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AUTOCLAVE - BIOHAZARD WASTE TREATMENT

This Standard Operating Procedure outlines necessary procedures regarding the autoclave-based disinfection of non-anatomical biohazardous waste. These procedures will ensure that the University of Guelph's autoclaves are following applicable guidelines and regulations. This SOP applies to the decontamination and disposal of solid and liquid biohazardous waste deemed to contain Risk Group (RG) 2 human and animal pathogens and/or materials including culture media or any solution that has been in contact with cells, viable organisms or their parts classified as RG2 human and animal pathogens in accordance to [ePATHogen](#) database.

1) Steam Sterilization (autoclave)

- ✓ Is suitable for bags of debris, plates, pipette tips, culture tubes, flasks, and aqueous liquids.
- ✓ It must be operated by trained personnel only.
- ✓ The load must be packaged carefully to allow maximum steam penetration.
- ✓ Appropriate PPE must be worn such as a buttoned lab coat, closed-toed shoes, eye protection, heat-resistant long-cuff gloves, rubber apron/sleeves may also be required.
- ✓ Always check the autoclave cycle log to ensure decontamination parameters have been effectively achieved.

Note: Refer to the [Lab Safety Manual](#) for further info on autoclave use.

Do not use steam sterilization (autoclave):

- for mixed waste (chemical + biohazard or radioactive + biohazard)
- for anatomical waste (anatomical biohazardous waste should be disposed via our biohazardous waste contractor for incineration)
- sharps (in sharps container) are to be disposed via our chemical waste system for further disposal by incineration.

Note: Do not use the same autoclave to process biohazard waste and to sterilize instruments or devices especially those used in a preclinical or clinical setting.

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a) Solid waste

- ✓ Only solid biohazard wastes should be steam sterilized (autoclaved) in the autoclave bags to prevent liquid leakage.

Note: Autoclavable bags filled with plasticware containing agar gel tend to leak fluids during and after the sterilization process.

The autoclaved solid biohazard waste bags (i.e. after steam sterilization) should be:

- double bagged or single sturdy autoclavable biohazard bag (options in the Appendix)
- tied properly to prevent leakage of contents (i.e., secured with an over-hand knot, gooseneck, zip tie, twist tie, or shut with nonporous tape) even if autoclaved bags are kept in plastic bins.
- tagged with a completed “Autoclaved non-hazardous waste tag” to confirm waste has been effectively decontaminated and is ready for removal from the autoclave room for disposal as non-hazardous garbage.

Note: Department transporting their own autoclaved waste do not need to tag their waste with autoclave waste tags. Tagging is required if custodial staff are picking up autoclaved waste. If autoclaved waste is not double bagged and/or tied improperly, the waste may leak onto hands during pick up. Custodial staff will not empty untagged, heavy, and/or leaking waste and the lab occupants will be informed for the unpicked waste.

b) Liquid waste

If steam sterilizing (autoclaving) liquid waste, then

- ✓ Do not collect liquid biohazard waste in waste bags.
- ✓ Liquid biohazard wastes must be collected in vessels or containers that are designed to withstand autoclaving temperatures.
- ✓ Liquid waste containers must be placed into an autoclavable tray or pan of sufficient capacity to contain all liquid.
- ✓ Treated biohazardous liquid waste can be disposed of via the sink. Please see the [Lab Safety Manual](#) for further information related to unacceptable sanitary sewer discharges and Biohazard manual for other options of liquid waste decontamination.

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Note: Liquids poured down the drain should not contain any gel that can block the drain. After pouring liquids, the drains should be flushed with copious amounts of water.

2) Verification

Autoclaves which are used to treat biohazardous waste must be capable of causing a 6 log₁₀ (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 10⁶). The indicators must be used either weekly or with each use if the autoclave is used less than weekly. Indicators should be placed in various positions within the autoclave chamber, outside of the waste load bag to avoid contact with infectious material for verification of the decontamination cycles. Copies of verification records must be annually forwarded to the Biosafety Officer and maintained for a minimum of **three** years.

For autoclave cycle verification, following can be used:

a) Biological Indicators (BI)

BI are test systems containing viable microorganisms for example, *G. stearothermophilus* spores used to confirm that a given autoclave cycle can decontaminate a load of waste.

b) Steam Chemical Integrators (SCI)

These are paper/polymeric films which allow steam to penetrate at a certain rate to monitor all three of the critical variables of the steam sterilization process (time, temperature, and pressure). These integrators can be used instead of BIs for autoclave cycle verification. There are 6 classes of chemical integrators available for use. Class 4, 5 and 6 are considered suitable to meet the requirements for autoclave validation and verification if they demonstrate the appropriate parameters for temperature and time. Specifically, classes 5 and 6 can be used to verify all critical parameters (temperature, time, and pressure) for an autoclave. Class 5 chemical integrators can verify critical parameters over a range of temperatures and closely resemble the performance of biological indicators. Class 6 are cycle specific monitoring devices that verify the critical parameters for a specific sterilization cycle.

Note: When purchasing chemical integrators, one must verify their limitations based on manufacturing specifications (e.g. whether it can be immersed in liquid) before deciding how to use it. Chemical indicators such as temperature-sensitive tape or strips verify temperature

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only and therefore cannot confirm verification of the autoclave cycle. So, chemical indicators are meant to be used in conjunction with biological indicators.

c) Parametric Monitoring Devices:

Parametric monitoring devices are data loggers that monitor and record parameters over a set time. Examples of these devices include thermocouples and gauges that capture cycle time, temperature, and pressure. Parametric monitoring devices may also be used to substitute biological indicators and, in some cases, are the most appropriate choice to verify/validate the decontamination of biohazardous waste. Unlike biological indicators and chemical integrators, the same parametric monitoring device can be used multiple times over a long period of time (i.e. some can last up to 10 years with proper upkeep).

Note: Parametric monitoring devices must be within calibration at the time of usage and calibration certificates are to be retained.

3) Validation

This process confirms the proper functioning of the equipment and the effective decontamination of materials prior to their disposal or removal from the containment zone. Placing indicators at various locations throughout the representative load will enable conditions in different parts of the load to be monitored and confirm that the load has achieved the temperature and time parameters necessary for successful decontamination. Copies of validation records must be forwarded annually to the Biosafety Officer and must be maintained for a minimum of 5 years.

✓ Validation can be achieved by employing either of the methods listed below:

1. **Three biological indicators** are placed on the top, middle and bottom of the representative load bag.

A representative load bag is a simulation batch of non-contaminated materials of a particular load (e.g., plastics, waste liquids etc.) or including mixed load types (e.g. containing pipette tips, agar plates and gloves). Even the quantity of non-contaminated material in the representative load should be similar to the routine biohazard waste load bag decontaminated using the autoclave. For instance., the quantity in a single load can be either a defined amount (e.g., 6 lab coats), size (e.g. 2/3 full autoclave bag) or weight (e.g., 5 kg)

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2. **Parametric monitoring devices** that accurately monitor the performance of decontamination equipment as listed above.

- ✓ Autoclaves should be validated on an annual basis or more frequently as indicated by:
 - a change, repair, or modification to the containment system
 - introduction of new pathogens
 - a request of the Public Health Agency of Canada (PHAC) or the Canadian Food Inspection Agency (CFIA).

Validations must be documented.

4) Common deficiencies in steam sterilization (autoclave) include:

- Validation or verification not performed.
- Validation or verification performed using expired biological indicators.
- Validation performed, but indicator is not placed within a representative load (e.g. indicator outside the bag or tested inside an empty chamber)
- Positive control for biological indicator is not used or is from a different lot number than the test biological indicator.
- The biological indicator used was not compatible with the cycle parameters or not used in accordance with its intended use.
- Validation records or procedure missing

5) Record Maintenance

All records of autoclave validation and cycles must be maintained on an ongoing basis and should include the following information:

- frequency and use of biological indicators or Steam Chemical Integrators
- results of biological indicators or other performance indicators as applicable
- daily use log, with the cycle used, exposure times, dates, user and nature of the load (cycle record printout if available should be stapled in the logbook)
- maintenance and troubleshooting records.
- validation procedure used and its frequency.

These records must be retained for at **least five years** in the autoclave room and must always be available for inspection.