Procedures for Decontamination by Autoclaving

Purpose:

Biohazardous waste material and sharps containers generated within research and teaching facilities are required to be decontaminated in laboratory (or departmental) autoclaves and disposed of using the appropriate waste streams.

The procedures below serve as guidelines to help autoclave users ensure safe and effective processing.

1. Select appropriate containers or bags for collecting materials to be autoclaved.

✸ **For biohazardous dry solid materials**

a. Collect in polypropylene AUTOCLAVE bags:

BSL-1 waste → Clear bags, no symbol
BSL-2 waste → Orange bags, symbol
BSL-3 waste → Red bags, symbol

b. **DO NOT** use the red bags that come with the Regulated Medical Waste (RMW) boxes for initial waste collection. They are not meant to be autoclaved.

c. Ensure that bags are free of sharp objects that may puncture bags. Autoclave bags are tear resistant, but can be punctured or burst in the autoclave.

d. Fill bags only 2/3 full.

e. Ensure adequate steam penetration by creating an opening of at least one inch in the bag's closed top.

f. On autoclaves which have no Prevacuum cycle, water can be carefully added to bags of waste run on Solids/Gravity cycle *if needed to achieve effective decontamination*. (Steam created inside the bag during processing aids in reaching appropriate temperature.)

✸ **For biohazardous sharps:**

a. Collect in commercially available Sharps containers with lids or closures. Containers must not be tightly sealed shut AND MUST NOT BE OVERFILLED.

☑ **POLYPROPYLENE AUTOCLAVE BAG**

⊗ **SHARPS PENETRATING BAG**

☑ **CLOSURE**

⊗ **CLOSURE**

⊗ **OVERFILLED**

✸ **For biohazardous liquids:**

a. Never autoclave plastic materials of uncertain heat stability. Collect liquid in glassware or plasticware that is suitable for autoclaving.

b. Do not fill containers more than 2/3 full.

c. Make sure that caps are loose or use vented closures.

d. Never put sealed containers in an autoclave. They can explode. Large bottles with narrow necks may also explode or boil over if filled too full of liquid.

e. Never put materials containing solvents, corrosives or radioactive materials in the autoclave (e.g., phenol, chloroform, pyridine, or bleach).

☑ **PROPER CLOSURES**

⊗ **NO PRESSURIZED VESSELS**

2. Place waste bags or containers with liquids in a secondary container.

a. Make sure your plastic secondary container is suitable for autoclaving. Polyethylene or HDPE cannot be autoclaved.

b. Polypropylene, polycarbonate or stainless steel pans are typically used for secondary containment. See Nalgene Labware's Autoclaving Web page for additional plastic considerations.

c. Select a container with the lowest sides possible for the autoclave. This will promote penetration of steam and will collect any leakage or overflow of liquids.

d. Make sure pan contains the entire volume of waste—no spilling over sides.

e. Leave space between items/bags to allow steam circulation.

f. Safely transport the material to the autoclave.

☑ **STAINLESS STEEL**

☑ **NALGENE PAN**

⊗ **OVERFILLED PAN**

3. Place a Class 5 Chemical Indicator (CI) in the waste load to check operating parameters.

☑ **5 Chemical Integrator with Extender**

a. If you are using a challenge test pack containing the CI, place it with the waste.

b. If you are using a CI with no pack, place it WITHIN the load of waste in a position where it will encounter the greatest challenge to steam penetration.

c. Avoid direct exposure to waste by using CIs with extenders, or make one yourself by straightening and trimming a coat hanger, and attach the CI to one end with autoclave tape. Place carefully to avoid puncture of bags.

d. Not every container of waste per load must receive a CI. Place CI in the container which occupies the most challenged position in the load (i.e., if running 3 bags, put CI in center bag).

4. Load the autoclave.

☑ **CHECK DRAIN SCREEN**

a. Review the Standard Operating Procedures (SOP) for the autoclave unit. Training must be provided for any new autoclave operators.

b. Check the drain screen at the bottom of the chamber before loading the autoclave.

c. Place a piece of autoclave tape (Class I Chemical Indicator) on the outside of the container or bag. Black stripes appearing on the tape give a visual verification that the material has been processed.

☑ **AUTOCLAVE TAPE**

d. If an autoclave is available, place the load + its secondary container in the autoclave chamber for processing.

- DO NOT OVERFILL THE CHAMBER!
- Load should not touch chamber walls
- DOOR should be clear of obstructions before closing

⊗ **OVERFILLED CHAMBER**

e. Whenever possible, autoclave the load immediately after preparation. Do not leave unprocessed items in the autoclave overnight.

f. If the autoclave is in use, store waste, in a secondary container, in a designated holding area, and decontaminate at the earliest possible time.

4. Choose an appropriate cycle.

CYCLE TYPE & TYPICAL PARAMETERS	RECOMMENDED FOR:
LIQUIDS **STERILIZE TEMP = 121° C** **STERILIZE TIME = 30-60 min.** **COOL TIME =40 min.** **RUN TIME = 70-100 min.**	• Type I borosilicate glass containers with vented closures; 2/3 full only • Liquid Media • Nonflammable liquids • Aqueous solutions • Liquid biowaste
	NOT RECOMMENDED FOR DRY ITEMS THAT DON'T REQUIRE A SLOW EXHAUST
SOLIDS / GRAVITY **STERILIZE TEMP = 121° C** **STERILIZE TIME = 30 to 40 min.** **DRY TIME =0 to 30 min.** **RUN TIME =45 to 80 min.**	• Glassware: -Type I borosilicate - empty & inverted - no tight or impermeable closures • Dry hard items, either unwrapped or in porous wrap • Metal items with porous parts • Other porous materials
	NOT RECOMMENDED FOR LIQUIDS OR MEDIA THAT REQUIRE A SLOW EXHAUST
PRE-VACUUM **STERILIZE TEMP121° C** **STERILIZE TIME = 30 to 45 min.** **COOL TIME = 2 to 5 min.** **RUN TIME 40 to 55 min.**	• Glassware that must be sterilized upright &/or can trap air • Wrapped dry items that can trap air • Pipette tip boxes • Sharps decontamination (in collection containers) • Biohazard waste decontamination (in autoclave bags; can be wet & dry tubes, plates, etc.)
	NOT RECOMMENDED FOR LIQUIDS OR MEDIA, LIGHTER WEIGHT PLASTIC CONTAINERS OR DRY ITEMS WHICH WILL COLLAPSE IN A VACUUM

☑ **LIQUID CYCLE**

⊗ **LIQUID RUN ON SOLIDS CYCLE—** (NOTE BOIL-OVER IN CHAMBER FLOOR PLUS NO SECONDARY CONTAINER)

☑ **PREVAC CYCLE**

5. Please note this important information:

a. For both DRY and LIQUID biohazardous waste materials, cycle times must be set for a minimum of 30 minutes @ 1210C, 15 psi.

b. LARGER VOLUMES OF LIQUIDS AND LARGER LOADS OF SOLIDS REQUIRE LONGER STERILIZATION TIMES.

c. LIQUIDS MUST BE AUTOCLAVED WITH SLOW EXHAUST.

RECOMMENDED STERILIZATION TIMES PER VOLUME FOR LIQUID CYCLES

Volume of Liquid in One Container	Minimum Recommended Sterilize Time at 121° C
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 + 10 min. / L

6. Fill out the autoclave use log (link) and be aware of required cycle times. Record your name, date, time, cycle to be run, etc. The results of the load verification results must also be recorded on this log.

☑ **SIGN AUTOCLAVE USE LOG**

7. Always employ the following safety guidelines when the autoclave cycle is finished:

a. Wear personal protection equipment:
- Lab coat
- Eye protection (when removing load)
- Closed-toe shoes
- Heat-resistant gloves to remove items, especially hot glassware

☑ **USE REQUIRED PPE**

b. Never open an autoclave unless the chamber pressure = 0.

c. Open the door cautiously. Stand behind the door or beside the unit and slowly crack it open no more than ½". Allow all steam to escape by waiting at least 10 minutes before unloading the material. CAUTION: Material will still be HOT!

d. Let liquids stand 10–20 minutes after the autoclave is opened. Superheated liquids can boil over and damage the autoclave and cause personal injury.

e. Do not override autoclave's built-in safety control features under any circumstances. If a problem occurs, contact the responsible technician.

8. **Verify operating parameters by checking for color change on Chemical Indicator strip.**

   - See example on right for 3M™ Comply Chemical Indicators.
   - See EHSS website to download SOP for Chemical Indicators (CI).

9. **Properly dispose of materials that have been successfully decontaminated as verified by Chemical Indicator strip.**

   a. Discard BSL-1 decontaminated waste (contained in clear bags with no biohazard symbol) into the regular trash.

   b. Place BSL-2 or BSL-3 decontaminated waste (contained in orange bags or bags with biohazard symbol) and ALL Sharps containers into Regulated Medical Waste boxes lined with red biohazard bags.

   c. Decontaminated biohazardous liquids may be poured down the drain.

   d. Loads that do not pass verification must autoclaved again and shown to be successfully decontaminated by CI verification before disposal.

   e. Causes of all CI verification failures must be determined and corrected, or reported to the responsible technician who will initiate corrective action.

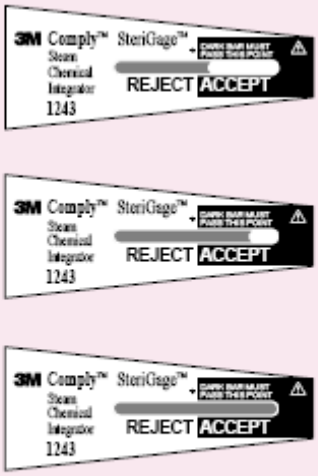
   NOTE: The stripes on autoclave indicator tape changing from light to dark does not ensure that decontamination conditions were successfully met, but serves only as a visual indicator of processed (heat-exposed) versus non-processed items.

10. **Perform required verification testing for your autoclave.**

    a. Use Biological Indicator (BI) testing for:
       - Verifying proper function of newly installed autoclaves

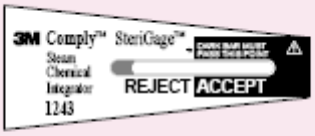
☑ **CHEMICAL INTEGRATOR VERIFICATION**

ACCEPT



The color bar has reached the ACCEPT window in all three samples shown above, indicating that the necessary conditions for sterilization have been met.

REJECT



The color bar is in the REJECT window, indicating that the necessary conditions for sterilization have not been met.

☑ **REGULATED MEDICAL WASTE**

☑ **BIOLOGICAL INDICATOR**

- A monthly check on proper function for all other autoclaves used to decontaminate waste

b. When the heat-resistant bacterial spores *(Geobacillus stearothermophilus)* in the BI vial are killed, definitive verification for decontamination was achieved by the autoclave.

c. Each specific cycle (type, time, temperature, etc.) used to decontaminate biohazardous waste must be verified with B.I. testing.

d. Label the BI with pertinent information (date, autoclave tested, location in chamber, etc.)

e. Place BI in the waste load in one of the following ways:
- Challenge test packs are placed with a waste load (such as between 2 bags of waste).
- BI vials (no packs) are positioned <u>within</u> a load, such as inside a Sharps container or bag of waste, to encounter the greatest challenge to steam penetration.
- For more thorough testing, additional vials can be placed in critical loads.

d. BI vials used alone can be taped to the same extenders used for CI strips to facilitate placement and avoid direct exposure to waste.

e. Upon completion of the cycle, follow BI manufacturer's instructions for activating and incubating test vials and positive control. Observe vials at specified intervals (such as 24 to 48 hours) for a color change indicating bacterial growth. If growth occurs, the autoclave tested has not met appropriate operating parameters.

f. Results must be recorded on the Biological Indicator Testing log.

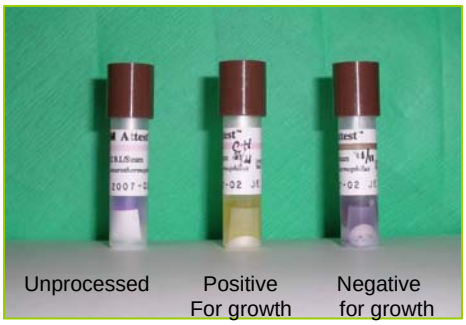
g. See the EHSS website to download SOP for Biological Indicators (BI).

<u>g.</u> <u>BI Failures:</u>

- All BI testing failures must be reported immediately to the technician responsible for the autoclave, who will investigate and take corrective action.
- Users of the autoclave also must be informed of any failure that may have affected runs processed in the autoclave at or near the time of testing.

☑ **B.I. INCUBATOR**

BI TEST RESULTS



☑ **RECORD IN BI STERILITY TESTING LOG**

- The autoclave in question must be taken out of service for decontamination of waste until the problem is found and proper function is restored as verified by repeat BI testing.

h. BI verification testing should also be performed:

- After a sterilizer has been repaired
- As required for research needs

☑ **SIGN ON OUT-OF-SERVICE AUTOCLAVE**

11. Keep autoclaves in good repair with preventive maintenance.

a. The responsible technician, the autoclave's manufacturer, or the autoclave's sales /service representative can provide more information.

b. If you suspect there is a problem with your autoclave's performance, contact the responsible technician for assistance.

☑ **UTILITIES SIDE OF AUTOCLAVE**

References

1. Le, R.N., et al (2005), Autoclave Testing in a University Setting. *Applied Biosafety*, 10(4), 248-252.

2. Centers for Disease Control and Prevention, Oral Health Resources "Sterilization – Monitoring FAQs," April 2005. www.cdc.gov/oralhealth/infectioncontrol/faq/sterilization_monitoring.htm

3. University of Ottawa Environmental Health and Safety Service, A Guideline for the Safe Use of Autoclaves, 9 July 2003, http://www.uottawa.ca/services/ehss/docs/autoclave.pdf

4. 3M™ Technical Information Sheet: 3M™ Comply ™ (SteriGage™ Steam Chemical Integrator, 1999, 70-2009-0710-6 (29.5) DPI

5. 3M™ Technical Product Profile: 3M™ ATTEST™ Biological Monitoring System, 1994