination method for routine loads. The quantity that would be decontaminated in a single load can be a defined amount (e.g., 6 lab coats), size (e.g., an autoclave bag 2/3 full) or weight (e.g., 5 kg)

Verification: Verification of autoclaves is intended to test processes between annual validations to detect process or equipment failures. The degree to which the autoclave is used largely determines the verification needs. Units used daily may consider weekly or bi-weekly verification runs. For less frequently used autoclaves, monthly verification may be sufficient. Biological indicators or Chemical integrators would be placed outside the load for verification to avoid potential worker exposure to infectious materials

Biological indicator**:** Biological indicators are used in the efficacy testing of the autoclave process to effectively sterilize the contents being treated. *Geobacillus stearothermophilus* spores are used, as they are the most resistant organism to steam autoclaving. The standard for testing requires a 6 log10 reduction of spores to ensure a level 4 treatment. This means the spore ampule must contain a 106 population of spores, and those spores must be killed by the steam or heat treatment. Since spore vial populations come in more than one population density, it is important to purchase the correct test vials.

Chemical integrator: Integrating indicators, generally known as chemical integrators, mimic the response of a biological indicator so closely that they allow you to confidently release biohazard waste loads before spore tests are received. They contain a steam sensitive material that sequentially moves across the strip into a SAFE ZONE indicating all parameters of sterilization (time, temperature, steam) have been met.

6.0 Safety Precautions

Since autoclaves utilize steam, heat and pressure, the risk of personal exposure and potential harm is great. The operator must wear the appropriate protective equipment. Often material to be loaded contains potentially infectious material, so the standard laboratory protective equipment must be worn. This includes:

- Safety eye and face protection (face shield minimizes the risk of facial steam burns when unloading the autoclave),
- Gloves (latex or nitrile gloves prevent contact with contaminated material), while heat resistant gloves (moisture proof preferred to protect hands from scalding liquids) must be used when loading and unloading the autoclave),
- Lab coats (long sleeves must be used to protect wrists and forearms, plus an apron if a spill hazard exists).

Remember that although the autoclave trays may be cool, the door and walls of the chamber may still be hot enough to cause a burn.

7.0 Work Practices

Autoclaves must be operated following the same safety precautions and procedures explained in the Autoclave Standard Operating Procedure (SOP-LAB-02).

7.1 Biohazardous Waste Preparation, Treatment and Removal

- Biohazardous waste to be autoclaved must be collected in plastic bags without the biohazard symbol (double bagged) in containers bearing an orange biohazard label. These labels can be obtained from the Department of Environmental Health and Safety.
- When the container is ¾ full, the bag can be closed and transported to the autoclave area using a puncture resistant secondary container with a lid (same used for holding the biohazard waste in the lab) on a cart. For CL2/CL2+ labs, the container must be surface disinfected using appropriate disinfectant concentration and contact time before leaving the laboratory.
- Wear a lab coat and glove one hand when transporting the waste to the autoclave room.
- In the autoclave room, biohazard waste bags can be removed from the container and placed into the autoclave tray.
- Remember to open the bag to allow steam penetration.
- Waste must be autoclaved using settings that have been proven by biological indicators to be consistently successful for sterilization. If biological indicators frequently show negative results for sterilization more rigorous settings must be used.
- Chemical indicators of sterility, such as temperature sensitive tape or strips, must be used on each bag of biohazardous waste that is treated by autoclaving.

- After successful autoclave treatment, these bags must then be placed inside a regular black garbage bag which is free of any markings or labels and then tied closed securely. The waste may then be disposed of through the municipal system.
- A record of the cycle logs produced by the autoclave must be maintained using the Autoclave Log Appendix B SOP-Lab-02. These records must be kept for a minimum of five years.

7.2 Pathological Waste

- If pathological waste, as defined in the Definitions section above, is produced in any significant amount then it must be treated either by incineration or by an autoclave with the appropriate Certificate of Approval issued by the Ministry of the Environment. The Certificate of Approval states that the waste which is treated by autoclaving no longer has

characteristics similar to the characteristics of the original pathological waste. More information on the Certificates can be found on the Ministry of the Environment website: http://www.ene.gov.on.ca/en/business/cofa/index.php.

- In practice, Queen's pathological waste is disposed of by incineration by placing in "burn barrels" obtained through the Department of Environmental Health and Safety, or by removal through KHSC waste treatment system.

7.3 Autoclaves Validation & Verification Testing

7.3.1 Validation

1. All autoclaves will be validated annually following the annual inspection and servicing or following any repair service which might have impacted the autoclaves' ability to meet run parameters.
2. Identify the load type for the cycle to be validated, e.g. liquid waste, mixed waste, reusable glassware, contaminated reusable PPE. If a cycle is used for more than one type of load, choose the most challenging load for validation purposes. This may require initially running validation cycles for all load types to determine which is most difficult to pass. This record should be kept for auditing purposes.
3. Determine the maximum quantity for each load type to be processed in a single run.
4. Note the cycle parameters in the logbook – cycle name, temperature, sterilization time, pressure.
   Note: Autoclaves which are used to treat biohazardous waste must be capable of causing a $6\log_{10}$ (99.9999%) reduction in spores of *Bacillus stearothermophilus* and should therefore be tested with an indicator which verifies this level of sterilization (i.e. an indicator with a spore population of 10^6). Indicators which are past their expiration dates must not be used.
5. Place the chosen efficacy monitor (Biological indicator or chemical integrator) in the most challenging area of the load to sterilize. For example, if the load consists of two autoclave bags ¾ full of plasticware, the monitor could be placed in the centre of each bag.
6. Load the autoclave and run the cycle.
7. Note the cycle parameters captured on the cycle print out or chart paper in the logbook. The printout or chart paper should also be stored with the logbook.
8. Retrieve the monitor (BI or CI) at the end of the cycle and process as appropriate.

- Read the CI
- Download temperature sensor data
- Incubated BI with a positive control from the same lot

9. Accept the cycle parameters for the load type if the efficacy monitor indicates sterilizing temperature and time were achieved.
10. Should both the test vial and positive control vial be yellow following incubation, then the load was not sterilized, and the load should be repeated with a longer run time with new bio-indicators.
11. · Should the bio-indicators 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.
12. ·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.
13. Upon successful completion of the validation test (colour change on chemical integrator and test vial is purple with positive control vial yellow upon incubation) fill and affix to the Autoclave Validation Record from (Appendix A2). The Biosafety Office (BSO) will review and sign the autoclave validation record during EHS annual autoclave inspections.

7.3.2 Verification

- Autoclaves which are used to treat biohazardous waste must be tested with a biological indicator or chemical integrators once a week, unless the autoclave is not being used to treat waste that week in which case this must be recorded. This is considered your weekly verification.
- The load for verification can be any size that does not exceed the load used for validation.
- Place the chosen efficacy monitor (check expiry date) outside of the load and in the centre of the autoclave chamber.
- Run the cycle with the validated parameters.
- Remove the monitor at the end of the cycle and process as appropriate.
- Record results in the Biological Indicator Testing Log (Appendix A1)
- Records of these tests must be kept for a minimum of five years.
- If any test fails, the load and any previous loads which remain on site are considered untreated and must be re-treated only after successful verification takes place.

7.4 Biological Indicators & Chemical Integrators record management

In addition to the required records mentioned in the SOP-LAB-02, a log of all validation and verification testing must be kept. The test logs must include the date, the cycle time and settings, the indicator information (brand, expiry date, and lot #) and the test results. Records must be kept for a minimum of five years. Appendix A2 Autoclave Validation Record

8. Training requirements

Training is absolutely required prior to using the autoclave. Training requirements as per SOP-LAB-02 Section 6.

9. References

- Information Sheet Autoclave Validation and Verification. Public Health Agency of Canada (PHAC)
- UBC Safety and Risk Services. Annual Autoclave Validation and Verification. BIO_GDL-014. Revised 03/20/2023
- Nipissing University, A guideline for the safe use of autoclaves. March 2022
- Biosafety Program—Autoclave Maintenance, Validation & Verification C. Williams February 2018

10. Revision History:

Date	Revised by	Changes
August 2009:		Initial release
October 2019		Updated to include chemical integrator verification and change record retention period to five years.
May 2025 (Rev 3.0)	Raico Lamela (BSO) Natalie Roy	Changes in format. SOP updated with validation and verification procedures. Biohazardous waste treatment and transportation updated.

APPENDIX A

Biological Indicator / Chemical Integrator Testing Log

Indicator Brand and Type: _____

Date and Initials	Cycle Setting (temp. °C)	Cycle Time	Indicator Expiry Date	Lot #	Test Result

Appendix A2 Autoclave Validation Record

Validation plans must be approved by EHS in advance. Complete each highlighted section and submit for approval to royn@queensu.ca.

Autoclave location (Building & Rm. #): _____

Person completing validation: _____

Date: _____

Load Type	Parameters (time/temp)	BI or CI (Type used & number)	BI or CI expiry date & load	Location(s) in	Pass/Fail				

APPENDIX B

Biological Indicator Testing Procedure

1. Read and follow the supplier's instructions.
2. Place *B. stearotheromophilus* in the centre of the representative test load, attached to a string or any other device for retrieval after autoclaving.
3. Process the load in a normal fashion
4. a) Extract and incubate the B. stearothermophilus sample as instructed by the manufacturer.
   b) Use another ampoule (same lot #) which is not autoclaved to act as a positive control.
5. Check for a colour change at regular convenient intervals during the incubation period (<18, 24, and 48 hours). If the media is yellow and turbid the autoclave process has FAILED. Re-run all waste bags which remain on site with new biological indicators immediately upon noting yellow colouration.
   - If failure continues to be noted, either increase the time of exposure or initiate repairs to the autoclave. Note the autoclave cannot be used again for biohazardous waste treatment until a validation procedure indicates that autoclave is now adequately sterilizing the material. Post a sign on or near the autoclave log indicating that it may not be used for waste treatment.
6. Record all results, positive and negative. If the test is positive for growth record all corrective actions taken.

APPENDIX C

Chemical Integrator Testing Procedure

1. Read and follow the supplier's instructions.