

Guideline for the Safe Use of Autoclaves

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University of British Columbia

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Acknowledgements

We would like to thank the University of Regina for permission allowing us to use and modify their manual, *Autoclave Program User Guide 2016*, making changes that are specific for the Department of Zoology and the Department of Botany at the University of British Columbia. We would also like to thank STERIS for use of their operating manuals and resources and UBC Safety and Risk Services (SRS) for the use of various guides and manuals. Thank you to SRS, and others at UBC, for their feedback on this manual.

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USER GUIDE

1. Introduction

Guideline For the Safe Use Of Autoclaves is intended to promote consistent and safe use of the autoclaves in the Biological Sciences Building within the Department of Zoology and the Department of Botany at the University of British Columbia.

This Program and corresponding standard operating procedures (SOPs) are not a substitute for training and/or reading the appropriate manuals before use. All Principle Investigators and Supervisors must document that training has been received by students and staff who will be using an autoclave.

For training, please contact Joanne Denny (email denny@zoology.ubc.ca or phone: 604-822-3389). A list of authorized users will be kept by Joanne Denny and the Department once training has been completed.

For research, there are 4 Steris Amsco Lab 250 autoclaves in the South Wing (rooms 3302-Zoology and 2302-Botany) of the Biological Sciences Building. For teaching labs, there is 1 Steris Amsco Lab 250 (room 2014B) and 2 Steris Amsco 250LS (rooms 2014A and 4012) autoclaves.

2. Background

An autoclave delivers heat and steam under pressure to decontaminate laboratory media, equipment, and waste. **Decontamination** occurs when the contamination level is reduced to a point where it is no longer a hazard to personnel or the environment (by removing or inactivating infectious materials or toxins); this can be accomplished by disinfection or sterilization.

Sterilization is the process of completely eliminating all living microorganisms, including bacterial spores. **Disinfection** is the process of eliminating most forms of living microorganisms; disinfection is much less lethal to infectious material than sterilization.

Sterilization will only occur if the material is heated to a specific temperature for a given period of time. There are four main parameters that affect successful steam sterilization: steam, pressure, temperature, and time.

Steam: Steam improves heat transfer and is ideally dry and saturated. **Pressure:** A high

pressure allows higher temperatures to be reached as required to properly decontaminate. **Temperature:** The accepted temperatures for steam sterilization are 121°C in a gravity sterilizer for a minimum of 15 minutes at 15psig, or 132°C in a prevacuum sterilizer for a minimum of 4 minutes at 15psig. **Time:** Time varies depending on type of item (solid or liquid, dense or porous, and total mass or volume), how the item is packaged, and type of sterilization applied.

3. Types of Steam Sterilization

Gravity Sterilization: Steam is admitted at the top or side of the chamber and because steam is lighter than air, it forces air out the bottom of the chamber through the drain vent. As a result, air can remain trapped in upright containers or bottles and lead to ineffective sterilization.

Prevacuum Sterilization: Is similar to gravity sterilization, except that the air is removed from the chamber by several vacuum pulses before the saturated steam enters the chamber. This resolves the problems of air entrapment that can occur when air is removed by gravity displacement. It increases the speed and efficiency of sterilization and is more effective on porous heat and moisture stable materials.

Types of Steam Sterilization Cycles

In addition, the building's two autoclave models are equipped with various "cycles" for appropriate material sterilization.

Gravity Sterilization Cycle: This type of sterilization cycle is suitable for sterilizing hard goods (e.g. empty glassware and nonporous materials); but is not suitable for liquids, or anything that will become liquid when heated.

Prevacuum Sterilization Cycle: This type of sterilization is suitable for sterilizing wrapped goods (e.g. instrument trays and containers) with the fast exhaust option. This type of sterilization is suitable for sterilizing liquids in heat-resistant containers with vented closures with the slow exhaust option.

Liquid Sterilization Cycle: This type of sterilization cycle is useful for sterilizing any liquid samples (e.g. reagents, media), wastes containing liquids, items that will become liquid when heated (e.g. agar plates). This cycle has a slow exhaust to minimize liquid boiling over in the autoclave.

Remember, not every item or material can be autoclaved, see *Section 9 – Autoclave*

Material Preparation for more information on what is safe for autoclave sterilization.

4. Verification of Autoclave Efficacy for Biological Waste Decontamination

The effectiveness of sterilization is assessed using both chemical and biological indicators.

Chemical Indicators: Chemical indicators, such as heat tape (aka **autoclave tape**) are affixed to the outside to monitor temperature only. Chemical indicators do not indicate if a load was successfully decontaminated, they simply confirm exposure of the load to a given temperature.

Biological Indicators (BI): The effectiveness of steam decontamination is monitored with a BI containing heat-resistant spores of *Geobacillus stearothermophilus*. If the autoclaved waste load does not reach the correct internal temperature for the correct length of time, the spores survive and germinate. Their metabolic by-products will change the colour of the pH sensitive media. BIs are used to develop the processing times for typical loads and monitor efficacy of decontamination processes.

It is mandatory to regularly verify the effectiveness of waste decontamination for every steam autoclave using *Geobacillus stearothermophilus* biological indicators.

Biological indicators at UBC are available as a self-contained unit.

Self-contained units: A sealed glass ampoule with recovery media is housed in a plastic tube along with a spore strip. After autoclaving and cooling, the glass vial is crushed, mixing the spores with the media for incubation. Biological Indicators are supplied by UBC Safety & Risk Services and are obtainable by emailing autoclave.report@ubc.ca. Check expiration date and that storage is per manufacturer recommendations.

When should biological monitoring occur?

Autoclave efficacy will be validated on a regular basis. The person in charge of the autoclaves, will send a department email when the autoclaves are set to be tested for the following reasons:

- Regular scheduled once per month testing.
- After an autoclave has been repaired or moved.

Who is responsible for efficacy testing?

The person responsible is Joanne Denny (denny@zoology.ubc.ca room 4042 East wing of the Biological Sciences Buildings, phone: 604-822-3389).

Efficacy Testing Bags of Waste:

A. A typical load for waste disposal is considered to be **one** 25" x 35" autoclave bag. The bag should not be more than **1/2 full**. If using a smaller bag, do not fill more than **3/4 full**. BI tests run in Biological Sciences Building use a bag of dry soil (approximately 1/2 full).

NOTE: Do not compress waste as steam will be unable to penetrate and the load **will not be decontaminated**.

B. Bags should be loosely tied, leaving the top open, at minimum a few inches, so steam can penetrate the load. The bag can also be fully opened prior to being autoclaved.

NOTE: Placing several small autoclaves bags within one large autoclave bag is prohibited, as waste will not be decontaminated at current autoclave parameters.

C. Validation test with biological indicators (UBC Autoclave Validation Protocol LAB-SOP-001). See Section 5 for appropriate PPE to be worn when carrying out validation test.



EZTest self-contained Biological Indicator (Mesa Labs)

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.
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.

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

3.1. If your representative load is dry material in a single bag (such as soil), tie a string to the vial and place the vial in the center of the soil/bag. Keep the string end outside of the bag opening and leave the bag open. Bag openings may be loosely gathered and pointed upwards but not sealed. Using soil is the preferred method for a representative load for running BI tests in the Departments of Zoology and Botany.

3.2. If your representative load is mainly agar plates (plates 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 and keep the string end outside the bag, and the bag open.

4. Record load in the required log book, noting that it is a validation test cycle.

5. Setup and ensure the parameters for the waste cycle are: temperature = 121°C, pressure = 15 psi, time = 60 minutes

Place tray containing the bag in the center of the autoclave and run a steam cycle of the parameters used for waste decontamination. For soil and agar plate waste use a liquid cycle.

6. Check the chart reader/recorder to verify that the cycle parameters were achieved. Chart recorder to be archived for review during annual audits by SRS.

7. Remove the waste at the end of the cycle, taking care to avoid burns by wearing autoclave gloves and allowing steam to vent out of the autoclave before reaching in to the autoclave to remove items.

8. Remove the experimental biological indicator and allow to cool 10 minutes at room temperature before proceeding. Note that the indicator strip on the outside of the BI will have changed color from blue to black if the temperature in the autoclave reached 121°C– indicating that it was exposed to the autoclave cycle. If the strip has remained blue, the autoclave has not reached sterilization temperature and an investigation as to why must be carried out.

9. Incubation of Biological Indicator.

- 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 (breaking the glass ampule inside the vial). This can be done by placing the vial perpendicular to the edge of a bench or table. Gently press on either end of the vial to break the glass ampule. Once broken, gently agitate the vial, being careful not to allow the liquid to come into contact with the lid.
- Place both vials at 57°C (or between 55-60°C) in the Mesa labs incubator heat block (this block is designed to fit the EZTest vials) for 24 hours
- At the 24 hour mark, remove vials for interpretation.

10. Interpretation of Results.

- A color change to yellow indicates growth of the spores.
- No colour change (purple) means spores are inactive/dead.
- See Table 1 for interpretation of results and next steps.

Table 1: Interpretation Guidance Table [UBC Safety and Risk Services: Autoclave Validation Protocol (LAB-SOP-001)]

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. <div style="border: 1px solid black; padding: 2px; width: fit-content; margin: 5px auto;">Report results to Safety and Risk Services</div> 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.

11. Place waste in a holding area until the results of the BI are known.

12. If the BI is negative (i.e. no growth) the waste can be discarded.

13. If the BI is positive (i.e. growth), run the load again changing the parameters of the cycle to increase either the temperature or time or both. Use another BI and follow all steps again. Inform users that this autoclave is not available for use until it has passed a BI validation test. If it fails again, contact engineer for repair. Once autoclave has been repaired, repeat autoclave test. A pass must be obtained before allowing users access to the autoclave.

14. Once you have the results from the BI test, Complete and submit the online Autoclave Facility Monthly Report via the Hazardous Waste Inventory System (HWIS). Note that the online report must be completed even if there was no BI testing during that month (e.g. no waste generated, autoclave under repairs, etc.)

<https://cwis.rms.ubc.ca/wis/UserApp/autoclave.aspx>

15. Autoclave any positive BIs prior to discarding them.

Other requirements:

- The autoclave room must be inspected regularly to ensure the area is kept clean and tidy (e.g. place waste bags in secondary containers) and is free of any spills.
- The autoclave cycle recordings must be kept for a minimum of 2 years plus the current year (for a total of 3 years). Cycle recordings are the long paper rolls on the autoclaves that document parameters reached during a cycle.
- A log book that contains all autoclave use, including waste cycles and efficacy testing, must be maintained and records are kept for a minimum of 3 years (2 years plus the current year).

5. Personal Protective Equipment

Often material to be loaded contains potentially infectious material or toxins, thus standard laboratory protective equipment must be worn.

- Nitrile or latex gloves
- Safety glasses or goggles; it is advisable to wear a full face shield if a splash hazard exists
- Laboratory coat
- Closed-toe and closed-heel shoes

In addition, since the interior of the autoclave is extremely hot, **heat resistant gloves** are required for loading and unloading autoclaved materials.



Do not wear previously used nitrile gloves inside the heat resisting gloves, since you risk contaminating the inside of the heat resistant gloves.

6. Training

Autoclave hands on training is required prior to using an autoclave for **all** Faculty, Staff, Postdocs and Students. Training will help minimize the risk of personnel being harmed and damage to the equipment, as well as proper decontamination/sterilization of material.

Currently, site-specific training is facilitated by Joanne Denny. A training session can be requested by emailing denny@zoology.ubc.ca or by phone 604-822-3389. This training covers general autoclave processes and procedures.

Note: This training **must** be complemented by additional research-specific training provided by the Supervisor.

Other mandatory safety training requirements include the SRS Biosafety Training, Hazardous Waste Training, and Autoclave Training (available on the UBC SRS website <https://srs.ubc.ca/training-and-general-education-courses/>).

7. Potential Hazards & Safety Advisories

The autoclave operates under high pressure and temperature; therefore, there is a significant danger of burns and scalds. See specific Autoclave Operator Manuals for a complete listing of warnings and cautions.

- **Heat burns** may occur when the operator comes in contact with hot materials or the autoclave chamber walls and doors.
- **Steam burns** can be caused by contact with residual steam coming out of the autoclave or autoclaved materials upon completion of a cycle.
- Do not attempt to open the sterilizer door unless the chamber pressure gauge on the front panel reads zero (0 psig).
- **Hot fluid scalding** can result from boiling liquids or spillage in the autoclave. Do not allow hot bottles to be jostled as this can cause the bottle to break or explode.
- When sterilizing liquids, the Liquid Cycles must only be used. Use only vented closures; do not use screw caps if possible (if using screw caps ensure they are very loose) or rubber stopped with crimped seals.
- **Explosion** can occur if flammable compounds are processed. Do not process flammable liquids, solvents, chlorinated compounds (e.g. HCl, bleach), oils, waxes, radioactive materials, or substances that may emit toxic fumes.
- Sterilization of >3% chloride-containing solutions (e.g. sodium hypochlorite (bleach), HCl, and NaOH) can cause chamber corrosion and is not recommended

by manufacture. **Please consider an alternative sterilization procedure such as vacuum filtration.** Sterilization of 1-3% saline and sodium chloride- containing solutions may be autoclaved with secure secondary containment.

- Autoclaves require a thorough preventive maintenance program to ensure safe and proper sterilizer operation. Load sterility may be compromised if the biological indicator indicated a potential problem.

8. Autoclave Spill and Incident Procedures

Report all incidents and spills **immediately** to your Supervisor (PI) and Departmental Administrators, Katie Pikor (Zoology zadmin@zoology.ubc.ca) and Jessica Trat (Botany jessica.trat@botany.ubc.ca). Also report to Safety & Risk Services (SRS) using **the** UBC Centralized Accident / Incident Reporting System (CAIRS) **within 24 hours of occurrence.** (https://www.cairs.ubc.ca/public_page.php)

a) Incidents

The most immediate concern following a spill of biologically hazardous materials or organisms is to contain the spill and treat any exposed persons. After this occurs, properly trained employees can begin the cleanup and decontamination process.

If hot liquids are spilled onto clothing, or clothing is soaked in steam:

1. Remove clothing and cool the injured part in cool water.
2. Seek medical attention, if necessary call 911 and/or the Occupational First Aid Team 604-822-4444.
3. Report the incident to your PI and or Laboratory Manager immediately.

Perform clean-up procedures only if:

- The appropriate spill control material, equipment and protective clothing are available.
- Personnel are familiar with equipment and clean-up procedures.
- More than one person is in the lab and available to participate. Work in teams. One person cleans the spill; the other should remain outside of the contaminated area and hand supplies to person cleaning.
- There are no ignition sources present.

Further information regarding chemical spills and biological spills can be found at the UBC Safety and Risk Services website under Research Safety Resources and Documents: <https://srs.ubc.ca/health-safety/research-safety/research-safety-resources-documents/>

b) Spills : General Procedures

i. Spills in Autoclave

The autoclave must be “cleaned” and/or “disinfected” following any spill. Spills in an autoclave may occur from a boil over or breakage of containers. No operation of the autoclave is allowed until the spilled is cleaned up. **Spill kits are located in Rooms 2014 (Zoology), 2303 (Botany), 2374 (Botany-need a key for this room) and the Zoology Office room 4213 (Zoology).**

- 1) Review the SDS (formally MSDS) and PSDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary.
- 2) Wear gloves, laboratory coat, safety glasses, pants, and appropriate foot protection (and any additional personal protective equipment indicated by the SDS and PSDS), and contain the spill material first using the Autoclave Spill Kit.
- 3) Report all spills to Jessica Trat and Katie Pikor.
- 4) Before cleaning spills:
 - a. **Turn off the autoclave.**
 - b. Put a sign on autoclave indicating that is not to be used until the cleanup is complete.
 - c. Wait until the autoclave and materials have cooled to room temperature, before completing clean up:

A. Small and Large Non-Hazardous Material Spill (*Spills you are comfortable cleaning up, examples: seawater, media*):

1. All persons should inform other personnel in the affected area not to enter.
2. Put up a sign indicating there is a spill and to not use the autoclave.
3. Lab PI, Lab Manager, or ‘spill buddy’ should be informed for cleanup assistance.
4. Review the SDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals and biological materials

involved.

5. Wear appropriate PPE.
6. Cover the spill with paper towels to contain it, or use an appropriate spill control material. Use Chemical Spill Kit if necessary. Create a 360° barrier around spill. You may need to remove the shelf from the autoclave (place in sink).
7. Do not allow any solid material to go down the chamber drain.
8. Clear away any materials like broken glass using forceps, or another mechanical device, and place in the appropriate broken glass disposal container (see section 8iv on glass clean up).
9. Clean the spillage area using paper towels other appropriate cleaning materials, moving from the outside to the inside of the spill. Remove the waste and paper towels to an appropriate container for disposal.
10. Wipe any remaining spilled material with Liqui-Jet detergent diluted 1:10 with tap water, using a coarse spray (to avoid creating a fine mist). Rinse inside of chamber with tap water at least 2 times to remove any remaining detergent, and dry with a soft, lint free cloth.
11. Clean the shelf using a soft/non-abrasive cloth and Liqui-Jet detergent diluted 1:10 with tap water. Rinse with tap water and dry with a lint free cloth. Return to autoclave.
12. Clean drain strainer by removing from drain, turn upside down under running tap water. Use a brush to clean out any material that may be stuck inside.
13. Dispose of the waste following the protocol appropriate for the material. If materials have mixed, follow the cleanup and disposal protocol for the most hazardous component of the mixture. This may require re-sterilization of the waste. DO NOT autoclave organic waste and oxidizing agents, such as bleach, before allowing 48 hours for oxidization.
14. Contact Environmental Services Facility (ESF) at 604-822-1285 or 604-363-4420 for information on disposal of waste.
15. Do not use the autoclave until Jessica Trat has given permission to do so.
16. Record the spill and cleanup procedures in the **Autoclave Logbook**

**B. Small Hazardous Material Spill (less than 1L) or Biological Spill (less than 100 ml)
(Spills you are comfortable cleaning up, example: waste RG1 organism in media):**

1. All persons should immediately leave the affected area and allow aerosols to

settle (~30 minutes).

2. Signs should be posted indicating that entry into area is forbidden. Post a sign stating “DO NOT ENTER, HAZARDOUS MATERIAL SPILL OR BIOLOGICAL SPILL”. Contact (name and phone #) for information.”
3. Any exposed person should seek **medical assistance immediately** (within **1-2 hours**) from a health care professional.
4. Lab PI, Lab Manager, or ‘spill buddy’ should be informed for cleanup assistance. Please contact Jessica Trat or Katie Pikor to inform of spill, also to assist in contacting the appropriate people in helping with spill cleanup if necessary.
5. Review SDS and PSDS to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemical and biological materials involved.
6. Wear appropriate PPE.
7. Cover the spill with paper towels to contain it or use an appropriate spill control material. Create a 360° barrier around spill. Use Chemical Spill kit if necessary. You may need to remove the shelf from the autoclave (place in sink).
8. Do not allow any solids to go down the chamber drain.
9. Spray or pour an appropriate disinfectant, or neutralizer, (according to the specific SDS or PSDS) moving from the outside towards the inside of the spill.
10. After the appropriate amount of time (see SDS/PSDS), clear away any materials like broken glass using forceps, or another mechanical device, and place in a sharps container/biohazard container. Set up a container or bag for disposal of waste material (if still need to autoclave materials then use an autoclave bag) (see section 8iv on glass clean up).
11. Clean and disinfect the spillage area using paper towels and other soft non-abrasive cleaning materials, moving from the outside to the inside of the spill. Place waste material into disposal bag. Rinse the area/inside of the chamber with tap water to remove traces of the disinfectant.
12. Wipe down the inside of chamber with Liqui-Jet detergent (diluted 1:10 with tap water) using a coarse spray (to avoid creating a fine mist). Rinse with water at least 2 times to remove any remaining detergent, and dry with a soft lint free cloth.
13. Clean the shelf using a soft/non-abrasive cloth and Liqui-Jet detergent diluted 1:10 with tap water. Rinse with tap water and dry with a lint free cloth.
Return to autoclave.

14. Clean drain strainer by removing from drain, turn upside down under running tap water. Use a brush to clean out any material that may be stuck inside.
15. Dispose of the waste following the protocol appropriate for the material. If materials have mixed, follow the cleanup and disposal protocol for the most hazardous component of the mixture. This may require re-sterilization of the waste or chemical waste disposal. DO NOT autoclave organic waste and oxidizing agents, such as bleach, before allowing 48 hours for oxidization.
16. Contact Environmental Services Facility (ESF) at 604-822-1285 or 604-363-4420 for information on disposal of waste, including broken glass waste.
17. Do not use the autoclave until Jessica Trat has given permission to do so.
18. Record the spill and cleanup procedures in the **Autoclave Logbook**.

C. Large Hazardous Material Spills (larger than 1L) or Large Biological Spills (larger than 100 ml) (example: waste RG1 organism in media):

1. All persons should immediately leave the affected area and allow aerosols to settle (~30 minutes).
2. Signs should be posted indicating that entry into area is forbidden; post a sign stating "DO NOT ENTER, HAZARDOUS MATERIAL SPILL OR BIOHAZARD SPILL. Contact (name and phone #) for information."
3. Any exposed person should seek **medical assistance immediately** (within **1-2 hours**) from a health care professional.
4. Immediately call 911. Be prepared to give them the following information:
 - Type of Chemical or Hazard
 - Amount spilled
 - Exact location of the spill (building and room number)
 - Do not hang up until the operator releases you
 - Inform the UBC Department of Health, Safety and Environment 604-822-2029. Inform your immediate supervisor as well as Departmental Administrator (mentioned at the start of this section).
 - Try to have SDS (PSDS) on hand for emergency personal.
5. Supervised decontamination should proceed.
6. Inform PI, Lab Manager, and Jessica Trat or Katie Pikor of spill.
7. Do not use autoclave until given permission to do so.
8. Record the spill and cleanup procedures in the **Autoclave Logbook**.

ii. Spills outside Autoclave

A. Small and Large Non-Hazardous Material Spill (Spills that you are comfortable cleaning up, **examples: seawater, media**):

1. All persons should inform other personnel in the affected area not to enter. Signs should be posted indicating that there has been a spill, include contact (name and phone #) for information.”
2. The Laboratory Supervisor, or a “Spill Buddy” should be informed for cleanup assistance.
3. Review the SDS to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals involved.
4. Wear appropriate PPE.
5. Cover the spill with paper towels to contain it, or use an appropriate spill control material. Create a 360° barrier around spill. Use Chemical Spill kit if necessary.
6. If needed, spray or pour an appropriate disinfectant over the paper towels and the immediate surrounding area (according to the specific SDS), apply from the outside and move inwards.
7. After the appropriate amount of time (~30 minutes), clear away any materials like broken glass using forceps or another mechanical device and place in a sharps container/biohazard container. Set up a container or bag for disposal of waste material (if still need to autoclave materials then use an autoclave bag) (see section 8iv on glass clean up). .
8. Clean and disinfect the spillage area using paper towels and other appropriate cleaning materials, moving from the outside to the inside of the spill. Wash the affected area with an appropriate cleaning solution (soap and water).
9. Place the waste material/paper towels into the disposal bag.
11. Dispose of the waste following the protocol appropriate for the material. If materials have mixed, follow the cleanup and disposal protocol for the most hazardous component of the mixture. This may require re-sterilization of the waste or chemical waste disposal. DO NOT autoclave organic waste and oxidizing agents, such as bleach, before allowing 48 hours for oxidization.
12. Contact Environmental Services Facility (ESF) at 604-822-1285 or 604-363-4420 for information on disposal of waste.

B. Small Hazardous Material Spills (less than 1 L) and/or Biological Spills (less than 100 ml) (Spills you are comfortable cleaning up, example: waste RG1 organism in media):

1. All persons should immediately leave the affected area and allow aerosols to settle (~30 minutes).
2. Signs should be posted indicating that entry into area is forbidden. Post a sign stating “DO NOT ENTER, HAZARDOUS OR BIOHAZARD SPILL. Contact (name and phone #) for information.”
3. Any exposed person should seek **medical assistance immediately** (within **1-2 hours**) from a health care professional.
4. The Laboratory Supervisor, or a “Spill Buddy” should be informed for cleanup assistance. Please contact Jessica Trat or Katie Pikor to inform of spill, also to assist in contacting the appropriate people in helping with spill cleanup if necessary.
5. Review the SDS and PSDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals and biological materials involved.
6. Wear appropriate PPE.
7. Cover the spill with paper towels to contain it, or use an appropriate spill control material. Create a 360° barrier around spill. Use Chemical Spill kit if necessary.
8. Spray or pour an appropriate disinfectant/neutralizer over the paper towels and the immediate surrounding area (according to the specific biological PSDS). Apply the disinfectant from the outside and move inwards.
9. After the appropriate amount of time (see PSDS or ~30 minutes), clear away any materials like broken glass using forceps or another mechanical device and place in a sharps container/biohazard container. Set up a container or bag for disposal of waste material (if still need to autoclave materials then use an autoclave bag) (see section 8iv on glass clean up).
10. Clean and disinfect the spillage area using paper towels and other appropriate cleaning materials, moving from the outside to the inside of the spill. Wash the affected area with an appropriate cleaning solution (soap and water).
11. Place the waste material/paper towels into the disposal bag.
12. Dispose of the waste following the protocol appropriate for the material. If materials have mixed, follow the cleanup and disposal protocol for the most

hazardous component of the mixture. This may require re-sterilization of the waste or chemical waste disposal. DO NOT autoclave organic waste and oxidizing agents, such as bleach, before allowing 48 hours for oxidization.

13. Contact Environmental Services Facility (ESF) at 604-822-1285 or 604-363-4420 for information on disposal of waste.

C. Large Hazardous Material (larger than 1 L) and Biological Spills (larger than 100 ml) (example: RG1 organism in media):

1. All persons should immediately leave the affected area and allow aerosols to settle (~30 minutes).
2. Signs should be posted indicating that entry into area is forbidden; post a sign stating “DO NOT ENTER, HAZARDOUS MATERIAL OR BIOLOGICAL SPILL. Contact (name and phone #) for information.”
3. Any exposed person should seek **medical assistance immediately** (within **1-2 hours**) from a health care professional.
4. Immediately inform 911. Be prepared to give them the following information:
 - Type of Chemical or Biological Material
 - Amount spilled
 - Exact location of the spill (building and room number)
 - Do not hang up until the operator releases you
 - Inform the UBC SRS 604-822-2029. Inform your immediate supervisor as well as the Departmental Administrator (mentioned at the start of this section).
 - Try to have SDS (PSDS) on hand for emergency personal.
5. Supervised decontamination should proceed.
6. Inform PI, Lab Manager, and Jessica Trat or Katie Pikor of spill.
7. Do not use autoclave until given permission to do so.
8. Record the spill and cleanup procedures in the **Autoclave Logbook**.

iii. Potentially Hazardous Aerosol Release

1. All persons should immediately leave the affected area and no one should enter the room for an appropriate amount of time (e.g. 30 minutes), to allow for aerosols to be carried away and heavier particles to settle. If the laboratory does not have a central air exhaust system, entry should be delayed (e.g. for 24 hours).

2. Signs should be posted indicating that entry is forbidden. Post a sign stating “DO NOT ENTER, HAZARDOUS MATERIAL OR BIOHAZARD SPILL. Contact (name and phone #) for information.”
3. Any exposed person should seek medical assistance immediately (within 1-2 hours) from a health care professional.
4. Call 911. Be prepared to give them the following information:
 - Type of Aerosol
 - Approximate amount released
 - Exact location of aerosol release (building and room number)
 - Do not hang up until the operator releases you
 - Inform the UBC Safety and Risk Service 604-822-2029. Inform your immediate supervisor as well as Departmental Administrator (mentioned at the start of this section).
 - Try to have SDS (PSDS) on hand for emergency personal.
5. Supervised decontamination should proceed.
6. Inform PI, Lab Manager, and Jessica Trat or Katie Pikor of aerosol release.
7. Do not use autoclave until given permission to do so.
8. Record the aerosol release and cleanup procedures in the **Autoclave Logbook**.

iv. Exploded Glassware (uncontaminated) (For contaminated, see above procedures for clean up of Non-Hazardous and Hazardous spills)

1. If glassware has exploded inside the autoclave chamber, turn off the autoclave and allow it to cool down.
2. Post a sign indicating the danger and your contact information.
3. The Laboratory Supervisor, or a “Spill Buddy” should be informed for cleanup assistance. Please contact Jessica Trat or Katie Pikor to inform of glass explosion, also to assist in contacting the appropriate people in helping with cleanup if necessary.
4. Wear appropriate PPE.
5. Place ‘Broken Lab Glassware’ (lined with heavy duty clear plastic bags) container nearby to dispose of broken glass as it is removed from the autoclave. These containers can be obtained from BioSci Shipping/Receiving Room 1015.
6. Gather appropriate materials to help with cleanup, ex: dustpan and brush, gloves, forceps and paper towel.
7. Place broken glass into the broken glassware container carefully as to avoid

cutting yourself.

8. When the glass container is 3/4 full, tie the bag closed and ensure that no glass protrudes out past the top of the container. Obtain a new broken glassware container if needed. Bring full broken glassware containers to Shipping/Receiving in Biosci 1015, during opening hours, for disposal.
9. Once glass has been removed, wet some paper towels with tap water and carefully wipe down the inside of the autoclave. Dispose of the paper towels in the broken glass container.
10. Remove the drain trap at the front of the chamber. Empty any broken glass into the broken glass container.
11. Rinse the drain trap with tap water and place back in the autoclave drain.
12. Do not use autoclave until given permission to do so.
13. Record the spill and cleanup procedures in the **Autoclave Logbook**.

Always contact [Safety and Risk Services \(604-822-2029\)](tel:604-822-2029) prior to wearing a respirator for the first time. You MUST be fit-tested.

9. Material Preparation

Table 2 – General Material Preparation for Autoclave Sterilization

ITEMS THAT CANNOT BE AUTOCLAVED	ITEMS THAT CAN BE AUTOCLAVED
- SEALED CONTAINERS	- CONTAINERS WITH LOOSENED CAPS OR LIDS (E.G. ALUMINUM FOIL CAP)
- OILS, WAXES	- CONTAMINATED SOLID ITEMS, SUCH AS: PETRI DISHES, EPPENDORF TIPS, PIPETTES, GLOVES, PAPER TOWELS
- FLAMMABLE MATERIALS	- ITEMS FOR STERILIZATION, SUCH AS: GLASSWARE, MEDIA, AQUEOUS SOLUTIONS, EQUIPMENT
- MATERIALS CONTAINING: SOLVENTS, OR CORROSIVE CHEMICALS (I.E. PHENOL, TRICHLOROACETIC ACID, ETHER, CHLOROFORM, ETHANOL)	- BIOHAZARD MATERIALS MUST BE LABELED AS SUCH AND SECURED IN CONTAINMENT VESSELS OR AUTOCLAVE BAGS
- MATERIALS CONTAINING: VOLATILE >3% ACIDS AND BASES (I.E. HCl, NaOH)	- MATERIALS CONTAINING: 1 -3% SALINE AND CHLORINATE COMPOUNDS (I.E. CaCl ₂ , NaCl, KCl, PBS)
POWERFUL OXIDIZERS (BLEACH)	
- RADIOACTIVE MATERIALS	- STAINLESS STEEL
- SOME BUFFERS (MOPS) MAY DEGRADE IN THE AUTOCLAVE	- BOROSILICATE GLASS (PYREX)
	- LOOSE OR DRY MATERIALS WRAPPED OR BAGGED IN STEAM-PENETRABLE PAPER OR LOOSELY COVERED WITH ALUMINUM FOIL
- SOME PLASTICS (POLYSTYRENE (PS), POLYETHYLENE (PE), AND HIGH DENSITY POLYETHYLENE (HDPE)) CANNOT BE USED AS SECONDARY CONTAINER	- SOME PLASTICS (POLYPROPYLENE (PP) AND POLYCARBONATE (PC)) CAN BE USED AT SECONDARY CONTAINER

1. Before preparing items for sterilization or decontamination, ensure that each item can be autoclaved (above **Table 2**).
2. Review the SDS and PSDS if you are unsure of the proper safety precautions and personal protective equipment required for the material to be autoclaved.
3. All items to be autoclaved should be placed inside a suitable **primary container** (*i.e.* flasks, tubes, beakers, biohazard bags, or wrapping paper or muslin for instruments), which in turn is put into a **secondary container** (*i.e.* autoclave pan).
 - a. The primary container should be heat resistant, thermally conductive,

puncture proof and waterproof. Suitable containers include:

- i. Borosilicate glass (Pyrex or Kimax)



- ii. Polypropylene (PP) and polycarbonate (PC) plastics



- iii. Teflon (PTFE)

- iv. Stainless steel



- v. Polypropylene bags

- b. **Do not fill primary containers beyond 75% of their holding capacity.**
- c. **All primary containers must be unsealed by loosening screw or vent caps,**

capping open containers with aluminum foil, opening plastics bags slightly (no less than three fingers width) prior to loading into autoclave.

- d. **DO NOT AUTOCLAVE SEALED CONTAINERS OR BAGS.**
 - e. Optional: It can be useful to add 250 mL of water to the contents of bags containing solids to create additional steam to displace any air in the bag during the cycle.
4. Place primary container (i.e. bag or flask), into a SOLID secondary container.
- a. Heat-resistant secondary containers must be large enough to contain any leaks in the primary containers.
 - b. **Plastic secondary containers must be (polypropylene (PP) and polycarbonate (PC)) only.**
 - c. Optional: If using gravity sterilization cycle, 1 -2 mL of water can be added to each item separately. This will fill insulating air pockets that may be generated when the glassware is placed in the pan.
 - d. Do not allow items to touch in pan (takes longer for items to reach required temperatures). Avoid crowding or stacking items.
 - e. Do not overload secondary container; leave sufficient space between each item for steam circulation. For large loads, if the space is greater than 6 inches between each item, the run time can be set for the volume/weight of the volume/heaviest item. If the space is smaller than 6 inches apart, items are considered to be one, and must be sterilized according to the mass of the two objects combined.
 - f. Place empty flasks, test tubes, or other non-porous containers on their sides with loose cover to prevent air trapping and air pockets.
5. Liquids and dry wastes should be processed separately.
6. Materials that are to be sterilized are separated from those to be decontaminated.
7. Temperature sensitive tape must be affixed to all bags and individual items to indicate that the material has been autoclaved. This tape does not prove that the item has been successfully sterilized or decontaminated; it simply indicates that a given temperature was achieved.

Examples of Correct Autoclave Material Packaging



A) Pyrex flasks are filled to only 75% of their holding capacity and placed inside appropriate plastic secondary container, with space between each item to allow steam to circulate.



B) 75% filled primary items are spaced in appropriate plastic secondary container.



C) Autoclavable bags are filled to only 75% of their holding capacity and placed inside appropriate plastic secondary container. The bag opening should be at least 3 finger widths to allow steam to penetrate inside the bag.



D) Appropriate plastic secondary containers are properly placed inside the autoclave equipment.

10. Steris Autoclave Standard Operating Procedures

a) Loading the Autoclave

1. Check the **Autoclave Logbook** first, to ensure that the autoclave is functioning properly (*i.e.* no problems are listed) and that the autoclave has been tested for efficacy in the month.
2. Wear heat resistant gloves, lab coat, and closed-toe shoes. Note: autoclave door and chamber may be hot from a previously run cycle.
3. If autoclave is in standby mode, press the screen to proceed to the main menu screen.
4. If not already logged on, enter Username: STERIS (all CAPS) and password: 1000.
5. Press foot pedal (bottom right side of autoclave) to open door (if no foot pedal, manually open the door by pulling it down). Stand back to allow any steam still in the chamber to escape. Hot steam can cause burns.
6. Check the inside of the autoclave to ensure that it is clean, and that the drain strainer is in place and not blocked. Carefully remove any debris if found and report to Jessica Trat (Jessica.trat@botany.ubc.ca) or Joanne Denny (denny@zoology.ubc.ca). Blockages in the strainer/drain will affect the ability of the autoclave to reach the appropriate temperature for sterilization. If you notice any spills inside the autoclave, do not use and contact Jessica or Joanne immediately.
7. Place materials in autoclave, observing the following precautions:
 - a. Do not overload secondary containers
 - b. Avoid touching, crowding, or stacking items.
 - c. Liquid loads should be a uniform volume and container size
8. Make sure contents are completely inside chamber before closing the door.
9. Press the foot pedal to close the door (or manually close the door if no foot pedal).

b) Cycle Section and Operation

1. Select an appropriate cycle based on the materials to be sterilized. See individual autoclave standard operating procedures for more information. Use arrow keys on the screen to scroll to the appropriate cycle and then press the cycle you want to run. See below for guidelines on cycle recommendations.

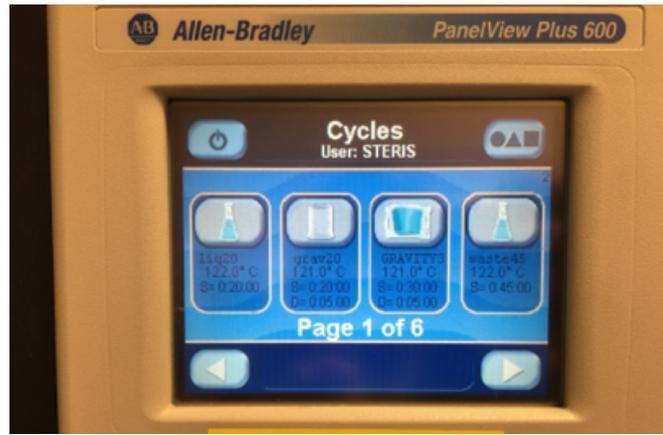


Table 3 – Gravity Cycle Recommendations

Items	Recommended Sterilize Time at 121°C (minutes)*	Dry Times (Minutes)
Glassware (empty, inverted, vented)	15	0
Instruments (metal combined with suture tubing or other porous materials (unwrapped))	20	0
Hard Goods (unwrapped)	15	0
Hard Goods (wrapped in muslin or equivalent)	30	30
Biological Waste	60 (unless exempt)	0

*Listed times include the combined time required to reach 121°C and the time required to achieve sterilization at 121°C. Cycles maybe longer than shown on display due to time to cool down and release pressure at the end of the cycle.

Table 4 – STERIS Liquid Cycle recommendations

Volume of Liquid in One Container (ml)*	Recommended Sterilization Time at 121°C (minutes)**
75	25
250	30
500	40
1000	45
1500	50
2000	55
>2000	55+10 min/L

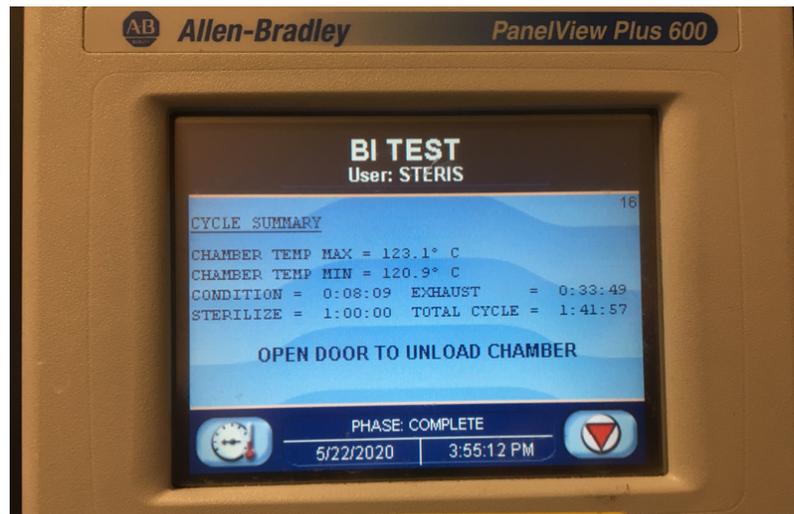
*This time may vary due to viscosity of liquids and other parameters.

**Listed times include the combined time required to reach 121°C and the time required to achieve sterilization at 121°C. Cycles maybe longer than shown on display due to time to cool down and release pressure at the end of the cycle.

2. Biological waste:
 - a. All biological waste must be autoclaved for a **minimum of 60 minutes at 15 PSI** using a liquid cycle before disposal, regardless of the volume/mass being sterilized (UBC Hazardous Waste Manual 2014). The Botany department has an exemption list for some RG1 (non-hazardous) Biologicals Waste that has been approved by SRS:
 1. transgenic *Arabidopsis thaliana* plants;
 2. transgenic *Helianthus annuus* plants;
 3. *Physcomitrella patens* moss plants;
 4. *Chlamydomonas reinhardtii* algae (plants);
 5. *Nicotiana benthamiana* plants;
 6. non-pathogenic, non-hazardous *Agrobacterium tumefaciens* (microbe);
 7. non-pathogenic, non-hazardous *Escherichia coli* bacterial strains (microbe); and
 8. *Saccharomyces cerevisiae* (microbe).
 - b. Please contact Jessica Trat or Joanne Denny for this *Department of Botany Standard Operating Procedures (SOP)*
3. Soil, vermiculite:
 - a. Anything particulate (soil, vermiculite, etc.) must be run in a liquid cycle only; the rapid exhaust of a gravity cycle could cause the particulate to “coat” the inside of the autoclave chamber. A pre-vacuum cycle should be used, where available.
4. Liquids:
 - a. All liquids and any media that will melt during sterilization must be processed using a liquid cycle.
 - b. Liquid cycles have slower exhaust rates to minimize boiling and evaporation of the material being autoclaved; there is no drying time associated with liquid cycles.
5. Press “[Start Cycle](#)”. Do not leave immediately; remain in room until the autoclave has started and the screen has transitioned to a time for the length of the cycle.
6. Record autoclave parameters in the Autoclave Logbook.



7. If the run doesn't start, the autoclave will set off an alarm.
 - a. Record the error message displayed on the screen.
 - b. Silence the alarm.
 - c. IMMEDIATELY report the alarm to Jessica Trat or Joanne Denny.
 - d. They will assess the situation and determine if a service call needs to be made.
 - e. Record problems in **Autoclave Logbook**.
8. Once the cycle is complete, try to unload material promptly. Others maybe waiting patiently for equipment. Screen below shows a complete cycle.



9. Do not attempt to open door while autoclave is operating. If you must access the materials in the autoclave you will have to abort the cycle by pressing the abort button on the screen (see below for aborting cycles 10e). Only use abort if it is an emergency. Only press the red Emergency abort button for emergencies also. This will lock the autoclave down and a service technician will need to be called.
10. If cycle alarms at any time during the run (or aborts), please record the problem in the **Autoclave Logbook** and contact Jessica Trat to report the problem.

c) Unloading the Autoclave

1. Wear heat resistant gloves, lab coat, shoes, and face shield. The greatest risk of personal injury occurs during autoclave unloading.
 - a. High risk of burns or scalds from autoclaved materials.
 - b. Exposure to vapors and gases due to inadvertent autoclaving of volatile chemicals.
2. Do not attempt to open the sterilizer door unless the chamber pressure gauge on the front panel reads zero (0 psig). If there is no gauge, check the screen. If the screen is on the main page of cycles then it is safe to open. If there is a run currently in the machine, there will be a countdown timer. The door will be locked and will not open. Do not attempt to force it open. If a run has finished, the screen will show a cycle summary and will say at the bottom 'open door to unload chamber' (see picture on previous page).
3. Stand away and to the side of the door, step on the foot pedal (bottom right if present) to open the door (or manually open if there is no foot pedal). Stand back from the door to avoid the escaping steam.
 - a. If samples are boiling or bubbling, wait until they subside before removing them.
 - b. Do not agitate containers of super-heated liquids or remove caps before unloading. Super-heated liquids can "bump" when they are removed from the autoclave causing a spray of boiling liquid, possibly resulting in serious burns.
4. Using heat resistant gloves, carefully transfer the autoclave trays to a cart.
5. Use the foot pedal (if present) to close the autoclave door (or manually close). Keep the chamber door closed between cycles and when not in use.
6. Verify that the temperature-sensitive tape has changed colour. If not, new tape needs to be applied and the material autoclaved again (ensure that the cycle is running before leaving). If the tape does not change colour a second time, and no alarm went off, chances are the tape is "bad". Confirm that the autoclave cycle temperature was reached for sufficient time by reviewing the autoclave paper printout if one is available, or check the 'completed cycle' screen before opening the door. This screen will show you the temperature achieved during the cycle. The autoclaves in the South Wing do not have working printers. If you are sure your cycle ran, please contact Joanne Denny (denny@zoology.ubc.ca) to run an efficacy (BI) test. Please supply a sample of your autoclave tape.

7. Record any problems in the **Autoclave Logbook**.
8. Transport items to a safe location and wait for the items to cool before storing or disposing.
9. When disposing of autoclaved biohazard waste, it must be placed inside designated biological waste receptacles in Biological Sciences Building Shipping/Receiving room 1015B (East wing). It may never be placed on ground or disposed of in your laboratory. See Department Waste Disposal Protocols for more information. Please contact Shipping/Receiving at shiprec@botany.ubc.ca or by phone, Tel: 604-822-5040 for opening times.

d) Standby and Shutdown

On Weekends, or weekdays after 6 pm and before 8 am, autoclaves may be in standby mode. To use, tap the screen and enter user name and password (this information is on the main screen). The main cycle screen will appear. Use arrows to scroll to the cycle you would like to use.

e) Aborting cycles

Aborting a cycle should only be used in an emergency. You can abort a cycle by pressing abort on the screen. Please note, this stops the cycle immediately, however, you will not be able to open the door until the pressure inside the chamber reaches 0 PSI. Due to stopping during the cycle, the pressure lowers slowly and it can take a while, often a few hours (could be longer than your original run would have taken) to reach 0 PSI depending on what the PSI was before aborting. This is a safety feature that cannot be bypassed. Only press the red Emergency abort button, on the outside of the machine, for emergencies also. This will lock the autoclave down and a service technician will need to be called. Examples of emergencies: hearing something exploding inside the autoclave during a run, and steam or a loud hissing coming from the steam generator underneath the autoclave chamber.

11. Reference Guides

2016 Autoclave Program User Guide-University of Regina www.uregina.ca

BOTANYwasteSOP2019MarFINAL-UBC Department of Botany

Steris-LS250 Operator Manual
Steris Operation Manual for AMSCO Lab Series Sterilizers

The following are from:

University of British Columbia (CA), Safety and Risk Management.

Available from: <https://srs.ubc.ca/>

UBC Biosafety Manual 4962880

UBC Biohazard Waste Treatment and Disposal Procedure 2020

UBC Biological Spill Cleanup (BIO-SWP-001)

UBC Autoclave Validation Protocol (LAB-SOP-001)

UBC Chemical Spill Cleanup (UBC-RMS-OHS-SWP-17-001)

UBC Hazardous Waste Manual 2014

UBC Laboratory Glass Disposal

Steris AMSCO 250LS (Teaching Labs)

Steris AMSCO 250 (Research Labs)

