



STANDARD OPERATING PROCEDURE: Autoclave Usage

1. Purpose:

To provide step by step instruction on how to safely use an autoclave.

2. Scope:

Applies to everybody using an autoclave.

3. Prerequisites:

WHMIS, Laboratory Biosafety course (EHS601), and autoclave training session

4. Responsibilities:

Principal investigators are responsible for enforcing this SOP and ensuring that those operating the autoclave are properly trained. Lab-personnel are responsible for complying with this SOP.

5. Personal Protection Equipment (PPE):

Eye/face protection (if necessary), nitrile gloves, insulated gloves, lab coat



Insulated gloves

6. Procedure:

Before Using Autoclave/Sterilizer

- 1) Prior to using the autoclave, verify that it has been functioning correctly by reviewing previous cycle log recordings (time, temperature, pressure), and the results of efficacy testing with biological indicators if available.
- 2) Follow the steps below before using autoclave:
 1. Open chamber door and check drain strainer is clean and in place.
 2. Verify chamber interior is clean and close chamber door. Refer to Section 8 of autoclave manual if cleaning is necessary.
 3. Open front cabinet panel on load end of autoclave. Verify steam and water supply valves to sterilizer are ON. Close cabinet panel.
 4. For *Manual Flush option only*, if autoclave is equipped with an integral electric steam generator, flush and start up generator as outlined in Section 4.7 of manual. This is done by opening the front panel of autoclave. Only manual flush when autoclave is cool.
 5. Open printer access door and verify amount of printer paper is sufficient. A colored warning stripe is visible when paper roll is near end. Refer to Section 8 of manual if paper roll needs replacement, or to [Replacing Paper Roll](#) section of this SOP.
 6. Close printer access door. Printer records sterilizer type.
 7. Enter Operator Mode (refer to Section 5.1 of manual). NOTE: Once operating mode is entered, steam enters sterilizer jacket and heats jacket to 115°C. Also, the isothermal mode does not turn jacket on.
 8. Load chamber as outlined below and in [Appendix 1](#).

Using Autoclave

- 1) Wear the appropriate PPE required to safely handle the material being loaded into autoclave
- 2) Prepare materials to be autoclaved following the guidelines shown in [Appendix 1](#)
- 3) Place material in autoclave ensuring it is evenly spaced out and not overloaded, or **fully sealed**. Containers holding liquids should not be more than 75% full.
 - a. Do not mix solid and liquid materials (they use different autoclave cycles)
 - b. Place packages on their edges, and empty flasks/tubes horizontally
 - c. Ensure all containers allow steam penetration (**slightly open autoclave bags and bottles**) (see [Appendix 1](#) for more information)
 - d. Primary containers must be placed into secondary containers, which must be made of a material that can withstand repeated autoclaving

- e. When sterilizing liquids, ensure only Type 1 borosilicate glass bottles are used, and not ordinary glass bottles

Types of Cycles

1. Pre-Vacuum Cycle
 - Rack item, peel pouches (wrapped)
 - Can extract air from inside
2. Gravity Cycle
 - Unwrapped items eg. Glass items
3. Liquid Cycle
 - Vented containers, loose caps
 - Exhaust slowly to ensure liquid doesn't turn to gas
4. Waste Cycle

Loading Autoclave

- a. Open chamber door.
 - b. Slide shelf half way out of sterilizer chamber.
 - c. Place load on shelf and slide shelf back into chamber. Ensure shelves are completely inside chamber before closing door.
 - d. Close chamber door. Sterilizer is now ready to run a processing cycle. Refer to appropriate Cycle Operation Instructions included in manual, for instruction on running the cycle. Ensure cycle is long enough to allow for it to reach appropriate temperature.
- 4) Close and latch autoclave door firmly
 - 5) Choose the appropriate cycle for the material (to determine which cycle and time is best consider):
 - a. Whether it is to be decontaminated or sterilized
 - b. Composition of the load (solid or liquid)
 - c. Density of material
 - d. Volume and viscosity of liquids (larger volumes require more time)
 - 6) Cycle selection includes: slow exhaust (for liquids), fast exhaust (for glassware), fast exhaust and dry (for wrapped items)
 - a. Consult autoclave manual for assistance in choosing a cycle
 - b. When sterilizing liquids, always use Liquid cycle**
 - 7) Do not open the door while the autoclave cycle is occurring. If a problem with the autoclave is perceived, abort the cycle and contact the person in charge immediately
 - 8) When unloading autoclave, wear necessary PPE (ex. Heat-insulating gloves)
 - 9) Ensure that cycle is complete and both the temperature and pressure have returned to a safe range. **Check chamber pressure gauge before opening door- it should be zero**

- 10) Carefully open door a little and avoid the steam. Allow steam to escape and the pressure within liquids and containers to stabilize.
- 11) Do not disturb containers of super-heated liquids or remove caps prior to unloading these materials. Gently transfer containers to trolley.
- 12) Check autoclave tape for colour change and cycle log recorder for time and temperature attained.
- 13) If disposing of biological liquid waste after autoclaving, first allow to cool, then pour down the drain.
- 14) Ensure door is closed after unloading autoclave.

Important Functions/Buttons

- Button on top left of screen puts unit on stand-by
- Inverted triangle at the bottom of the screen is the abort button
- Red button on right is emergency button, which needs key
- Emergency exhaust is the red handle, which should only be used if the autoclave needs to be opened in an emergency

Replace Printer Paper Roll

- Replace the roll whenever a colored stripe is visible on one or both edges of the printout paper.
1. Open thermal printer front cover. Note: cover is magnetically held closed.
 2. Lift Inner cover.
 3. Position paper roll as follows:
 - a. Position paper roll in bottom of printer compartment, paper coming from bottom of roll.
 - b. Ensure at least 8 inches of paper extends from printer compartment.
 4. Close inner cover.
 5. Remove take-up spool from printer and carefully pull apart.
 6. Place paper between take-up spool; then push spool halves together.
 7. Tightly wind paper around spool two to three times; then place spool back in original position (at top of printer compartment).
 8. Close printer cover.

Clean Chamber Drain Strainer

Allow autoclave to cool before performing any maintenance.

1. Remove drain strainer from drain in chamber bottom.
2. Remove any obvious debris from strainer. If necessary, clear screen in strainer using a brush, wire, or similar tool.
3. Once strainer has been cleared of obvious debris, reverse/rinse strainer under running water.
4. Replace strainer in chamber drain.

Flush Chamber Drain

Flush chamber drain as follows whenever line becomes clogged:

1. Turn OFF steam supply valve. Wait until jacket pressure is zero. Wait until chamber has cooled to room temperature.
2. Remove chamber drain strainer. Clean strainer using procedures given above, if necessary.
3. Pour hot solution of 15mL of tri-sodium phosphate to 500mL of hot water.
4. Open door and return strainer to drain.

7. Procedure for Injuries/Spills:

- 1) All incidents, including spills must be reported to the supervisor and department (Please refer to the [Spill Reporting Procedure](#) webpage on the EHS website)
- 2) If any injury occurs, seek first aid and/or medical assistance depending on injury
 - a. Accident report must be filled out in the event of injury (Please refer to either the [Accident Form for Students](#) webpage or the [Accident Form for Employees](#) webpage on the EHS website)
 - b. If clothing absorbs hot water/steam, remove clothing and apply cool water/ice to affected area. Use safety shower if necessary (use with clothes on).
- 3) Notice must be placed on autoclave to indicate unit is out of service until the cause of incident is identified, pro-active measures are taken to prevent future accidents, and autoclave is deemed safe for operation (see [Appendix 2](#) for sign to place on autoclave)
- 4) No operation of the autoclave is allowed until the spill is cleaned up, investigation of the incident has been completed, and the autoclave has been found safe to use
- 5) Operator is responsible for the clean-up. Wait until the autoclave and materials have cooled down to room temperature before attempting to clean-up.
- 6) If spill of biological material occurred before autoclaving (during loading), follow [biological spill SOP](#). If spill is found after autoclaving, then any biological material should no longer be hazardous.
- 7) Dispose of any cracked glassware properly

Appendix 1:

All containers must have a loose seal, which can be achieved by:

- Loosening screw caps or using self-venting caps
- Capping open containers for sterilization with aluminum foil (see Image 1 below)
- Opening plastic (autoclave) bags slightly prior to loading them into the autoclave
- Using envelope folds for wrapping Kraft paper or muslin

Indicators

- Physical Indicators: pressure and temperature recording devices. Thermocouples can be placed inside the load to determine the temperature achieved in the bag itself.
- Chemical Indicators: change colour after being exposed to specific temperatures e.g. heat sensitive tape.
- Biological Indicators: bacterial spores are autoclaved along with the load, and incubated for period stated by the manufacturer, and observed for any sign of growth that would indicate that autoclave is not sterilizing properly. *Bacillus stearothermophilus* spores are used, as they are most resistant to steam autoclaving.

Autoclave Tape

- Temperature sensitive tape should be affixed to each item to be autoclaved (see Image 2 below)
- Lines will appear when this tape is exposed to high temperatures (see Image 2 below)
- **It is not proof that the autoclave cycle was successful at decontaminating or sterilizing the contents** (just means the outside of the container got hot)
- A biological indicator or other means should be used to validate the efficacy of the sterilization procedure



Less than
75% full

Image 1

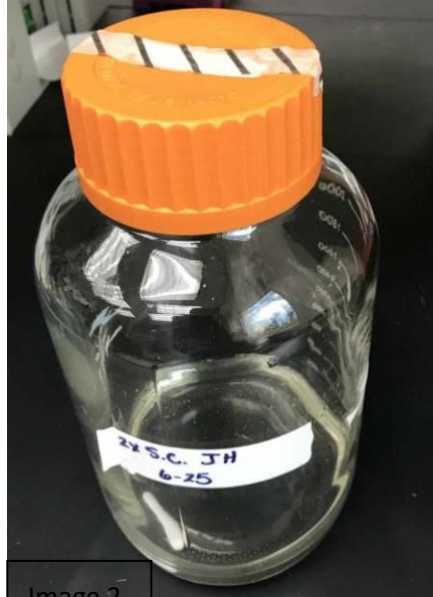


Image 2

Appendix 2:

AUTOCLAVE IS OUT OF SERVICE

