



Autoclave Operation and Safety

Training

If an autoclave is not operated correctly, your research or clinical materials could be compromised, the unit can become damaged and taken out of service, or worse, you could be badly injured. Autoclave training is given by the EH&S Biological Safety Office upon request. The training is conducted at the autoclave and is geared for research staff. The proper use and maintenance of autoclaves as well as safety training is covered. Please contact the Biosafety Office at 392-1591 to schedule a training session.

Standard Procedures

- **Collect biohazardous waste in autoclavable bags.** Close the bags and place them in a sturdy, leak-proof tray or container for transport to the autoclave. The tray or container is used as secondary containment; do not overload these. Use a wheeled cart to transport bags/trays to and from autoclave. ALWAYS bring autoclave gloves with you to the unit.
- Autoclave bags or must be closed, yet vented, to allow proper sterilization. Loosen the closure on the bags as you load the autoclave; sealed bags will not allow penetration of steam and may explode during exhaust step.
- The inner contents of tightly sealed or covered containers will not be adequately sterilized. Sealed containers may also explode in the autoclave or when you attempt to remove them.
- **Do not place items directly on racks.** Use autoclave trays for all sterilization of biohazardous waste. Trays must be designated for use at 250°F.
- **Autoclave trays, Sharps boxes & biohazardous waste bags must be labeled with PI name** and laboratory room number.
- **Do not leave biohazardous waste unattended.** If the sterilizer is in use, please return the biohazardous waste to your laboratory.
- DO NOT change autoclave temperature to >250°F.
- DO NOT turn off the autoclave at the end of your cycle.
- DO NOT adjust water/steam settings or valves.
- DO NOT autoclave liquids containing bleach, formalin or glutaraldehyde.
- DO NOT autoclave flammable or volatile liquids.
- DO NOT autoclave radioactive materials.
- DO NOT autoclave items containing corrosives (acids, bases, phenol).
- **Warning! Burn Hazard** – clogged lines, equipment malfunction or a failure in the steam supply may cause the autoclave chamber to fill with scalding water. **If water leaks from front of the autoclave, DO NOT OPEN the chamber door.** Failure to heed this warning may result in second- or third-degree burns from scalding water!
- IMPROPER USE OF THE AUTOCLAVE IS DANGEROUS TO YOU & CAN RESULT IN EXPENSIVE REPAIRS.
- **Training** is essential to use the autoclave and minimize the risk of injury. Training is given by EH&S Biological Safety Office at the autoclave. Please contact the Biosafety Office at 392-1591 to schedule a training session.
- **EMERGENCIES:** During work hours, call EH&S at 352-392-1591 & speak with Idania Alvarez regarding any mechanical problems/failures. EH&S will notify the approved vendor for service. *Please contact Physical Plant after hours or weekends at 352-392-1121.*



Biological Safety Office

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Carr 406-Specific Instructions for Steris Pre-Vacuum Autoclave:

- **BEFORE LOADING THE STERILIZER**

- Carefully and gently open the vertical glide chamber door.
- Check the drain strainer is clean and in place.
- Check the chamber interior is clean.
- Check gasket is fully retracted and seated within track.
- If the autoclave is in **Standby Mode**, you must enter username and password for access.

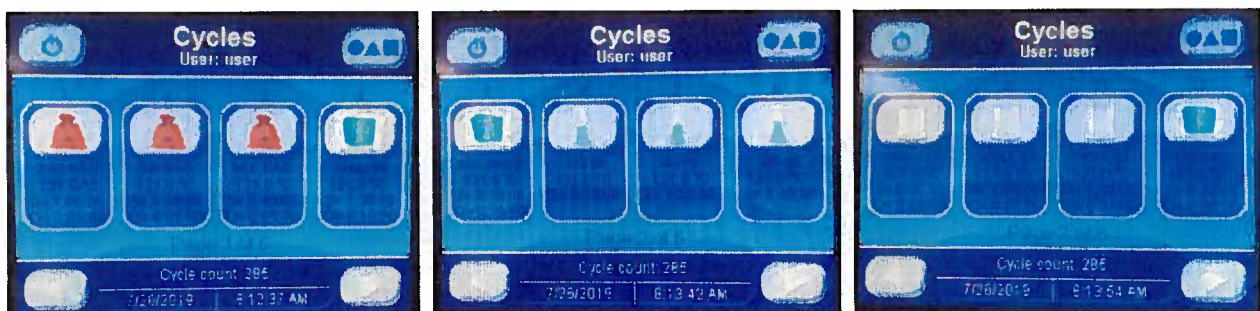


- **LOAD STERILIZER**

- Slide shelf halfway out of the chamber and place autoclave pan(s) containing items to be sterilized on shelf and slide shelf back into chamber. **Do not place items directly on racks.**
- Make certain that red biohazardous bags are as far back in the unit as possible.
- Manually close the autoclave door. Be careful to NOT pinch your fingers!

- **OPERATION**

- There are numerous pre-set, password-protected programs for this unit.



- On the Touch Screen, all the rectangular boxes with icons (**buttons**) are the touch-sensitive areas.
 - Icons indicate the type of cycle: Biowaste, Pre-Vacuum, Liquid, and Gravity.
- Touch/press the RIGHT ARROW to advance to the next screen. Touch/press the LEFT ARROW to return to previous screen.
- Touch/press the CYCLE button to select a cycle. Upon selecting a cycle, the cycle parameters are displayed, including sterilization and drying times. To select a different cycle, press 'Previous' to take you back to the cycle selection screen.
- Touch/press the CYCLE button again to seal chamber and begin sterilization. This is an ACTIVE seal unit, meaning that the door gasket will be pushed out against the door interior by steam/air to create the sterilization chamber.
- Screen timer will indicate how long the total run will be.
- Log Book must be filled out for each autoclave run.
- Please return promptly to retrieve your items.
- At the end of the cycle, the buzzer will sound. **Make certain chamber pressure is at zero PSI.**
- To retrieve sterilized items, open the chamber door. Allow the steam to escape (2-3secs) before reaching in to retrieve your items. Wear heat-protective (autoclave) gloves and avoiding touching hot metal parts.
- HOW TO AVOID DAMAGE
 - Do NOT try to open the door if there is **PRESSURE IN CHAMBER**.
 - Do NOT try to open door if **WATER IN CHAMBER ALARM** is shown on screen.
 - NEVER use a wire brush, abrasives, or steel wool on door and chamber.
 - Do NOT use chlorine-based cleaners (bleach) on door and chamber.
 - IMMEDIATELY wipe up saline solution spills.

Successful Biowaste Containment for Sterilization



FAILED Biowaste Containment for Sterilization:



Autoclave FAQs

- What plastics are autoclavable?
 - Polypropylene and polycarbonates.
 - *Polyethylene and high density polyethylene are not autoclavable.*
 - How long should I autoclave my liquids?
 - Generally, the liquid volume determines the length of sterilization needed.
LIQUIDS (bottles with vented* caps, ½ full)
 - 75mL — 25 MINUTES
 - 250mL — 30 MINUTES
 - 500mL — 40 MINUTES
 - 1000mL — 45 MINUTES
 - 1500mL — 50 MINUTES
 - 2000mL — 55 MINUTES
 - Other factors to consider are the viscosity of your solution, the material of your container [glass, plastic or metal], the packing density [separate bottles to allow steam to surround each bottle], and the location in the autoclave [close to the wall vs center of unit, over the drain].
- * Never autoclave a sealed container of liquids as this may result in an explosion of superheated liquid during the cycle or when the container is opened.*
- Why is my Liquid cycle taking so long? It's only set for 30 minutes.
 - Depending on the unit, liquid cycles can take ~20-30 minutes longer to run than the set sterilization time due to slow charge and slow exhaust steps.
 - Which autoclave program should I use?

Program	Description	Items
Pre-Vac	Air is actively removed from chamber by a series of vacuum and pressure pulses using a vacuum pump.	Biohazardous waste , items wrapped in porous material (surgical trays wrapped in muslin or towels; surgical instruments in sterilization pouches), pipette tip boxes, soils, etc.
Gravity	Because steam is lighter than air, as it enters at the top rear of the chamber, it passively "pushes" the air out of the chamber down the drain port in the front of the unit.	Non-porous items such as glassware (for example, media bottles, beakers, and flasks), spatulas, magnetic stirrers, etc., and biohazardous waste (if Pre-Vac cycle is unavailable).
Liquid	Gravity cycle with slower charge and exhaust steps, designed to prevent boil-over of liquids.	Media, buffers, water, contaminated agar plates, etc. <i>Do NOT autoclave flammable or volatile liquids (solvents, ether, chloroform, etc.), corrosives (acids, bases, phenol), liquids which will emit toxic fumes (such as bleach, formalin or glutaraldehyde).</i> <i>Do NOT autoclave radioactive materials.</i>

- Why does EH&S have the autoclave reserved for testing?
 - Various colleges have contracted with EH&S to monitor the autoclaves for proper function. To minimize disruption to researchers, all units in a building are tested at a preset day/time. Per contract agreement, EH&S will test the units weekly using a biological indicator vial. The vial contains a thermophilic organism (*Geobacillus stearothermophilus*; ATCC® 7953) on paper strip and growth media.
- Why does EH&S test the autoclaves if we use autoclave tape on all items?
 - Indicator tape does not measure/guarantee effectiveness of sterilization; only spore testing does. Use indicator tape with each load to verify it has been autoclaved.
- Why is steam pouring out the door gasket?
 - Some units have an interior hinge on the radial-arm door. Make certain this hinge is flat as you close the door. If this hinge is askew, though the door will feel locked, it will leak steam when the unit is started.
 - Some autoclaves are 'passively' sealed units, where the door is manually closed by the user. The door handle must be turned to the right until it will no longer rotate. Failure to tighten door completely will allow steam to leak during charge step.
- Can an autoclave really explode?
 - **Yes!**

