

## SCU Autoclave Safety Procedure

**PLEASE NOTE:** Autoclave use is regulated by the Santa Clara County Department of Environmental Health. All users are required to be trained on autoclave use and this procedure.

### Elements Required for Effective Autoclave Use

Autoclaves must be used properly to effectively decontaminate potentially biohazardous materials, sterilize materials, and prepare media. Temperature, time, and contact all contribute to autoclave effectiveness:

- **Temperature** - Required use temperatures may vary depending on the nature of the materials being autoclaved. A minimum of 121 °C (250 °F) is considered the minimum required temperature for general sterilization of materials and for treatment of biohazardous materials.
- **Time** - Adequate autoclaving time is procedure dependent. For example, a minimum of 30 minutes, measured after the temperature of the material being sterilized reaches 121 °C (250 °F) and 15 psi pressure, is **REQUIRED** when treating bio-hazardous materials prior to disposal. The tighter the autoclave is packed, the longer it will take to reach 121 °C (250 °F) in the center of the load.
- **Contact** - Steam saturation of the load is essential for effective sterilization. Air pockets or insufficient steam supply will prevent adequate contact. To ensure adequate steam contact, do not pack items too tightly or overcrowd the autoclave with excessively large loads.

**Note:** Tape indicators can only verify that the autoclave has reached normal operating temperatures for decontamination. Most chemical indicators change color after being exposed to 121°C (250 °F) but cannot measure the length of time spent at 121 °C (250 °F).

### Autoclave User Safety Precautions:

Because an autoclave uses saturated steam under high pressure to achieve sterilizing temperatures, proper use is important to ensure operator safety. Prevent injuries when using the autoclave by observing the following rules:

1. **Required Personal Protective Equipment:** Heat resistant gloves, safety glasses with side shields, no shorts or exposed legs, shoes that fully enclose the foot and a lab coat - especially when unloading the autoclave.
2. **Items that CANNOT be autoclaved:** Sealed containers (they can explode), large bottles with narrow necks (may explode if filled too full of liquid), solvents, volatile, or corrosive chemicals (such as phenol, chloroform, bleach, etc.), or radioactive materials.

3. **Inspect autoclave prior to use:** If you find a problem, notify your supervisor or the Laboratory Manager/Technician. Do not operate the autoclave until it has been properly repaired.

### Autoclave Use Instructions for Sterilizing Media or Research Materials:

1. Refer to Equipment SOP for specific operating instructions as needed.
2. Prepare research materials for sterilization:
  - Glassware:
    - Cap empty or full Pyrex bottles loosely (to prevent explosions due to expansion).
    - Use aluminum foil to cover bottles not made of safety glass (Pyrex)
  - Liquids: Fill containers only half full.
  - Apply a temperature indicator if desired.
  - Combination loads: Do not combine strong oxidizing material (such as dry hypochlorites) with organic materials (such as paper, cloth, or oil).
3. Use **secondary containment** for ALL items being autoclaved. Trays may be used for materials such as instrument packs, racked pipette tips, etc.
  - Use only polypropylene or stainless steel for secondary containment. Polypropylene can be identified by PP, recycle #5.
  - Provide sufficient space between items for proper steam circulation.
  - Do NOT load more than 3 liters of liquid media at one time.
4. Close and ensure the door is sealed before selecting the cycle and select the correct cycle:
  - For liquids, choose the liquid or "slow exhaust" cycle.
  - For sterilizing other materials, use the Gravity cycle, or contact your Laboratory Manager or Technician for guidance.
5. Suggested Sterilization Temperature: The autoclaves are pre-set to 121 °C (250 °F), the temperature used for most situations. Certain sensitive materials may require a lower temperature. Be sure to follow manufacturer's instructions and protocols and adjust the temperature if necessary. Be sure to RESET the temperature to 121 °C when you are finished.
6. Suggested Sterilization times:
  - a. Liquids - Generally 15-20 minutes. Follow manufacturer instructions or protocols. Large volume loads may require a longer time!
  - b. Plastics - No longer than 20 minutes.
  - c. Other Materials - Discretionary, as needed.
7. Push the "start" button on the control panel to initiate the cycle.

8. Before opening the autoclave after use, wait for the pressure gauge to drop to zero with zero time remaining before opening the door (to prevent steam burns and shattered glassware):
  - a. Slowly crack the seal and open the autoclave door and allow the steam to escape gradually.
  - b. Never open an autoclave set for "Liquid Cycle" until the cycle is complete. Superheated liquids can boil over and damage the autoclave and the operator.
  - c. Open the door cautiously. Stand behind the door and slowly open it. Allow all steam to escape before reaching inside.

### Autoclave Use Instructions for Sterilizing Biohazardous Waste:

1. Refer to Equipment SOP for specific operating instructions.
2. Ensure autoclave is calibrated and permitted to process biohazardous waste.
3. Prepare and label biohazardous waste for autoclaving:
  - Ensure that dry waste is free of liquids and sharp objects that could puncture the waste bag. Any biohazardous waste that could puncture a bag must be placed in a sharps container.
  - Use ONLY red bags (not clear or orange) that are marked with the universal biohazardous materials symbol.
  - Label waste in red biohazardous waste bags by marking on a piece of tape with the organism name if known (if unknown, label 'unknown' and placing on the bag). Bags should be no more than two-thirds (2/3) full. If material is dry, add 10-20 mL of water to the bag. If materials are moisture-containing (e.g. Agar plates), this step is not necessary. Bags should then be closed off loosely with wire ties or tape.
  - Place bags in a secondary container - use only polypropylene or stainless steel for secondary containment. Polypropylene can be identified by PP, recycle #5.
  - Cover the bag's biohazard symbol with a sufficient amount of autoclave indicator tape (minimum 4 inches or more of tape). Each bag or vessel must have its own indicator tape. In the case of racked test tubes, place a 4-inch strip of indicator tape across the tops of the tubes in the center of the rack. NOTE: Best practice is to use indicator tape that the word 'autoclave' shows after treatment.
  - Autoclave the load immediately after preparation. Never leave items in the autoclave overnight waiting to be autoclaved or after treatment as others may want to use the autoclave.
4. Confirm temperature and set decontamination time:
  - Decontamination requires temperatures of at least 250 - 255 °F (121-124 °C). The chamber should reach 90% of its full temperature within 5 minutes.

- The autoclave must run for at least 30 minutes and should run longer for large loads (add 10 - 20 minutes for crowded items).
  - Sterilization time for liquids:
    - i. Less than 500 milliliters (mL): 30 minutes
    - ii. 500 mL - 1 liter: 40 minutes
    - iii. 2 - 4 liters: 55 minutes
    - iv. More than 4 liters: 60 minutes
5. Close and ensure the door is sealed before selecting the cycle and select the correct cycle:
    - For liquids, choose the liquid or "slow exhaust" cycle.
  6. Push the "start" button on the control panel to initiate the cycle.
  7. If at the end of the cycle the running time or the temperature is incorrect, or temperature indicators fail to indicate minimum temperature was achieved, rerun the load. If the autoclave fails to maintain the correct time or temperature again, contact the Laboratory Manager or Technician.
  8. Before opening the Autoclave after use, wait for the pressure gauge to drop to zero with zero time remaining before opening the door to prevent steam burns and shattered glassware:
    - Slowly crack the seal and open the autoclave door and allow the steam to escape gradually.
    - Never open an autoclave set for "slow/liquid exhaust" until the cycle is complete. Superheated liquids can boil over and damage the autoclave and the operator.
    - Open the door cautiously. Stand behind the door and slowly open it. Allow all steam to escape before reaching inside.
    - Let liquids stand another 10 minutes after the autoclave is opened to avoid any movement that could cause them to boil. Remove items carefully.
    - Check temperature indicators to ensure minimum temperature was achieved.
  9. After the run parameters were maintained and indicators functioned properly, you must fill out the autoclave log accordingly (do not have to complete the log if the load has to be rerun).
  10. Remove autoclaved waste immediately after the cycle is completed and liquids have stood for 10-20 minutes. Never leave items in an autoclave overnight.
    - Dispose of waste as prescribed in your training.
    - Remember: red biohazardous bag waste must have a sizable (4 inches or greater indicator tape) that has changed color. Best practice is to use the indicator tape that shows the word 'autoclaved' after treatment. The treated red biohazardous bag waste must be sealed in black trash bags prior to disposal.

## Autoclave Laboratory Manager Responsibilities

- Anyone under your responsibility must be trained prior to using an autoclave.
- Autoclaves are classified as pressure vessels, and must be inspected at least annually according to federal and state regulations.
  - Only authorized autoclave contractors are permitted to inspect and/or repair autoclaves.
- Once a month, use either a biological indicator (such as *Geobacillus stearothermophilus* spore ampules) or a chemical indicator that measures both time and temperature (such as Sterigage).
  - Bury the indicator in the center of the load to validate adequate steam penetration.
  - Keep a log book to record the results.

## References

1. California Health and Safety Code Sections 117600 - 118360 (Medical Waste Management Act)
2. Santa Clara University Biosafety Program