

Autoclave Program

This Autoclave Program (User Guide and Maintenance Guide) was written in collaboration with the Autoclave Working Group, Faculty of Science, Biosafety Advisory Committee, Health, Safety & Environment, and Facilities Management.

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USER GUIDE

1. Introduction

This *Autoclave Program User Guide* (Version 1, 2015) was not developed for use or maintenance of University of Regina autoclave equipment prevacuum options/ cycles. If this need is recognized in the future, additional equipment testing and maintenance will need to occur prior to use of options/cycles.

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.

A list of authorized users will be kept by the Microbiology Technician in LB432.1.2 and Health, Safety & Environment in AH435.

This Program is intended to promote consistent and safe use of the autoclaves within the University of Regina.

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 two models of autoclaves 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 are available in a variety of formats, as explained below. Your waste production will dictate what type and size of BI that should be used. Please consult with the Biosafety Advisor to find the BI best suited to your needs. Always follow the manufacturer's instructions for safe handling, incubation temperature, and time.

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.

Spore strips: Spores inoculated onto filter paper and packaged in a paper pouch or envelope. They must be aseptically transferred to growth media for incubation. Media must be made or purchased separately. These are best to use with dry goods and are not recommended for waste or liquids.

Sealed ampoules: Spores suspended in recovery media inside a glass ampoule. No crushing or transfer is required. These are appropriate to use with bags of waste or liquid waste.

When should biological monitoring occur?

- Autoclave efficacy should be validated on a regular basis with biological indicator testing on an “as used” basis. The person in charge of the autoclave will determine the frequency of the testing. Consider regular as:
 - Weekly if one or more waste autoclave loads are run daily
 - Every two weeks if 2-3 loads/week
 - Monthly if \leq one load/week
- Whenever a new type of material is decontaminated and when any change to standardized waste loads.
 - Users must inform the person in charge of the autoclave when changes occur.
- After an autoclave has been repaired.
 - The person in charge of the autoclave is responsible for arranging this testing.

Who is responsible for efficacy testing?

The person in charge of the autoclave will organize and communicate with users when efficacy testing needs to be done and what steps in the following procedures each user is required to assist with.

Efficacy Testing Bags of Waste:

1. A typical load for waste disposal is considered to be **one 25" x 35"** autoclave bag. The bag should not be more than **½ full**. If using a smaller bag, do not fill more than **¾ full**.

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

2. 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 autoclave bags within one large autoclave bag is prohibited, as waste will not be decontaminated at current autoclave parameters.

3. Procedure:

- a. Using long forceps, insert BI **into the middle of the load in the centre of the bag** to be decontaminated.

- i. If using a self-contained BI:

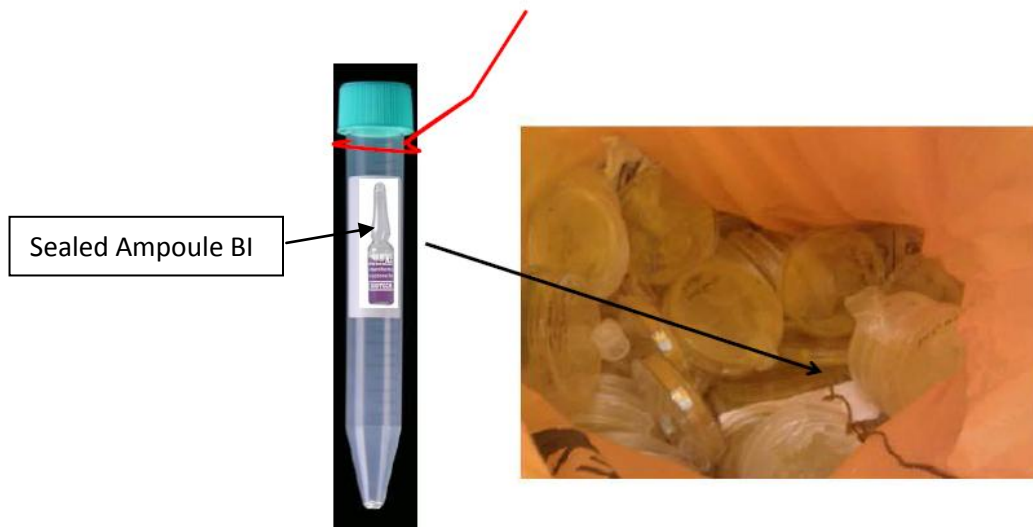
1. Tie a long string to the BI as this will allow easy removal.

- ii. If using a sealed ampoule BI:

1. Insert the sealed ampoule into a test tube, container, or some other device that has a cap and fits approximately 15-100 ml of water. Fill with water. Loosely cap.

2. Tie a string around the tube for easy retrieval

3. Place upright in bag.

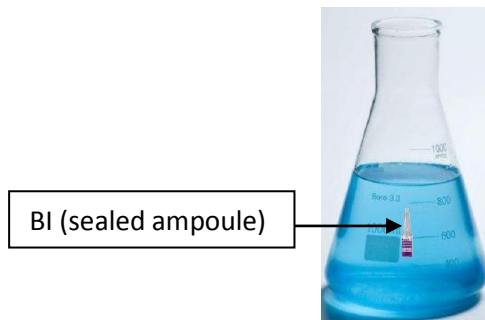


- b. Autoclave the load for a **minimum of 60 minutes** using the WASTE liquid cycle.
- c. Optional for validation tests: Run a representative test load the first time to ensure parameters are correct for actual waste loads.
 - i. To make a representative test load: in an autoclave bag, place agar plates, gloves, paper towel, or pipette tips. Use anything that you would place in the bag under normal circumstances.
- d. Allow the load to cool for at least 10 minutes.
 - i. If using a self-contained BI, disinfect the outside of the vial as soon as you remove it from the autoclaved load (always assume it is contaminated).

- ii. If using a sealed ampoule, remove the test tube from the load and disinfect the outside. Allow the liquid in the tube to cool before handling. Retrieve the ampoule using forceps.
- e. Follow manufacturer's instructions for how to activate and incubate the BI. A **control** from the same lot number must be incubated at the same time. The control should always show a positive (i.e. growth) result. If it does not, there is something wrong with the lot.
- f. Place waste in a holding area until the results of the BI are known.
- g. If the BI is negative (i.e. no growth) the waste can be discarded. Deface the biohazard symbol on the bag and place into the regular garbage cans.
- h. 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 steps a through f again.
- i. Autoclave any positive BIs prior to discarding them.

Efficacy Testing for Liquid Waste

1. Secure the appropriate BI (sealed ampoule) to a thin gauge wire and secure the wire to the container containing the waste to keep the BI submerged in the center of the liquid.



The volume of liquid being autoclaved will dictate what size of sealed ampoule you will need. Talk to the manufacturer of the BI or the Biosafety Advisor to determine the correct size for your needs.

- a. **Autoclave for the appropriate amount of time** (see Table 4 – STERIS Liquid Cycle Recommendations)
- b. Allow the liquid to cool before retrieving the ampoule.
- c. Follow manufacturer's instructions for how to activate and incubate the BI. A **control** from the same lot number must be incubated at the same time. The control should always show a positive (i.e. growth) result. If it does not, there is something wrong with the lot.
- d. Place waste in a holding area until the results of the BI are known.
- e. If the BI is negative (i.e. no growth) the waste can be discarded.
- f. 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 steps a through f again.
- g. Autoclave any positive BIs prior to discarding them.

Other requirements:

- The autoclave cycle recordings must be kept for a minimum of 5 years. Cycle recordings are the long paper rolls on the autoclaves that document parameters reached during a cycle.
- If no cycle recorder is on the autoclave, you must validate the predetermined cycle parameters using chemical sterilization integrators. If this is the case, please contact the Biosafety Advisor for assistance.
- 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 5 years.

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 Training is absolutely required prior to using an autoclave for **all** Faculty, Staff, Students, and Visitors. Training will help minimize the risk of personnel being harmed and damage to the equipment.

Currently, site-specific training is facilitated by the Faculty of Science. A list of upcoming training sessions can be found on the Human Resources website: <http://www.uregina.ca/hr/training-events/index.html> or can be requested by contacting the Microbiology Technician (306-585-4892). This training covers general University autoclave processes and procedures.

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

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 if a "Water in Chamber" (Steris) or "Water in Drain" (Getinge) alarm condition exists.
- 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 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

a) Incidents

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. Aspen Medical Centre is open Monday – Friday.
3. Report the incident to the Microbiology Technician (306-585-4892) and/or Health, Safety & Environment (306-585-4776) immediately.
4. A sign will be placed on the autoclave indicating that it is not to be used until the accident investigation is complete and autoclave is deemed safe for operation.

All incidents must be reported to Microbiology Technician, Health, Safety & Environment, and if applicable, a student's Supervisor within 24 hours of occurrence.

b) Spills

i. Spills in Autoclave

The autoclave must be “cleaned” and/or “disinfected” following any spill. Spills in autoclave may occur from a boil over or breakage of containers. No operation of the autoclave is allowed until the spilled is cleaned up.

1. Review the 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 MSDS and PSDS), and contain the spill material first using the Autoclave Spill Kit.
3. Report the spill to the Microbiology Technician (306-585-4892) and/or Health, Safety & Environment (306-585-4776), who will advise the user on the best way to clean up the spill.

This may involve the following:

- 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:

i. Small Non-Hazardous Material Spill (Spills you are comfortable cleaning up):

1. All persons should inform other personnel in the affected area not to enter.
2. Review the MSDS and PSDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals and biological materials involved.
3. Wear gloves, laboratory coat, shoes, pants, and other appropriate personal protective equipment (i.e. face and eye protection).
4. Cover the spill with cloth or paper towels to contain it.

5. Spray or pour an appropriate disinfectant over the paper towels and the immediate surrounding area (according to the specific biological PSDS; generally, Oxivir TB or 70% ethanol solutions are appropriate).
6. Start applying the disinfectant from the outside and move inwards.
7. After the appropriate amount of time (5-10 minutes), clear away any materials like broken glass using forceps or another mechanical device and place in the appropriate broken glass disposal container.
8. Clean and disinfect the spillage area using paper towels and other appropriate cleaning materials.
9. Remove the chamber shelf assembly and wipe any remaining spilled material.
10. Clean the inside of the chamber and the shelf using a soft/non-abrasive cloth and Liqui-Jet detergent diluted 1:10 with tap water.
11. Rinse all of the detergent out of the autoclave before it is restarted.
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.
13. Or place contaminated materials into a labelled, leak-proof, puncture-resistant waste disposal container and dispose of waste appropriately. Contact Health, Safety & Environment (306-585-4776) for waste disposal assistance.
14. Complete an **Incident Report Form** and forward to Health, Safety & Environment within 24 hours. Forms can be found online www.uregina.ca/hr/hse or by contacting health.safety@uregina.ca.
15. Do not use the autoclave until the Microbiology Technician (306-585-4892) has given you permission to do so.
16. Record the spill and cleanup procedures in the **Autoclave Logbook** (Appendix 1).

ii. Large Non-Hazardous Material Spill (Spills you are not comfortable cleaning up):

1. All persons should inform other personnel in the affected area not to enter.
2. Review the MSDS and PSDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals and biological materials involved.
3. The Laboratory Supervisor and UR Hazardous Material Spill Response Team (via Campus Security (306-585) 4999) should be informed for cleanup assistance.

iii. Small Hazardous Material Spill (Spills you are comfortable cleaning up):

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, 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, Health, Safety & Environment (306-585-4776), or a "Spill Buddy" should be informed for cleanup assistance.
5. Wear gloves, laboratory coat, shoes, pants, and eye/face protection.
6. Spray or pour an appropriate disinfectant (according to the specific biological PSDS; generally, Oxivir TB disinfectant should be sufficient.)
7. After the appropriate amount of time (see PSDS), clear away any materials like broken glass using forceps or another mechanical device and place in a sharps container/biohazard container.
8. Clean and disinfect the spillage area using paper towels and other soft non-abrasive cleaning materials.
9. Remove the chamber shelf assembly and wipe any remaining spilled material.
10. Clean the inside of the chamber and the shelf using a soft/non-abrasive cloth and Liqui-Jet detergent diluted 1:10 with tap water.
11. Rinse all of the detergent out of the autoclave before it is restarted.
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.
13. Or place contaminated materials into a labelled, leak-proof, puncture-resistant waste disposal container and dispose of waste appropriately. Contact Health, Safety & Environment (306-585-4776) for waste disposal assistance.
14. Complete an **Incident Report Form** and forward to Health, Safety & Environment within 24 hours. Forms can be found online www.uregina.ca/hr/hse or by contacting health.safety@uregina.ca.
15. Do not use the autoclave until the Microbiology Technician (306-585-4892) has given you permission to do so.
16. Record the spill and cleanup procedures in the **Autoclave Logbook** (Appendix 1).

iv. Large Hazardous Material Spill (Spills you are not comfortable cleaning up):

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, 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 and UR Hazardous Material Spill Response Team (via Campus Security (306-585) 4999) should be informed for cleanup assistance.
5. Supervised decontamination should proceed.

ii. *Spills outside Autoclave*

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, a properly trained employee can begin the clean up and decontamination process.

a. **Small Non-Hazardous Biological Spill**

(Spills that you are comfortable cleaning up)

1. All persons should inform other personnel in the affected area not to enter.
2. Review the MSDS and PSDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals and biological materials involved.
3. Wear gloves, laboratory coat, shoes, pants, and other appropriate personal protective equipment (i.e. face and eye protection).
4. Cover the spill with cloth or paper towels to contain it.
5. Spray or pour an appropriate disinfectant over the paper towels and the immediate surrounding area (according to the specific biological PSDS; generally, 10% bleach or 70% ethanol solutions are appropriate).
6. Start applying the disinfectant from the outside and move inwards.
7. After the appropriate amount of time (5-10 minutes), clear away any materials like broken glass using forceps or another mechanical device and place in a sharps container/biohazard container.
8. Clean and disinfect the spillage area using paper towels and other appropriate cleaning materials.
9. Place contaminated materials into a labelled, leak-proof, puncture-resistant waste disposal container and dispose of waste appropriately. Contact Health, Safety & Environment (306-585-4776) for waste disposal assistance.
10. Complete an **Incident Report Form** and forward to Health, Safety & Environment within 24 hours. Forms can be found online www.uregina.ca/hr/hse or by contacting health.safety@uregina.ca.

b. **Large Non-Hazardous Biological Spill**

(Spills you are not comfortable cleaning up by yourself)

1. All persons should inform other personnel in the affected area not to enter.
2. Review the MSDS and PSDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals and biological materials involved.
3. The Laboratory Supervisor and UR Hazardous Material Spill Response Team (via Campus Security (306-585) 4999) should be informed for cleanup assistance.

c. **Small Hazardous Biological Spill**

(Spills you are comfortable cleaning up)

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, 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, Health, Safety & Environment (306-585-4776), or a “Spill Buddy” should be informed for cleanup assistance.
5. Wear gloves, laboratory coat, shoes, pants, and eye/face protection.
6. Cover the spill with cloth or paper towels to contain it.
7. Spray or pour an appropriate disinfectant over the paper towels and the immediate surrounding area (according to the specific biological PSDS; generally, 10% bleach solutions are appropriate).
8. Start applying the disinfectant from the outside and move inwards.
9. After the appropriate amount of time (see PSDS), clear away any materials like broken glass using forceps or another mechanical device and place in a sharps container/biohazard container.
10. Clean and disinfect the spillage area using paper towels and other appropriate cleaning materials.
11. Place contaminated cleaning materials into a labelled, leak-proof, puncture-resistant waste disposal container and dispose of waste appropriately. Contact Health, Safety & Environment (306-585-4776) for waste disposal assistance.
12. Complete an **Incident Report Form** and forward to Health, Safety & Environment within 24 hours. Forms can be found online www.uregina.ca/hr/hse or by contacting health.safety@uregina.ca.

d. Large Hazardous Biological Spill

(Spills you are not comfortable cleaning up)

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, 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 and UR Hazardous Material Spill Response Team (via Campus Security (306-585) 4999) should be informed for cleanup assistance.
5. Supervised decontamination should proceed.

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, 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 and UR Hazardous Material Spill Response Team (contacted via Campus Security (306-585) 4999) should be informed for cleanup assistance.
5. After the appropriate amount of time (~30 minutes – 24 hours), supervised decontamination should proceed.

Always contact Health, Safety & Environment (306-585-4776) prior to wearing a respirator for the first time. You **MUST** be fit-tested.

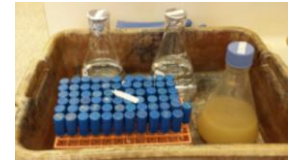
9. Material Preparation

Table 1 – 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>i.e.</i> 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% saline and chlorinate compounds (<i>i.e.</i> HCl, NaOH or bleach)	- Materials containing: 1 -3% saline and chlorinate compounds (<i>i.e.</i> CaCl ₂ , NaCl, KCl, PBS)
Contact Microbiology Technician at 306.585.4892 if you need to autoclave >3% saline and chlorinate compounds for assistance	
- 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
- 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

1. Before preparing items for sterilization or decontamination, ensure that each item can be autoclaved (above **Table 1**).
2. Review the MSDS 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 water proof. Suitable containers include:
 - i. Borosilicate glass (Pyrex)

- ii. Polypropylene (PP) and polycarbonate (PC) plastics
 - iii. Teflon (PTFE)
 - iv. Stainless steel
 - v. Polypropylene biohazard 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 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 room for steam circulation. For large loads, if a spacer greater than 6 inches is between each items, the run time can be set for the volume/weight of the volume/heaviest item. If the space is smaller than 6 inches apart, they 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. Liquid and dry wastes need to 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 indicated that a given temperature was achieved.



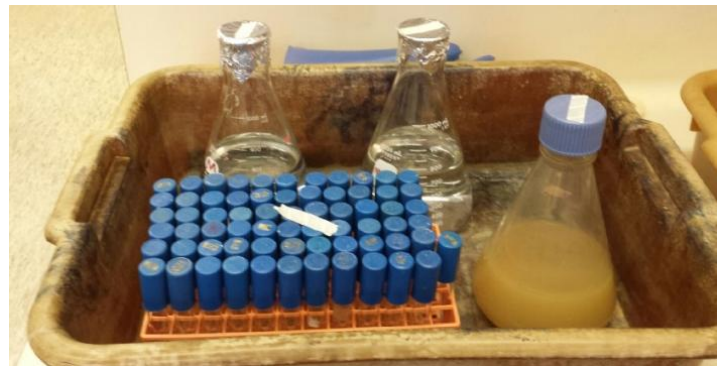
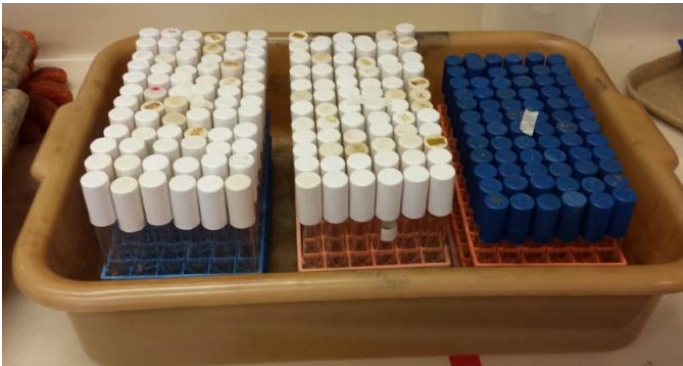
Examples of Correct Autoclave Material Packaging



Pyrex flasks are filled to only 75% of their holding capacity and placed inside appropriate plastic secondary container



Polypropylene biohazard bags are filled to only 75% of their holding capacity and placed inside appropriate plastic secondary container



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



Appropriate plastic secondary containers are properly placed inside the autoclave equipment

Table 2 - List of University of Regina Autoclave Models

Location	Autoclave Model
Laboratory Building 306	Market Forge Sterilmatic
Laboratory Building 306	Barnstead
Laboratory Building 407	Market Forge Sterilmatic
Laboratory Building 432	Steris Amsco Eagle Century
Laboratory Building 432	Getinge Model 533LS
Research and Innovation Centre 240	Steris Lab Series 250
Research and Innovation Centre 240	Steris Amsco Century
Research and Innovation Centre 539	Steris Lab Series 250

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 all appropriate efficiency that last 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 centre of the screen to proceed to the main menu screen.
4. Choose your lab for operator access, enter your code, and press enter. (If the autoclave is logged in, select “[Option](#),” “[Next](#),” and then choose “[Standby](#)”. Touch screen and proceed to log in as usual).
5. Press foot pedal to open door.
6. Check the inside of the autoclave to ensure that it is clean, and that the drain strainer is in place.
7. Slide shelf half way out of sterilizer chamber.
8. Place materials in autoclave, observing the following precautions:
 - i. Do not overload secondary containers
 - ii. Avoid touching, crowding, or stacking items.
 - iii. Liquid loads should be a uniform volume and container size
9. Make sure shelves are completely inside chamber door before closing door.
10. Press the foot pedal to close the door.



b) Cycle Section and Operation

1. Select an appropriate cycle based on the materials to be sterilized. See individual autoclave standard operating procedures (below) for more information.

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	20
Biological Waste	30 (minimum)	0

*Listed times include the combined time required to reach 121°C and the time required to achieve sterilization at 121°C.

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.

2. Biological waste:
 - a. All biological waste must be autoclaved for a **minimum of 60 minutes** using a liquid cycle before disposal, regardless of the volume/mass being sterilized (Le *et al.*, 2005; Rutala, W.A *et al.*, 1982).
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 slow exhaust cycles to minimize boiling and evaporation of the material being autoclaved; there is no drying time associate with liquid cycles.
5. Record autoclave parameters in the **Autoclave Logbook.**

6. Press "[Start Cycle](#)". Do not leave immediately; remain in room until the autoclave has started. If the cycle is going to fail (usually due to incomplete warm-up) it will happen in this first step.
7. If run doesn't start, the autoclave will begin to alarm.
 - b. Record the error message displayed on the screen.
 - c. Abort the cycle by selecting the abort button on the screen. DO NOT press the large red Emergency abort button.
 - d. IMMEDIATELY report the alarm to the Microbiology Technician (306-585-4892).
 - e. The Microbiology Technician will assess the situation and determine if a service call needs to be made.
 - f. Record problems in **Autoclave Logbook**.
8. Once cycle is complete, try to unload material promptly. Others are waiting patiently for equipment.
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. DO NOT press the red Emergency abort button. This will lock the autoclave down and a service technician will need to be called.

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 vapours 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).
3. Stand away and to the side of the door, step of the foot pedal to open the door. 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 if proper containers are not used.
4. Using heat resistant gloves carefully transfer the autoclave plans to a cart.
5. Use the foot pedal to close the autoclave door. Keep 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. 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.
7. Record any problems in the **Autoclave Logbook** and notify the Microbiology Technician (306-585-4892).
8. Return autoclave to Standby Mode by selecting "[Options](#)" (from the main menu), followed by "[Standby](#)".



9. Transport items to a safe location and wait for the items to cool before storing or disposing.
 - a. Biohazardous materials must be labelled as “decontaminated” prior to placing in garbage waste containers. This can be accomplished by placing “decontaminated” heat sensitive tape on autoclave bags or using VWR/Fisher autoclave bags that have the word “decontaminated” become visible (heat activated) once autoclaved.
10. When disposing of autoclaved waste, waste must be placed inside designated garbage receptacles within the autoclave room. It may never be placed on ground or disposed of in your laboratory.



d) Standby and Shutdown

After a run is complete, the autoclave should always be left in standby mode.

11. Getinge 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 all appropriate efficiency that last month.
2. Wear heat resistant gloves, lab coat, and closed-toe shoes: autoclave door and chamber may be hot from a previously run cycle.
3. Note: To stop or reverse the direction of a moving door, press one of the following: “[Open/Close Door](#)” (opposite direction), “[Clear Alarm](#),” or “[Controls Off/On](#).”
4. If the LED on the “[Close Door](#)” switch is illuminated, press “[Open Door](#)” to unseal door.
 - a. If door does not open, press “[Unseal](#)” and wait 15-20 seconds for door to unseal. Then press “[Open Door](#).”
5. Stand away from door and press “[Open Door](#)” again to open completely.
6. Check the inside of the autoclave to ensure that it is clean, and that the drain strainer is in place.
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 shelves are completely inside chamber door before closing door.
9. To close door, press “[Close Door](#).” Stand away from door area while door is in motion. The door seals when the cycle starts.



b) Cycle Section and Operation

2. Select an appropriate cycle based on the materials to be sterilized. See **Table 3** and **Table 4** of this Autoclave Manual for guidance.
3. Biological waste:
 - a. All biological waste must be autoclaved for a **minimum for 60 minutes** before disposal, regardless of the volume/mass being sterilized (Le *et al.*, 2005; Rutala, W.A *et al.*, 1982).
4. 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.
5. Liquids:
 - a. All liquids and any media that will melt during sterilization must be processed using a liquid cycle.
 - b. Liquid cycles have slow exhaust cycles to minimize boiling and evaporation of the material being autoclaved; there is no drying time associated with liquid cycles.
6. Use an effluent cycle when aerosolized pathogens and organisms must be contained until terminal sterilization is achieved (see Getinge Manual for more information).
7. Record cycle parameters in the **Autoclave Logbook**.
8. Check that the “Start” button LED is flashing. This indicates that you can start the sterilizer.
9. From the process screen press “[Select Cycle](#).”
 - a. Use the up and down arrows to scroll the list of cycles.
 - b. Pressing “[Enter](#)” displays information about the selected cycle at the top of the screen.
 - c. Press “[Cancel](#)” anytime to return to the process screen without selecting a cycle.
10. Press “[Enter](#)” and then “[Ok](#)” to confirm cycle.
11. Press “[Start](#).”
 - a. The doors sealed and the “Door Sealed” LED illuminates.
 - b. During the cycle, the “In Process” LED illuminates.
 - g. Do not leave immediately; remain in room until the autoclave has started. If the cycle is going to fail (usually due to incomplete warm-up) it will happen in this first step. If run doesn’t start, the autoclave will begin to alarm. Record the error message displayed on the screen.
 - h. Abort the cycle by selecting the abort button on the screen. DO NOT press the large red Emergency abort button.
 - i. IMMEDIATELY report the alarm to the Microbiology Technician (306-585-4892).
 - j. The Microbiology Technician will assess the situation and determine if a service call needs to be made.
12. At the end of the cycle, the “Process Complete” LED illuminates and an alarm sounds.
13. Determine the effectiveness of your cycle using printout (see Getinge Manual).
 - a. Successful Cycle: The LED of the “Process Complete” indicators is flashing **green**. “Cycle Complete” appears against a **green** background in the display window. Doors unseal but remain closed.

- b. **Unsuccessful Cycle:** The LED of the “Process Complete” indicators is flashing *red*. The LED of the “Process Failure” indicators is flashing *red*. “Standby” appears against a flashing *red* background in the display window. Doors remain sealed.
- 14. Record any problems in the **Autoclave Logbook** and contact Microbiology Technician (306-585-4892).
- 15. Once cycle is complete, try to unload material promptly. Others are waiting patiently for equipment.
- 16. Do not attempt to open door while autoclave is operating.

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 vapours and gases due to inadvertent autoclaving of volatile chemicals.
2. Do not attempt to open the sterilizer door unless the CHAMBER pressure gauge and JACKET chamber gauge on the front panel reads zero (0 psig) or negative.
3. If a “Water in Drain” message displays, do not operate the chamber door. Wait until message clears before attempting to open the door.
4. If “Process Failure” indicator is flashing, the cycle must be considered incomplete and the load must be reprocessed.
5. If the LED on the “Close Door” switch is illuminated, press “[Open Door](#)” to unseal the door. Stand away and to the side of the door, wait a minute for the steam to vent before opening door.
6. Using heat resistant gloves carefully transfer the autoclave plans to a cart.
 - 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 if proper containers are not used.
7. Verify that the temperature-sensitive tape has changed colour. If not, new tape needs to be applied and the material autoclaved again. 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.
8. To close door, press “[Close Door](#).” Stand away from the door area while door is in motion.
9. Record any problems in the **Autoclave Logbook** and contact Microbiology Technician (306-585-4892).
10. Transport items to a safe location and wait for the items to cool before storing or disposing.
 - a. Biohazardous materials must be labelled as “decontaminated” prior to placing in garbage waste containers. This can be accomplished by placing “decontaminated” heat sensitive tape on autoclave bags or using autoclave bags that have the word “decontaminated” become visible (heat activated) once autoclaved.

11. When disposing of autoclaved waste, waste must be placed inside designated garbage receptacles within the autoclave room. It may never be placed on ground or disposed of in your laboratory.



d) Standby and Shutdown

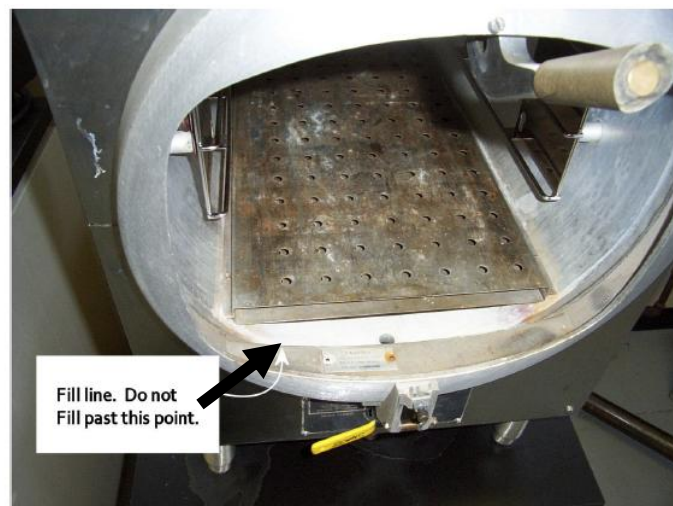
1. Close sterilizer doors.
2. Do not turn off the sterilizer, if you require operational readiness (avoids the 20-30 minute warm-up period).

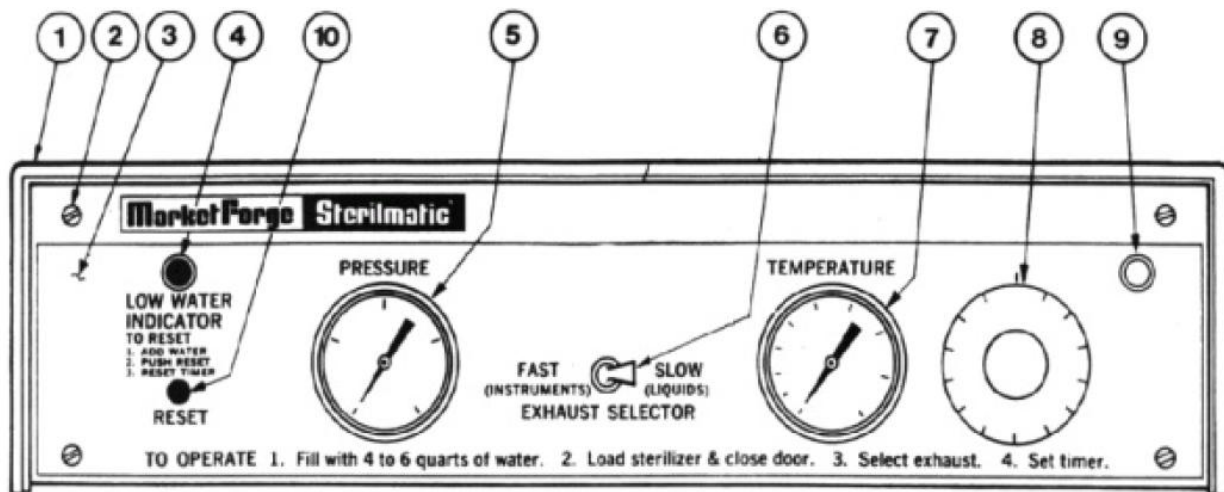
12. Market Forge Sterilmatic STM-E Standard Operating Procedures

Note: As of January 2015, no biological (hazardous or non-hazardous material) can be decontaminated using these autoclaves until the Microbiological Technician tests and approves use.

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 all appropriate efficiency that last month.
2. Wear heat resistant gloves, lab coat, and closed-toe shoes: autoclave door and chamber may be hot from a previously run cycle.
3. Check that the drain valve is closed. Fill bottom of sterilizer chamber with approximately six quarts of water or just below ledge at bottom of door opening.





CONTROL PANEL STM-E AND STM-EX

ITEM	STM-E	STM-EX	DESCRIPTION
1	10-0489		Bezel
2	10-1722		Round Heat Machine Screw 6-32
3	10-9280		Control Panel
4	10-5052	10-6669	Pilot Light (Red)
5	10-9267		Pressure Gauge
6	10-5999	10-9271	Switch
7	10-9268		Temperature Gauge
8	10-6290	10-0870	Timer
9	10-5940	10-6873	Pilot Light (White)
10	10-5990		Low Water Cut-Off

4. 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
5. Grasp handle, and holding it in vertical position, pull door down until bottom of door rests in the bottom of door opening.
6. Rotate handle forward, engaging the lower curved portion under the horizontal rod in the casting at the bottom of the door opening. Push handle all the way down and back until door is locked securely in position.

b) Exhaust Selector and Sterilization Times

1. Set the exhaust selector to the correct position. This is located at centre of the control housing mounted on the top of the unit; which is marked "Fast (Instruments)" or "Slow (Liquids)." See **Table 5 – MarketForge Sterilizer STE Exhaust Selector Options** for guidance. Sterilize liquids separately from other supplies or materials.

Table 5 – MarketForge Sterilizer STE Exhaust Selector Options

Exhaust Options	
Instruments	All items, other than liquids
Liquids	Liquids; slow exhaust

2. Select an appropriate cycle based on the materials to be sterilized. In no case should timer be set to less than 15 minutes as sterilization will not be accomplished. See **Table 6 - MarketForge Sterilizer STE Sterilization Recommendations** for guidance (below).

Table 6 – MarketForge Sterilizer STE Sterilization Recommendations

Items	Recommended Sterilize Time (Minutes)
Glassware (empty, inverted); metal instruments; pipettes	15
75 – 250 ml Flasked Liquids; instruments wrapped in muslin	20
500 – 1000 ml Flasked Liquids	30
1500 – 2000 ml Flasked Liquids	45

3. Record cycle parameters in the **Autoclave Logbook**.
4. Timer is located at upper right front of the sterilizer. Select desired length of sterilizing periods. This turns power supply on and starts the cycle after pressure-temperature combinations has been reached. The amber pilot light indicates that the timer is running. **DO NOT ATTEMPT TO OPEN THE DOOR OR OPEN THE DRAIN VALVE DURING THE OPERATION OF THE AUTOCLAVE.**
5. When sterilizer chamber reaches selected temperature, the time exposure will begin.

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 vapours and gases due to inadvertent autoclaving of volatile chemicals.
2. When the chamber pressure gauge located at the top of the control housing reads “0,” the door may be opened.
3. Release handle and let go to avoid possible contact with remaining steam. When opening the door, allow 15- 20 minutes for steam to escape from chamber before opening completely.
4. Open the drain valve to allow the water to drain from the autoclave.
5. Record any problems in the **Autoclave Logbook** and contact Microbiology Technician (306-585-4892).
 - a) **LOW WATER INDICATOR:** If the water runs dry in the autoclave during operation, the temperature of the unit will rise too high, which automatically stops the heating cycle. Allow the pressure to return to “0” and temperature to decrease. Open door and refill the autoclave with water. Then close door. To restart the machine, you will need to push the manual “reset” button.

d) Standby and Shutdown

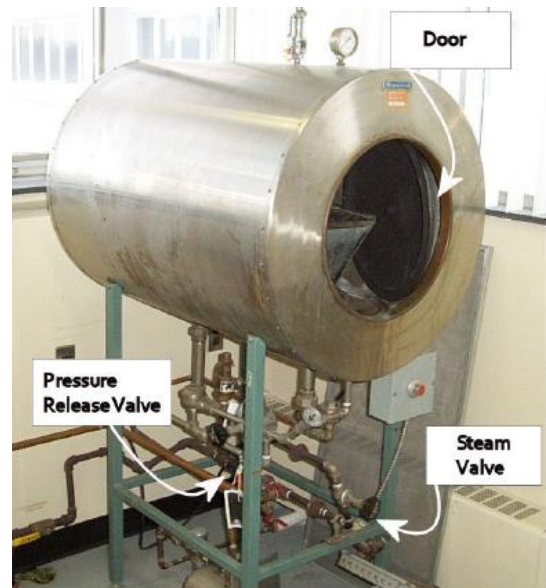
1. Remove bottom splash baffle and wash wetted portion of the cylinder thoroughly adding dilute (1:10) Liqui-Jet detergent.
2. Clean with a soft cloth or brush with dilute (1:10) Liqui-Jet detergent. After washing thoroughly rinse with clean water.
3. Dry cylinder and leave door open overnight.

13. Barnstead Standard Operating Procedures

Note: As of January 2015, no biological (hazardous or non-hazardous material) can be decontaminated using these autoclaves until the Microbiological Technician tests and approves use.

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 all appropriate efficiency that last month.
2. Wear heat resistant gloves, lab coat, and closed-toe shoes: autoclave door and chamber may be hot from a previously run cycle.
3. The pressure release valve should be closed in position (perpendicular to line).
4. Place materials in autoclave, observing the following precautions:
 - d. Do not overload secondary containers
 - e. Avoid touching, crowding, or stacking items.
 - f. Liquid loads should be a uniform volume and container size
5. Close door by rotating door until the door locks into the closed position.
6. Turn steam valve counter-clockwise to open steam valve. (You will hear noises coming from autoclave.)



b) Exhaust Selector and Sterilization Times

1. It will take time for the correct pressure and temperature to be reached. Pressure can be read from the top gauge while temperature can be read on the gauge just above the steam valve. It is recommendation to run autoclave for at least 1 hour but sterility has not been tested.
2. **DO NOT ATTEMPT TO OPEN DOOR OR PRESSURE RELEASE VALVE WHILE AUTOCLAVE IS RUNNING.**

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 vapours and gases due to inadvertent autoclaving of volatile chemicals.
2. The autoclave is not on a timer and must be shut off manually by turning the steam valve clockwise.
3. When the pressure gauge on the top of the autoclave reaches "0" the door may be opened. When opening door, open pressure release valve first then step back. Allow a few seconds for steam to escape from chamber before opening completely.
4. Record any problems in the **Autoclave Logbook** and contact Microbiology Technician (306-585-4892).

Appendix 1 – Autoclave Log

Date	Name	Phone #	Supervisor	Time In	Material Sterilized	Cycle/Type of Run	Time Out	Problems/ Comments

