

STANDARD OPERATING PROCEDURE: AUTOCLAVE (ESB)

Purpose of this Instrument: The autoclaves sterilize equipment and supplies, killing biological contamination and denaturing proteins.

Location: WVU – Engineering Sciences Building – Room: G75E

Primary Staff Contact: Dr. Huiyuan Li (304) 293-0747

The Shared Research Facilities are operated for the benefit of all researchers. If you encounter any problems with this piece of equipment, please contact the staff member listed above immediately. There is never a penalty for asking questions. If the equipment is not behaving exactly the way it should, contact a staff member.



Figure 1. Steris 250 Autoclave in G75E ESB.

1) INITIAL CHECK

This text is a guide to operating the **Steris 250**, edited based on the “Operator Manual, AMSCO Lab series, life sciences small sterilizer”. A more detailed and thorough account of these procedures can be found in the Operator Manual.

1. Log in your “BNRF ESB Autoclave” session on the FOM. (fom.wvu.edu/fom). You can use any computer in the BNRF lab.
2. Check log sheet to see the notes of previous users and check the print out from the autoclave for the information of last run. This will inform you about anything unusual that occurred before.
3. Write down your name, supervisor, start and end time (same as FOM), and items information on the log sheet.

2) PREPARATION BEFORE STERILIZATION

The purpose of packaging and wrapping items for sterilization is to provide an effective barrier against contamination during storage, once the items have been sterilized. Instruments to be sterilized must be free from all residual matter, such as blood or organic tissue. Instruments must also be dry and free from mineral deposits. Such substances may cause damage to the instruments themselves or the Sterilizer.

1. Solid:

- a. Be sure that instruments of dissimilar metals (stainless steel, carbon steel, etc.) are separated. Carbon steel instruments should be bagged or placed on autoclavable towels and not directly on stainless steel trays (mixing will result in the oxidation of these metals).
- b. Do not place materials to be sterilized against the chamber's wall, as the hot metal can damage the item. Place the material only on the tray or rack.
- c. When using a paper / plastic bag, the plastic side should always be down.
- d. Items must be sterilized in an open position. Surfaces that are hidden because the item is in a closed position will not be exposed to the steam and will not be sterilized.
- e. Make sure that all instruments remain apart during the sterilization cycle. Surfaces that are hidden because items are covering other items will not be exposed to the steam and will not be sterilized.
- f. Empty canisters should be placed upsidedown in order to prevent the accumulation of water.
- g. Do not overload the sterilizer trays. Overloading will cause inadequate sterilization & drying.
- h. Allow a distance of approximately 1" between trays to permit steam circulation.
- i. Wrapped instruments should be placed in material which will allow steam penetration and promote drying, such as autoclave bag, autoclave paper, or muslin towels.
- j. Tubing should be rinsed after cleaning. When placed in the tray, make sure that both ends of the tubing are open and there are no sharp bends or twists.

2. Liquids:

- a. Can be autoclave: 1) most buffers and salt solutions. 2) undefined bacterial and yeast media
- b. **Do not autoclave:** 1) buffers with detergents (SDS)
 - 2) organic solvents (ethanol, acetone, phenol, chloroform)
 - 3) heat labile ingredients (vitamins, hormones, antibiotics, proteins.)
 - 4) HEPES containing solutions
 - 5) DTT (dithiothreitol) or BME (beta-mercaptoethanol)
- c. **Use only Type 1 borosilicate glass bottles. Do not use ordinary glass bottles or any container not designed for sterilization.**
- d. **Leave at least a quarter of the container volume as free space** (otherwise your solution will boil over)
- e. Place your liquid bottles in a second autoclavable pans to catch liquids from any broken bottles.
- f. **Keep the caps loose or use vented cap.**

3. **Use autoclave tape to tape on any items you autoclave.** It will change color during autoclaving, thus will tell the future user that the item has been sterilized.
4. Tighten autoclave doors thoroughly.

3) INITIAL CHECK BEFORE OPERATION

1. Open chamber door and check drain strainer is clean and in place (Figure 2).



Figure 2. The location of chamber drain strainer.

2. Check chamber interior is clean and close chamber door. Make sure door opening and closing is clear of any obstruction.



WARNING-PERSONAL INJURY HAZARD

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

4) OPERATION

1. Load your samples
 - Open chamber door.
 - Place load on shelf. Make sure shelves are completely inside chamber before closing door. If you autoclave liquid, the liquid must place in a second container (Figure 3).



Figure 3. The position of sample loading and second container for liquid sample.

- Close chamber door. Sterilizer is now ready to run a processing cycle.



WARNING-PERSONAL INJURY HAZARD

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

- Based on your sample type and quantity, choose your cycle.

Gravity

Gravity cycle is for sterilizing hard goods (e.g., empty glasses, nonporous loads)

Table 1: The item types and autoclave settings for Gravity cycle.

Items	Minimum recommended sterilize time at 121 °C (Min)	Minimum recommended sterilize time at 132 °C (Min)	Dry time (Min)
Glassware (Empty, inverted vented)	15	3	0
Instruments (metal combined with suture, tubing or other porous materials (unwrapped))	20	10	0
Hard Goods (unwrapped)	15	3	0
Hard Goods (wrapped in muslin or equivalent)	30	15	30

Prevacuum

Prevacuum cycle is for sterilizing wrapped goods (e.g., instrument trays, textile packs, and instrument containers) with fast exhaust option, also for sterilizing liquids in heat-resistant containers with vented closure with slow exhaust option.

Table 2: The item types and autoclave settings for Prevacuum cycle.

Items	Minimum recommended sterilize time at 121 °C (Min)	Minimum recommended sterilize time at 132 °C (Min)
Wrapped goods (e.g., instrument trays, textile packs, and instrument containers)	15	4

Liquid

The liquid cycle is used for sterilizing liquids in heat-resistant containers with vented closures.

Table 3: The amount of liquid and its corresponding sterilize time for Liquid cycle.

Volume of liquid in one container (mL)	Minimum recommended sterilize time at 121 °C (Min)
75	25
250	30
500	40
1000	45

1500	50
2000	55
>2000	55+10 min/L

3. Choose the cycle and click the button of the selected cycle (Figure 4).

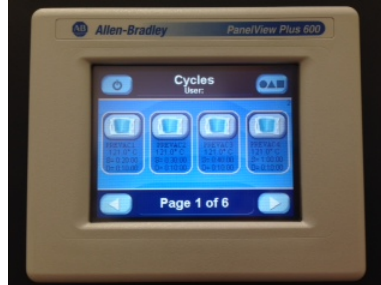


Figure 4. Available cycles to be chosen.

4. Press cycle symbol to start the cycle (Figure 5). If CLOSE DOOR button is shown, close the front door.



Figure 5. Start the cycle.

5. The cycle will start as shown in Figure 6. The remaining time is displayed on the screen.

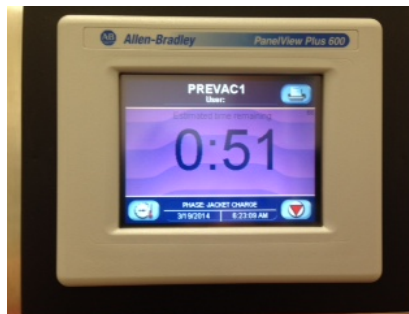


Figure 6. Running a cycle.

6. Wait until the cycle ends and completely cool down. Open door and unload your samples.
7. Once door is open, display returns to main operating mode screen.



WARNING- BURN HAZARD

1. Sterilizer, rack/shelves, and loading car will be hot after cycle is run. Always wear protective gloves and apron when removing a processed load. Protective gloves and apron must be worn when reloading sterilizer following the previous cycle.

2. *Steam may be released from the chamber when door is opened. Step back from the sterilizer each time the door is opened to minimize contact with steam vapor.*
3. *Do not attempt to open the sterilizer door if a WATER IN CHAMBER ALARM condition exists. Report to BNRF staff.*
4. *After manual exhaust, steam may remain inside the chamber. Always wear protective gloves, apron, and a face shield when following emergency procedure to unload sterilizer. Stay as far back from the chamber opening as possible when opening the door.*
5. *Allow sterilizer to cool to room temperature before performing any cleaning or maintenance procedures.*
6. *Failure to shut off the steam supply when cleaning or replacing strainers can result in serious injury.*
7. *Proper testing of the safety valve requires the valve to be operated under pressure. Exhaust from the safety valve is hot and can cause burns. Proper safety attire is required. Testing is to be performed by qualified service personnel only.*
8. *When sterilizing liquids, to prevent personal injury or property damage resulting from bursting bottles and hot fluid, you must observe the following procedures:*
 1. *Use LIQUID cycle only; no other cycle is safe for processing liquids.*
 2. *Use only vented closures. Do not use screw caps or rubber stopper with crimped seal.*
 3. *Use only Type 1 borosilicate glass bottles. Do not use ordinary glass bottles or any container not designed for sterilization.*
 4. *Do not allow hot bottles to be jolted. This can cause hot-bottle explosions. Do not move bottles if any boiling or bubbling is present.*



WARNING- EXPLOSION HAZARD

This sterilizer is not designed to process flammable compounds.



WARNING- SLIPPING HAZARD

To prevent falls, keep floors dry by immediately wiping up any spilled liquids or condensation in sterilizer loading or unloading area.

Table 4. The steps of each cycle:

Gravity cycle	Prevac cycle	Liquid cycle
Jacket charge phase	Jacket charge phase	Jacket charge phase
Active seal phase	Active seal phase	Active seal phase
Purge phase	Purge phase	Purge phase
	Pulse exhaust phase	
	Pulse evacuate phase	
	Pulse charge phase	
Charge phase	Charge phase	Charge phase
Sterilize phase	Sterilize phase	Sterilize phase
Fast exhaust phase	Fast exhaust phase	Slow exhaust phase
Vacuum dry phase	Vacuum dry phase	Vapor removal phase
Dry phase	Dry phase	

Air break phase	Air break phase		
Deactivate seal phase	Deactivate seal phase	Deactivate seal phase	Deactivate seal phase
Complete phase	Complete phase	Complete phase	Complete phase

4) OPTIONAL ISOTHERMAL CYCLE

Isothermal

The isothermal cycle is designed for low temperature processing (e.g., fractional sterilization, pasteurization) at 78 to 110 °C (172 to 230 °F).

1. After fully close the door, in the operating mode screen. There is a ISO MODE button at the bottom right.
2. Click the ISO MODE button.
3. After the cycle is done, click STANDARD MODE button at the bottom left to return to standard mode.

Table 5. The steps of Isothermal cycle:

Isothermal cycle
Active seal phase
Charge phase (45 °C)
Sterilize phase (45 °C)
Slow exhaust phase
Vapor removal phase
Deactivate seal phase
Complete phase

5) OTHER OPTIONAL CYCLES

Call Steris to get the Test pack.

Table 6. Optional test cycles


Cycles	Description
DART warmup cycle	The cycle is used to warm up sterilizer shelves, door, and jacket before a DART or Bowie-Dick cycle is run.
DART cycle (Only on prevacuum sterilizers)	The cycle is designed to document the removal of residual air from a sample challenge load.
Bowie-Dick cycle (Only on prevacuum sterilizers)	The cycle is designed to document the removal of residual air from a sample challenge load.
Leak test cycle (Only on prevacuum sterilizers)	The cycle measures the integrity of the sealed pressure vessel and associated piping to verify air is not being admitted to the sterilizer during vacuum drawdowns.

	Once a week by the B NRF staff.
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Table 7. The steps of optional test cycles

DART warmup cycle	DART cycle	Bowie-Dick cycle	Liquid cycle
Jacket charge phase	Jacket charge phase	Jacket charge phase	Jacket charge phase
Active seal phase	Active seal phase	Active seal phase	Active seal phase
Purge phase	Purge phase	Purge phase	Purge phase
	Pulse exhaust phase	Pulse exhaust phase	Pulse exhaust phase
	Pulse evacuate phase	Pulse evacuate phase	Pulse evacuate phase
	Pulse charge phase	Pulse charge phase	Pulse charge phase
Charge phase	Charge phase	Charge phase	Evacuate phase
Sterilize phase	Sterilize phase	Sterilize phase	Sterilize phase
Fast exhaust phase	Fast exhaust phase	Fast exhaust phase	Leak test phase
Vacuum dry phase	Vacuum dry phase	Vacuum dry phase	
Dry phase	Dry phase	Dry phase	
Air break phase	Air break phase	Air break phase	Air break phase
Deactivate seal phase	Deactivate seal phase	Deactivate seal phase	Deactivate seal phase
Complete phase	Complete phase	Complete phase	Complete phase

6) MAINTENANCE

Frequency	Maintenance
Daily	<ol style="list-style-type: none"> Clean the chamber drain strainer: Inspect and remove any debris from strainer, clear screen with brush or wire, rinse under running water and replace.  <ol style="list-style-type: none"> Flush the steam generator: Follow operator manual generator flush procedure to prevent mineral scaling and/or carryover into the chamber. Conduct Bowie-Dick test: Run test each day before using the sterilizer. If prevac cycles are used, run DART or AAMI ST8 B-D test pack to detect air leaks.
Weekly	<ol style="list-style-type: none"> Flush the Chamber Drain Cool sterilizer completely, shut off steam supply, flush drain with mild detergent or TSP solution as described in the operator manual. Inspect printer paper and ribbon: Verify adequate paper supply and determine if ribbon is printing clearly; replace if needed.

Monthly or as needed	Chamber must be at room temperature before washing. Wash the inside of the chamber and shelf assembly (plus any other loading equipment) with a mild detergent solution such as STERIS Liqui-Jet 2 Detergent. Never use a wire brush, abrasives or steel wool on door and chamber assembly. Do not use cleaners containing chloride on stainless-steel surfaces. Chloride based cleaners will deteriorate stainless steel eventually leading to failure of the vessel.
Annually	1. Have chamber professionally cleaned annually or as needed. STERIS's patented chamber cleaning system removes substances on chamber walls that can contaminate packs and hinder sterilizer performance. Elimination of chamber residue may result in: <ul style="list-style-type: none">• Reduced contaminated and rejected packs• Less potential for drain system blockage• Easier routine operator cleaning• Improved sterilizer chamber aesthetics

Reference:

1. STERIS operator manual AMSCO Lab series, life sciences small sterilizer. Rev. 4.