



SEB Autoclave Training



Autoclave Details

- 3 Autoclaves located in SEB, one on each upper floor.
- Expensive pieces of equipment (\$70,000 each).
- Essential for several groups research.
- Most malfunctions are from misuse.
- Misuse Results:
 - Research grinds to a halt.
 - We have to pay for costly repairs.
- Autoclave reaches 121oC and 15psi - killing all bacteria and spores.
- Operating an autoclave requires caution and adherence to strict regulatory requirements.
- Two main uses:
 - Sterilizing equipment and research material
 - Sterilizing bio-hazardous waste
- Machines have a log sheet attached. It is important that you sign in and describe any problems you experience when using the autoclave.
- Any problems should be reported immediately to Eric Knight at 895-2020.

How to Use the Autoclave Overview

- Logon by using your Username and Password.
- Our autoclaves contain Cast Iron Steam Generators. Each steam generator MUST be flushed daily in order to keep them working properly and avoid costly repairs.
- If you are the first person using the autoclave that day you may be prompted to flush the generator after Logon. If prompted always complete the operation. Do NOT cancel unless the pressure gage is above zero.
- Check the drain screen and if necessary clean before loading autoclave.
- Appropriately prepare your items for autoclaving.
- Always use Secondary Containment.
- Select the appropriate cycle for your items.
- Close the autoclave door securely and start the cycle.
- After the cycle is fully finished safely remove your items and Logoff

How to Flush the Steam Generator

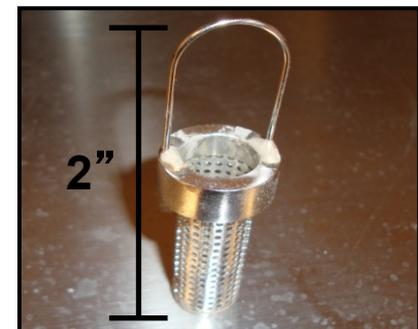
If and when prompted at Logon always flush generator following the onscreen instructions.

- Close the chamber door.
- Open MACHINE panel door by turning the key dial and pulling on the left side at about the center of the machine's left side.
- Confirm Generator is at zero PSI. Do NOT turn the orange knob if this is above zero!
- Locate the orange knob, which is located low toward the left inside of the machine (see picture to the right).
- **Open the flush valve by turning the knob Counterclockwise.**
- **Press/Touch FLUSH GENERATOR command on screen**
- **When the 5 minute cycle ends close the valve by turning the orange knob Clockwise and close the machine front panel.**



Before Starting the Autoclave Cycle

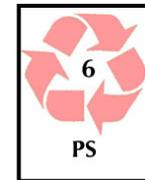
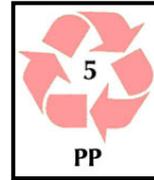
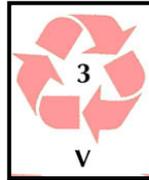
- Check that the autoclave drain screen is clean.
- When the autoclave is hot you must wear heat resistant gloves to check the autoclave drain.
- Remove the lower shelf from the autoclave.
- Reach into the autoclave to grab the drain loop in the front of the autoclave chamber.
- Clean the drain if fouled (if necessary use a paperclip or wire brush).
- Replace the cleaned drain screen.
- The autoclave is now ready to be used.
- For efficient heat transfer, steam must flush the air out of the autoclave chamber. If the drain screen is blocked with debris, a layer of air may form at the bottom of the autoclave and prevent proper sterilization.
- **Neglecting this Step may Result in the Autoclave Overheating, Downtime, and Costly Repairs.**



Secondary Containment

- **USE SECONDARY CONTAINMENT FOR ALL ITEMS BEING AUTOCLAVED !!!(Liquids, Solids, and Biohazardous Waste)**
 - Secondary Containment is required to catch any broken glass pieces and chemical spills. This will ensure protection of the autoclave cavity, tedious clean-up, and reduce the potential of expensive repairs.
- **Typical Secondary Containment:**
 - Polypropylene or stainless steel tubs.
 - Make sure all your plastic containers are suitable. Not all plastics can be autoclaved.
 - Plastic types can be identified by looking for initials imprinted on the container bottom.
 - **Use:** Polypropylene (PP) or Polycarbonate (PC)
 - **Do NOT Use:** Low Density Polyethylene (LDPE) or High Density Polyethylene (HDPE) – [for melting point specificity, see next page]
- IF YOU NOTICE EVIDENCE OF SECONDARY CONTAINMENT LEAKAGE, CHECK AND CLEAN AUTOCLAVE DRAIN AND CHAMBER BOTTOM.
- Select a container with the lowest sides and widest diameter possible to contain any breakage or spill over.
 - If you are unsure about a new container, place it in an autoclave safe container the first time.
 - See Nalgene Labware's Autoclaving Web page for additional plastic considerations
 - <http://www.nalgenelabware.com/techdata/care/steril-autoclaving.asp>

Plastic Types and Melting Points (MP)



Autoclavable:

- Polypropylene (PP) MP: 130-171°C
- Polystyrene (PS) MP: 240°C
- Polycarbonate (PC) MP: 267°C
- Polytetrafluoroethylene (PTFE) MP: 327°C
- Perfluoroalkoxy (PFA) MP: 302-310°C
- Polypropylene Copolymer (PPCo) MP: 160°C

Non-Autoclavable:

- High Density Polyethylene (HDPE) MP: 120-130°C
- Low Density Polyethylene (LDPE) MP: 105-115°C

Sterilization of Equipment & Reagents

- **PREPARATION OF RESEARCH MATERIALS FOR STERILIZATION**
 - **Glassware:**
 - Cap Pyrex bottles loosely- whether empty or filled – to prevent explosion due to expansion.
 - Cover bottles that are not made of safety glass (e.g. Not Pyrex) with aluminum foil.
 - Leave space between items to allow steam circulation.
 - **Liquids:**
 - Fill containers only half full and cap loosely.
 - **Combination Loads:** Do not combine strong oxidizing material (such as hypochlorites) with organic material (such as paper, cloth, or oil).
 - Adding a small amount of water to autoclave trays may reduce breakage.
 - **Do not autoclave items containing corrosives, solvents or volatiles or radioactive materials.**

Choosing the Correct Cycle

- **For Liquids**: Always choose a Liquid or “Slow Exhaust” cycle. You have 3 cycles to choose from: Liq 20, Liq 30 and Liq 45. Use these guidelines to help you decide which cycle to use if you are autoclaving bottles with the following filled volumes per piece:
 - Less than 500ml: Use Liq 20
 - 500ml-1L: Use Liq 30
 - 2-4L: Use Liq 45
 - Add 10-15 minutes for crowded items
- **Nonhazardous Dry Goods**: Always choose a Gravity Cycle, G20 Dry or G20 Wet. Both cycles run for 20 minutes, but G20 Dry includes a 20 min drying cycle that will evaporate water vapor off of your items. This is preferable when autoclaving enclosed items such as pipette tips or bottles with lids.
- When autoclaving agar plates always select the L45 cycle.
- When autoclaving sharps or dry biohazardous waste always select L45.
- If you are unsure of the cycle to choose, ask your lab manager or PI which cycle they recommend or parameters required for effective sterilization.
- If a custom cycle is required please contact Eric Knight at 5-2020 so one can be programmed.

PRECAUTIONS TO FOLLOW WHEN AUTOCLAVE CYCLE IS FINISHED

- **Wear personal protection equipment:**
 - Eye protection.
 - Heat-resistant gloves to remove items.
 - A rubber apron and rubber sleeve protectors are also recommended.
- **Wait for the pressure to drop to zero with zero time remaining before opening the door.**
 - **Never open an autoclave set for “slow exhaust” until the cycle is complete.** Superheated liquids can boil over and damage the autoclave and the operator.
 - **Open the door cautiously.** When the cycle is complete, open the door slowly. Allow the steam to escape in a controlled manner. Keep your head, face, and hands away from the opening.
 - **Let liquids stand another 10-15 minutes** after autoclave is opened to avoid any movement that could cause them to boil. Remove items carefully.

STERILIZATION OF BIOHAZARDOUS WASTE

- Decontamination requires temperatures of at least 250-255°F (121- 124°C).
- Properly Prepare Waste: Bag All Waste Using **RED** Autoclavable Bags.
- All materials must be placed in secondary containment when autoclaved.
- Ensure that dry waste (including agar plates) is free of liquids and sharp objects that could puncture the waste bag.
- Fill bags only **Half Full**. Ensure adequate steam penetration. Seal each bag with autoclave tape.
- If the bags don't have an autoclave indicator section, cover the bag's biohazardous waste symbol with autoclave indicator tape.
- Autoclave the load immediately after preparation. Never leave items unattended or in the autoclave overnight.
- When autoclaving sharps or dry biohazardous waste always select L45.



STERILIZATION OF BIOHAZARDOUS WASTE (cont.)

- **DISPOSAL OF BIOHAZARDOUS WASTE AFTER AUTOCLAVING:**
 - Your properly and completely processed bags can be placed inside another sturdy **black/opaque bag** for disposal in **regular domestic trash**.
 - Do NOT overfill the secondary trash bag or place items in them that would cut or puncture them.
- If in the rare event ALL SEB autoclaves are down or malfunctioning:
 - Take bio-hazardous waste to the SEB Stockroom for disposal.

STERILIZATION OF BIOHAZARDOUS WASTE (cont.)

• PREPARATION OF BIOHAZARDOUS SHARPS CONTAINERS FOR AUTOCLAVING

- When autoclaving sharps or dry biohazardous waste always select L45.
 - Use only autoclavable sharps containers.
 - If the containers don't have an autoclave indicator section, cover the container's biohazardous waste symbol with autoclave indicator tape.
 - Make sure the autoclavable sharps container is properly secured.
- Take all **sharps** Biohazardous waste to the **SEB Stockroom** for proper disposal.
 - All sharps biohazardous waste will then be picked up by UNLV Risk Management and Safety to be transported into our official medical waste service provider for proper treatment and disposal.



ENFORCEMENT & PROBLEMS

- **Enforcement:**

- Autoclave users **MUST** be trained prior to autoclave use. Those caught using the autoclave without having been previously trained, will be unable to use any autoclaves in the building until proper training by Eric Knight is completed.
- Trained individuals caught misusing the autoclave will be required to demonstrate proper autoclave use. For any egregious misuse the user may be suspended from autoclave use in SEB indefinitely.

- **Problems:**

- If you have problems or notice unusual autoclave behavior please report it immediately to Eric Knight @ 895-2020 and leave a note on the machine describing the fault to other users.