

Marine Biological Laboratory

Autoclave Standard Operating Procedures

Initiated by: **MBL Safety Office**
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1.0 Purpose

The purpose of this SOP is to ensure that all the research and teaching laboratories follow uniform procedures for using the autoclave to effectively decontaminate biological waste in accordance with the Marine Biological Laboratory (MBL) policy and the Massachusetts Department of Public Health biological waste disposal regulations.

Additionally, this SOP provides details for autoclaving media and sterilization for laboratory instruments or glassware.

2.0 Scope

This SOP applies to all research and teaching laboratories at the MBL where autoclaving is used to decontaminate potentially infectious waste prior to disposal as non-hazardous waste or for sterilization of goods.

Individuals must be trained with both the procedures and hazards of an autoclave prior to proceeding with work. Training should be provided by laboratory supervisor or contact the Safety Office at x7424.

Quarterly MBL staff performs certification for each autoclave unit. Certification is conducted using biological indicators to confirm each autoclave meets sterilization requirements. Results of the testing are documented. Appendix B describes procedures for the quarterly certification.

3.0 Preparing the Load for Autoclaving

- 3.1 Verify material to be autoclaved is compatible with the autoclave process.
- 3.2 Never autoclave materials containing hazardous chemicals such as organic solvents and corrosives (phenol, chloroform, pyridine or bleach) or radioactive materials in the autoclave. These materials must be disposed as hazardous waste.
- 3.3 Non-Liquid Biohazardous Material
 - 3.3.1 Use clear autoclave bags for autoclaving biohazardous materials such as live cultures, solid biological waste, plastics or glassware. Larger materials to decontaminate such as devices or animal cages should not be bagged.

- 3.3.2 Ensure that contaminated materials do not contain sharp objects that may puncture autoclave bags.
- 3.3.3 Only fill autoclave bags to a maximum of 75% of their holding capacity.
- 3.3.4 Add about 50-100 ml of water to each bag of dry solid waste to facilitate steam penetration in the bag.
- 3.3.5 A Class 5 Steam Sterilization Integrator strip is added to each autoclave bag as follows (The strip evaluates both that the proper time and duration of temperature in the presence of steam has been achieved):
 - 3.3.5.1 Position the integrator into the bag such that the extender tip protrudes slightly from the bag. This allows the chemical integrator to be removed and examined without disturbing the load contents.
 - 3.3.5.2 Close the bag loosely to ensure adequate steam will penetrate the load. Leaving a small opening, slightly close bag using autoclave indicator tape.

3.4 Liquid Material

- 3.4.1 Liquids should be placed in glass or plastic containers which are suitable for autoclaving. Containers should have caps loosened or use vented closures.
- 3.4.2 Only fill containers to a maximum of 75% of their holding capacity.
- 3.4.3 Never autoclave sealed containers of liquid as this could result in an explosion of superheated liquid.
- 3.4.4 Place the liquid containers in a stainless steel or polypropylene tray containing $\frac{1}{4}$ to $\frac{1}{2}$ inch of water to allow the bottles to heat evenly.
- 3.4.5 Add a strip of autoclave tape to the side of each container.

3.5 Glassware, Metal Instruments, and Cages

- 3.5.1 Place materials in a shallow stainless steel or polypropylene tray.
- 3.5.2 Large glass vessels must not be capped or covered with a non-porous material such as an inverted plastic beaker as risk of glass fracture during either heating process (during autoclave) or during cooling process (after autoclaving).
- 3.5.3 Add a strip of autoclave tape to the side of each container.

4.0 Loading the Autoclave

- 4.1 Personal protective equipment is required when using the autoclave. The PPE should include a minimum of laboratory coat and safety glasses (see Appendix A for additional safety considerations).
- 4.2 Check the drain screen for debris at the bottom of the autoclave chamber before using the autoclave. Clean out any debris if present.
- 4.3 Place the items, autoclave bags or containers into a secondary containment such as a polypropylene, polycarbonate or stainless steel pan.
- 4.4 If multiple items are in pan, leave space between items to allow proper steam circulation.
- 4.5 Place the secondary container with the load onto the shelf in the autoclave chamber.
- 4.6 Do not overfill the chamber. Inspect that the load is not touching the interior autoclave chamber walls.
- 4.7 Close autoclave door and turn crank until hand tight. If during start cycle steam emerges around door, immediately turn autoclave off and re-tighten. For the automatize unit (Primus or digital Consolidated unit), once the door is closed and program started, the unit performs an automatic gasket seal during the initial part of the run cycle.

5.0 Selecting Autoclave Cycle

5.1 Consolidated Sterilizer Operations:

- 5.1.1 The autoclave's temperature is preset at 250 °F (121 °C), which is not user changeable.
- 5.1.2 The "READY" light will be lit when the autoclave chamber jacket nearly reaches the operating temperature. When this occurs, the [START] button is activated.
- 5.1.3 Choose appropriate cycle DRY, FLUIDS or FAST. FAST is for metal instruments or cages; FLUIDS is for liquids; and DRY is for glass, cardboard or solid waste.
- 5.1.4 Open the electronic timer cover and set the two thumbwheel digits above "STERILIZE" to desired cycle time. Set the thumbwheel above "DRY" to desired time.

- 5.1.4.1 For non-liquid biohazardous material (autoclave bags or cages), set sterilization time for 60 minutes, using a DRY CYCLE.
- 5.1.4.2 For liquid biohazardous material, set the sterilization time based on table below:

Volume of Liquid in One Container	Minimum Recommended Sterilization Time at 250 °F
75 mL	25 minutes
250 mL	30 minutes
500 mL	40 minutes
1000 mL	45 minutes
1500 mL	50 minutes
2000 mL	55 minutes
>2000 mL	55 minutes + 10 min/L

- 5.1.5 Press the RED **“ON/OFF”** button to start the sterilization.
- 5.1.6 The full sterilization and exhaust cycle will begin once the autoclave chamber attains a set temperature of 250 °F and a minimum operating pressure of approximately 15 psi.

5.2 Primus Sterilizer Operations:

- 5.2.1 The available CYCLE NUMBERS with the corresponding cycle type and parameters are posted on the front panel of the autoclave just above the touchpad screen. See table below:

Cycle Number	Cycle Description	Sterilize Time (Minutes)	Exhaust and Dry Time (Minutes)
1	Hard Goods (Dry)	45	5
2	Hard Goods (Dry)	60	5
3	Hard Goods (Dry)	75	5
4	Liquid – 75mL	20	10
5	Liquid – 250 mL	30	10
6	Liquid – 1 L	45	10
7	Liquid – 2 L	60	10
8	Glassware (Dry)	30	15
9	Pipette Tips (Dry)	20	20
10	Biological Waste	60	5
11	Liquid – 5 L	90	10

5.2.2 Press the [SELECT CYCLE] button. The 10-key touchpad overlay appears on the touchscreen. Select the CYCLE NUMBER you want to run by pressing the corresponding numbered button(s) on the touchpad and press [ENTER]. The DOOR CLOSED screen appears, displaying the CYCLE NUMBER you selected.

5.2.3 Press the [START CYCLE] button. The START CYCLE screen appears and again press the [START CYCLE] button on this screen to start. The autoclave now proceeds automatically, with the touchpad screen displaying the phases and conditions throughout the run.

5.2.4 The thermal printer also records the details of the CYCLE. At the completion of a cycle, the CYCLE COMPLETE screen appears. Press the retract gasket [RETR GSKT] touchscreen button, to start gasket retraction to allow for opening of the autoclave.

5.3 Digital Consolidated Sterilizer Operations:

5.3.1 The available CYCLE NAMES are posted on the front panel of the autoclave just above the touchpad screen. The options are consistent with those on the Primus unit listed above.

- 5.3.2 After selecting [FAVORITES], [GRAVITY], [LIQUID] or [VACUUM] tab near top of screen, press the desired [CYCLE NAME] button.
- 5.3.3 Press the green [PRESS TO START CYCLE] button. The autoclave now proceeds automatically, with the touchpad screen displaying the phases and conditions throughout the run.
- 5.3.4 The printer records the details of the CYCLE. At the completion of a cycle, the CYCLE COMPLETE screen appears.

6.0 Unloading the Autoclave

- 6.1 When autoclaving cycle has completed, wait until the autoclave chamber pressure gauge reads zero before opening the autoclave door (or CYCLE COMPLETE for Primus).
- 6.2 Personal protection equipment is required for working with the autoclave which includes a laboratory coat safety glasses and heat-resistant gloves to remove items (see Appendix A for additional safety considerations).
- 6.3 Open the autoclave door slightly to allow steam to escape, if necessary. Wait 5 minutes.
- 6.4 After a minimum of 5 minutes, carefully remove the secondary container with the autoclaved material as not to spill the added and now hot liquids.
- 6.5 Allow items to completely cool down outside of the autoclave unit. Wait a minimum of 15 minutes.
- 6.6 Verify that the added indicator tape on the exterior of each item has black angled strips across the tape. Liquids and large items are now sufficiently decontaminated.
- 6.7 For material in autoclave bags, remove the Class 5 Steam Sterilization Integrator Strip and verify the indicator as follows:
 - 6.7.1 Examine the integrator located at the tip of the extender. Tip should have turned to a dark brown or black color, if not then the load did not process for sufficient temperature. Reprocess the load in the autoclave.
 - 6.7.2 If the indicator passed, remove the strip from bag by holding the extender tip.
 - 6.7.3 Now examine the other end of the integrator to verify that the dark bar has moved from the FAIL area to the PASS area showing appropriate sterilization conditions (temperature and time) have been met. Otherwise, conditions for appropriate sterilization have not been met. Reprocess the entire load again with a new integrator strip.
- 6.8 Complete the "Daily Autoclave Use Log" (see Appendix C) for each cycle as described below:

- 6.8.1 Each autoclave is assigned a log for tracking usage which is readily available in a binder at each autoclave.
- 6.8.2 User must record the following: Date, Time, Users Name, Origin of Waste, Cycle performed, Cycle Parameters (PSI, °F, Cycle Time) and Indicator Results (Pass or Fail).
- 6.8.3 The Safety Office shall maintain and file the Daily Autoclave Use Logs and results for the quarterly biological indicator tests.

7.0 Disposal of Autoclaved Biological Waste

- 7.1 After verifying that either the autoclave tape and/or Class 5 Steam Sterilization Integrator strip has passed, the materials may now be disposed.
- 7.2 Seal the autoclave bag and dispose of bag in outside dumpster. Do not leave bag behind in autoclave room or in trash within room.
- 7.3 For decontaminated biohazardous liquids, the liquid may now be poured down the laboratory drain.

APPENDIX A

ADDITIONAL SAFETY CONSIDERATIONS

HAZARD	SPECIFIC SOURCE	CONTROL MEASURES
DIRECT CONTACT BURNS	<ul style="list-style-type: none"> Hot autoclave chamber Hot autoclave walls and door Hot autoclaved trays and items 	<ul style="list-style-type: none"> Wear heat/water proof gloves when operating the autoclave. If liquids still boiling, allow extra time for cooling Use only Pyrex glass for liquids sterilization Do not shake hot glass containers Place glass containers with liquids in secondary tray
STEAM BURNS	<ul style="list-style-type: none"> Residual steam in the chamber and materials immediately after cycle completion, especially with liquid cycles 	
HOT FLUID SCALDS	<ul style="list-style-type: none"> Spillage of boiling liquids 	
CUTS	<ul style="list-style-type: none"> Broken glass Shattering containers due to pressure build up 	<ul style="list-style-type: none"> Do not cap containers with tightened lids or non-porous materials Place glass containers with liquids in secondary tray Carefully unload hot liquids Check for pieces of glass and remove them using a prong, before cleaning the strainer
HAND AND ARM INJURIES	<ul style="list-style-type: none"> Closing and opening the autoclave door 	<ul style="list-style-type: none"> Use caution when opening or closing the autoclave door
BODY INJURY	<ul style="list-style-type: none"> Equipment explosion 	<ul style="list-style-type: none"> Ensure that the autoclave is functioning correctly
INFECTIONS (PERSONAL AND ENVIRONMENTAL)	<ul style="list-style-type: none"> Biohazardous waste handling or failed decontamination 	<ul style="list-style-type: none"> Handle biohazardous waste with caution and wear recommended PPE to prevent exposure to potential aerosols Verify autoclave effective performance at least quarterly using biological indicators
SLIPS, TRIPS AND FALLS	<ul style="list-style-type: none"> Wet floor 	<ul style="list-style-type: none"> Do not walk on a wet surface; dry spills as they occur

APPENDIX B

QUARTERLY BIOLOGICAL INDICATORS SOP

Each quarter, the MBL Staff certifies each autoclave by validating autoclave performance using purchased biological indicators. Procedures are as follows:

1. Place one or more biological indicator ampule(s) (*Geobacillus stearothermophilus*) into the center of an autoclave bag to be processed.
2. Autoclave the waste following standard procedures as outlined in the MBL Autoclave SOP.
3. Once the cycle has been completed and contents have cooled, remove the indicator ampule(s) from the autoclave bag.
4. Prepare and incubate the indicator along with a control (biological indicator vial which was not autoclaved) at 55 to 60 °C for 24-48 hours as recommended by the manufacturer.
5. Read back incubated vials upon completion of the 24-48 hour time period.
6. Interpretation of biological indicator test ampules as follows:
 - i. **Control:** The control ampule should exhibit a color change from purple to yellow and/or turbidity. If the control ampule shows no signs of growth, the test is considered invalid.
 - ii. **Test:** For a passing test, the ampule should remain a dark purple color. If an intense color change from purple to bright yellow occurs, this indicates surviving spores in the ampule and thus a failing result.
 - iii. When an autoclave problem is suspected or the quarterly biological indicator test fails, the autoclave must be taken out of service and POM shall be contacted immediately for repair.
7. Record the results of the biological indicator test in the "Biological Indicator Test Results Log".

APPENDIX C

DAILY AUTOCLAVE USE LOG

AUTOCLAVE LOCATION (Bldg/Room): _____ AUTOCLAVE ID #: _____

Date	Time	Name of Autoclave User	Waste Origin Bldg/Room	CYCLE		CYCLE PARAMETERS			Is this Waste? [Y/N]	Indicator Results [P/F]	Corrective Actions or Comments
				Fast/Fluid/Dry	PSI	°F	Time(min)				

APPENDIX D REFERENCE INSTRUCTIONS FOR CONSOLIDATED STERILIZER

Please follow the instructions below for autoclaving materials. This document serves as a reference only for autoclave procedures. All users must read and understand requirements as defined in the MBL Autoclave Standard Operating Procedures, current version. Only trained individuals should proceed with processing materials in MBL autoclaves. Proper Personal Protection Equipment must be worn when handling autoclaves and biohazardous material at a minimum; laboratory coat, safety glasses and nitrile gloves (heat-resistant gloves for removing/handling heated materials).

1. Choose the appropriate exhaust selector for proper mode of operation. FAST is for metal instruments, FLUID is for liquids, and DRY is for glass or cardboard. Temperature is fixed at 250°F (this is not adjustable).
2. Open the electronic timer front and set the two thumbwheel digit wheels above "Sterilize" to 60 minutes for non-liquid biohazardous material. For liquids use the following minimum set times based on volume of liquid:

75 mL	25 minutes
250 mL	30 minutes
500 mL	40 minutes
1000 mL	45 minutes
1500 mL	50 minutes
2000 mL	55 minutes
>2000 mL	55 minutes + 10 min/L

3. Place items in secondary containment pan and load into autoclave. Items should not touch interior chamber walls. Close the sterilizer door until firm, but not tight.
4. Start autoclave by depressing the RED "On/Off" button. Sterilization process begins once set temperature of 250°F is reached.
 - a. Pilot light marked "Sterilizing" will light up showing timed function has started.
 - b. Digital read out will begin a count down from set time to zero.
 - c. When zero reached, "Sterilizing" pilot light will go OFF and the "Exhausting" pilot light will come ON and the digital display will now count down from 20 to zero.
 - d. Once cycle completed, the Green light will turn ON.
5. Push the "On/Off" button to turn off the green light and stop the Automatic Controlling System.
6. Open sterilizer door about 1/2 inch, to allow steam to escape. Wait 5 minutes before unloading the autoclave. Allow load to cool an addition 15 minutes before handling.
7. Please remove your items promptly from autoclave area. Bagged autoclaved waste must be disposed in outside dumpster.

For questions or assistance, please contact POM at x7776.