

TBEP Autoclave (Steris/Amsco Lab 250 Life Sciences Steam Sterilizer)

1. Definition

- a. Autoclaves can be used for the sterilization of reagents and labware used in laboratory procedures.
- b. Principles of sterilization, disinfection, decontamination procedures, and validation are listed in Chapter 15 of the CBH.
[\[https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition/chapter-11-15.html#ch15\]](https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition/chapter-11-15.html#ch15)

2. Training and Use:

- a. Before using the autoclave, new users must be trained by a Steris service technician or by a previously trained and experienced user in TBEP. Contact admin.tbep@utoronto.ca to arrange a training session.
- b. Book your timeslot in Booked Scheduler [\[https://tbep.bookedscheduler.com\]](https://tbep.bookedscheduler.com).

3. Precaution:

- a. This autoclave uses high temperature steam to sterilize various kinds of materials.
- b. Always use personal protective equipment (PPE) – heat resistant gloves, eye protection, lab coat, and closed-toe shoes – when operating the autoclave.
- a. Refer to the operator manual for more details related to safety
[\[https://labs.tbep.utoronto.ca/wp-content/uploads/2022/04/Autoclave-Operating-Manual.pdf\]](https://labs.tbep.utoronto.ca/wp-content/uploads/2022/04/Autoclave-Operating-Manual.pdf)

4. Preparation:

- a. Before using the autoclave, check the **drain strainer/plug** at the bottom of the chamber and clean it if blocked.
- b. If the machine is in “standby” mode, log in and wait for at least 15 minutes before starting a cycle.
- c. If the machine runs out the printing paper or does not print clearly, email admin.tbep@utoronto.ca to request a replacement paper roll or ink cartridge.
- d. Decide what autoclave cycle you need to use according to the materials you are going to sterilize.

5. Cycle Selection:

- a. Refer to the operator manual for additional cycle details
[\[https://labs.tbep.utoronto.ca/wp-content/uploads/2022/04/Autoclave-Operating-Manual.pdf\]](https://labs.tbep.utoronto.ca/wp-content/uploads/2022/04/Autoclave-Operating-Manual.pdf)

- **PREVACUUM** cycle: For fast and efficient sterilization of porous, heat- and moisture- stable materials, and the wrapped items (surgical tools) and any items that air can be trapped inside (such as tubes).

Prevacuum Cycle The Prevacuum cycle is recommended to process heat- and moisture-stable goods, except liquids, which are capable of being sterilized with steam. This cycle can also be used to decontaminate wastes, including wastes containing liquids, provided the materials are properly contained.

Refer to **Table 3-1** for recommended Prevacuum cycle parameters.

Table 3-1. Prevacuum Cycle Parameters

| Temperature | Pressure Point Psig (psia) | Minimum Recommended Sterilize Time* Minutes at Temperature |
|---------------|----------------------------|--|
| 121°C (250°F) | 12-14 (27-29) | 15 |
| 132°C (270°F) | 26-28 (40-42) | 4 |

* Minimum sterilize times are based on obtaining a 10⁶ Sterility Assurance Level (SAL) with standard test loads. Your specific loads may require different sterilize times to achieve this level of sterility, or you may require a different SAL.

- **GRAVITY** cycle: For sterilization of non-porous and heat- and moisture-stable goods, such as glassware, metal instruments, or hard goods.

Gravity Cycle Refer to **Table 3-2** for the type of items which can be processed in a Gravity cycle and the recommended parameters.

Table 3-2. Gravity Cycle Parameters

| Items | Minimum Recommended Sterilize Time at 121°C (250°F) (minutes) | Minimum Recommended Sterilize Time at 132°C (270°F) (minutes) | Dry Time (minutes) |
|---|---|---|--------------------|
| Glassware Empty, inverted, vented* | 15 Min. | 3 Min. | 0 Min.** |
| Instruments metal combined with suture, tubing or other porous materials (unwrapped) | 20 Min. | 10 Min. | 0 Min.** |
| Hard Goods Unwrapped | 15 Min. | 3 Min. | 0 Min.** |
| Hard Goods Wrapped in muslin or equivalent | 30 Min. | 15 Min. | 30 Min. |

* If items which can trap air must be sterilized upright, they should be sterilized in a prevacuum cycle.

** Goods will be wet when removed from sterilizer.

*** Dry time can vary for wrapped goods depending on pack density, weight of goods, pack preparation techniques including type of wrapping material used, and sterilizer loading procedures.

- **LIQUID cycle:** for sterilizing liquid materials. To prevent personal injury or property damage resulting from bursting bottles and hot fluid, you must observe the following procedures:
 - i. Use only containers resistant to thermal shock/thermal expansion (such as type 1 borosilicate glass bottles). Do not use ordinary glass bottles or any container not designed for sterilization.
 - ii. Fill liquid containers only half full.
 - iii. Use only vented closures. Do not use screw caps or rubber stoppers with crimped seal. This is to let air get out and hot steam get in and to avoid pressure building up inside containers.
 - iv. A stainless steel container, or a polypropylene container/tray is required to be put underneath the liquid containers to catch spills.
 - v. Leave space between items/containers to allow hot steam to circulate around.
 - vi. Use the racks to move the loads into the chamber.
 - vii. Use LIQUID cycle only. No other cycle is safe for processing liquids.
 - viii. Do not allow hot bottles to be jolted. This can cause hot-bottle explosion. Do not move bottles if any boiling or bubbling is present.

Liquid Cycle Refer to **Table 3-3** for recommended Liquid cycle parameters. The recommended times indicated in **Table 3-3** assume the use of vented bottles or Erlenmeyer flasks. The *minimum sterilization time* includes the time required to bring the solution up to the sterilization temperature plus the time required to achieve sterilization.

NOTE: Use load probes and F₀ option to optimize cycle times.

Table 3-3. Liquid Cycle Parameters - No Load Probes

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

* Minimum sterilize times are based on obtaining a 10⁶ Sterility Assurance Level (SAL) with standard test loads. Specific labs may require different sterilize times to achieve this level of sterility, or may require a different SAL.

6. Loading the Items:

- a. Do not raise or lower the door rapidly, as fast operation may damage the manual door mechanism.

- b. When closing the chamber door, keep hands and arms out of the door opening and make sure the opening is clear of obstructions.

7. Setting/Running the Selected Cycle:

- a. On the color touch screen, select the proper cycle for your items/loads.
- b. When selecting time for sterilization, take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 mL flasks each containing 250 mL of liquid.
- c. Material with a high insulating capacity (high sided polypropylene containers) increases the time needed for the load to reach sterilizing temperatures.
- d. There are pre-set programs for prevacuum, gravity, and liquid cycles. You can also program a new setting based on your need – contact admin.tbep@utoronto.ca for assistance with custom programming.

8. Removing the Processed Load:

- a. Sterilizer, rack, shelves and processed items will be hot after the cycle is complete. Always wear personal protective equipment (lab coat, eye protection, heat insulating gloves, and closed-toe shoes) when removing a processed load, and reloading sterilizer following the previous cycle.
- b. Check that the chamber pressure is zero. Steam may be released from the chamber when the door is opened. Step back from the sterilizer each time the door is opened to minimize contact with steam vapor.
- c. After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.
- d. Always close the door after unloading.
- e. To prevent falls, keep the floor dry by immediately wiping up any spilled liquids in the loading/unloading area.
- f. Allow sterilizer to cool to room temperature before performing any cleaning or maintenance procedures.

9. References:

- a. <https://labs.tbep.utoronto.ca/wp-content/uploads/2022/04/Autoclave-Operating-Manual.pdf>
- b. <https://ehs.utoronto.ca/our-services/biosafety/biosafety-manual/autoclaves-steam-sterilizers/>
- c. <https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition/chapter-11-15.html#ch15>