Autoclave Validation Protocol

SCOPE

This document provides written procedures (outlined in section 8 of this document) for validation of UBC Vancouver Point Grey Campus autoclaves used for decontamination of Risk Groups 1 &2 biological waste. As outlined in this procedure, the exact autoclave parameters must be established for each autoclave individually and validated monthly as part of the required monthly inspection. Local variation in biological indicators used as well as autoclave model will influence the customization of the procedure used. Note that more stringent testing is required for autoclaves used to destroy Risk Group 3 agents.

PURPOSE

To provide a consistent, institutional protocol for the validation of autoclaves used for decontamination of biological waste. Note that the contents of this protocol are available as a video at https://srs.ubc.ca/training-and-general-education-courses/environmental-training/.

BACKGROUND

Treatment and disposal of RG1/RG2 Biohazardous waste on UBC Vancouver's Point Grey Campus, including microorganisms, tissue culture waste or other materials contaminated with these biological substances will follow the UBC Procedures for Treatment & Disposal of Biohazardous Waste as per UBC's waste delisting permit from the Ministry of the Environment. The autoclave validation program is audited annually by both the UBC RMS Environmental Protection staff and by an external company. Annual reports on submitted to the Ministry of the Environment as required by the terms of the permit.

Sites using an authorized, certified disposal service are strongly recommended to establish an autoclave validation protocol as a matter of best practice.

RESPONSIBILITY

Individual Biosafety Permit Holders bear responsibility for:

- ensuring personnel handling, receiving, documenting and/or disposing of hazardous or potentially hazardous substances are adequately trained and supervised;
- ensuring to the best of their abilities, that all hazardous or potentially hazardous substances entering or leaving their inventories do so in accordance with applicable international, federal and provincial regulations and standards;

- ensuring all members of their study teams abide by the procedures outlined in section 8; and
- Procurement and use of materials, PPE and services listed in sections 6, 7 and 8 as necessary to comply with the procedures in section 8.
- All UBC members designated as responsible for the validation of autoclaves used for RG1&2 waste on Point Grey Campus:
- ensuring they are adequately trained prior to handling, storing, disposing and/or transporting hazardous or potentially hazardous substances;
- reporting all unsafe conditions including breaches of containment; and
- abiding by the procedures outlined in section 8.

All UBC Members bear responsibility for:

- ensuring they are adequately trained prior to handling, storing, disposing and/or transporting hazardous or potentially hazardous substances;
- reporting all unsafe conditions including breaches of containment.

DEFINITIONS

Autoclave or autoclaving: A chambered instrument used to effect the complete destruction of microorganisms. It employs saturated steam under a pressure of 103 kPa (15 psi) to achieve a chamber temperature of at least 121C for a minimum of period of time (but the time is based on the size of the load and the organism being autoclaved). The time is measured after the temperature of the material being sterilized reaches 121C.

Biological Indicator: a vial of *Geobacillus stearothermophilus* which, if sterilization occurs, will be unable to grow. Viability of the autoclaved vial is determined after incubation under supportive conditions and comparison to a control vial. Growth is detectable by color change. It is critical that the vial be placed in the most difficult areas for the steam to reach in your autoclave batch to be a valid test.

Biological Material: Pathogenic and non-pathogenic bacteria, viruses, fungi, prions, toxins, genetically engineered organisms, nucleic acids, tissue samples, toxins, diagnostic specimens, live vaccines and isolates.

Chemical Indicators: contain chemicals that change color if the correct temperature has been reached. Concerns with the use of Autoclave Tape are that the color change is not time dependent and it does not ensure that all ‘difficult to reach areas’ of the autoclave load are reaching the required temperature and pressure.

Containment: The combination of physical space delineation and operational practices that protect personnel, the immediate work environment, and the community from exposure to biological material.

Contamination: the undesired presence of infectious material or toxins on a surface or within materials.

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.

Disinfectant: A chemical used for the decontamination of surfaces and equipment that cannot be autoclaved. Correct selection and use of disinfectants is critical for effective decontamination. Note: a table is provided in section 6 of this document that outlines advantages and disadvantages of some common disinfectants.

Disinfection: Process that eliminates most forms of living microorganisms. The effectiveness of the disinfection process is affected by a number of factors including the nature and quantity of microorganisms, the amount of organic matter present, the type and state of items being disinfected, and the ambient temperature.

Exposure: Contact with, or close proximity to, infectious materials or toxins that may result in infection or intoxication, respectively. Routes of exposure include inhalation, ingestion, inoculation and absorption.

Gross Contamination: The accumulation of organic material on a surface that can be removed by physical methods such as scraping, wiping and brushing.

Sterilization: Process that eliminates all living microorganisms, including spores.

MATERIALS/EQUIPMENT

1. Testing Supplies

- Minimum of 2 vials of Geobacillus stearothermophilus biological indicators – obtainable by emailing autoclave.report@ubc.ca. Check expiration date and that storage is per manufacturer recommendations.
- Representative waste (in volume & composition) material
- Autoclave tape or equivalent chemical indicator strip
- Autoclave safe, clear bags
- Autoclave tape or equivalent chemical indicator strip
- Heat block or water bath stably set to between 55 and 60C
- Biological waste disposal tags
- Monthly autoclave inspection report template

2. Personal Protective Equipment (PPE)

- Protective clothing (lab coat or isolation gown)
- Gloves (nitrile)
- Autoclave Gloves (insulated for thermal protection)
- Eye/face protection – goggles or face shield
- Long, loose-fitting pants
- Fully covering shoes

PROCEDURE

Autoclave Validation with Geobacillus stearothermophilus biological indicators provided by Risk Management Services as part of the monthly inspection procedure. Note that validation is only established for loads using the same time, pressure, temperature and autoclave as used in the test cycle.

1.1. Validation test with biological indicators.

1.1.1. Exposure.
Note: At minimum, a lab coat, long pants and fully covering shoes must be worn when handling any biological materials. For Risk Group 2 waste, work in a certified Class II biological Safety Cabinet wearing any additional PPE necessary as per local area risk assessment.

1.1.1.1. Obtain a representative waste load bagged in one or two autoclave-safe clear bags. Note that if your representative load is mainly agar plates, it is easiest to use two bags stacked vertically. The bag(s) should be placed in an autoclave safe tray able to accommodate the bag(s) comfortably.

1.1.1.2. Each test will require a minimum of 2 biological indicator vials. 1 vial will be the positive control and must be kept at room temperature while the experimental vial(s) must be inserted into the center of the load. The experimental indicator should be in the most difficult area for the steam to penetrate and never at the bag surface.

1.1.1.3. Insertion of the experimental vial into the center of the load.

1.1.1.3.1. If your representative load is dry materials in a single bag (as with tissue culture waste), tie a string to the vial. Place the vial in the bag center, keeping the string end outside of the bag opening. Add ~125 ml of water to the bag to ensure adequate steam penetration. Leave the bag open.
1.1.1.3.2. If your representative load is mainly agar plates, split the load across 2 bags, neatly stacked one atop the other. Tie a string to the indicator for easy handling after autoclaving. Place the indicator between the bags in the center of the load. Bag openings may be loosely gathered and pointed upwards but not sealed.
1.1.1.3.3. Record load in the required log book, noting that it is a validation test cycle.
1.1.1.3.4. Place tray containing the bag(s) in the center of the autoclave and run a steam cycle of the parameters used for waste decontamination. Note that initial efforts to validate an autoclave will require testing various conditions to establish these parameters. (Recommended starting conditions are 121C, 15 psi for 60 minutes but these must be verified for each autoclave using this test procedure)
1.1.1.3.5. Check the chart reader/recorder to verify that the cycle parameters were achieved. Chart recorder to be archived for review during annual audits by RMS or 3rd parties.
1.1.1.3.6. Remove the waste at the end of the cycle, taking care to avoid burns by wearing autoclave gloves and allowing steam to vent before reaching inwards.
1.1.1.3.7. Remove the experimental biological indicator and allow to cool 10 minutes at room temperature before proceeding. Note that the indicator will have changed color from blue to black – indicating that it was exposed to the autoclave cycle.

1.1.2. Incubation of Biological Indicator.

1.1.2.1. For each of the cooled experimental indicator vial and the control indicator vial, squeeze the vial laterally to bring the medium into contact with the spores.

1.1.2.2. Place both vials at 55-60C for 24 hours.

1.1.2.3. At the 24 hour mark, remove vials for interpretation.

1.1.3. Interpretation of Results.

1.1.3.1. A color change to yellow indicates growth of the spores.

Table 1: Interpretation Guidance Table

Experimental Vial	Positive Control	Interpretation	Result	Next Step
No color change	Color change	Autoclave validation achieved for this load type with these cycle parameters in this autoclave	Pass	Complete Monthly Inspection Report.
Color change	Color change	Autoclave validation failed for this load type with these cycle parameters in this autoclave	Fail	Label as autoclave out of order. Biological waste must be processed in an alternate autoclave or segregated for disposal by a certified contractor. Arrange for servicing before retesting. Report test results to Risk Management. Retest after servicing.
No color change or Color change	No color change	Validation test failure. Autoclave may or may not be functioning correctly.	Fail	Obtain fresh indicators and retest.

1.2. Completion of Monthly Inspection Checklist

1.2.1. After running the validation test, complete the "Autoclave Facilities Monthly Inspection Checklist" available as Appendix 2 the Hazardous Waste Manual.

1.2.2. Email completed checklists to autoclave.report@ubc.ca.

REVIEW AND RETENTION

This Guideline is reviewed as deemed necessary by Safety & Risk Services.