

## ENVIRONMENTAL HEALTH & SAFETY

### Standard Operating Procedures for Safe Autoclave Operations

The purpose of this document is to provide standard operating procedures for the safe use of autoclaves. Autoclaving is a process used to destroy microorganisms and decontaminate biohazardous waste and microbiological equipment used at Biosafety Level 1 and 2.

#### Hazards

Autoclaves use high pressure and high temperature steam for sterilization. The potential safety risks for the operators include:

- Heat burns from hot materials and autoclave chamber walls and door.
- Steam burns from residual steam coming out from autoclave and materials on completion of cycle.
- Hot fluid scalds from boiling liquids and spillage in autoclave.
- Hand and arm injuries when closing the door.
- Body injury if there is an explosion.

#### Safety

- To insure the health and safety of personnel using the autoclave, it is important for each department to maintain autoclaves and to train personnel in their proper use.
- It is the supervisor's responsibility to ensure employees are trained before operating any autoclave unit.
- Procedural and instructional documents provided by the manufacturer must be followed.
- Personal protective clothing and equipment must be worn when loading and unloading the autoclave.
- Autoclaves must be inspected at least annually. A basic visual inspection should be performed monthly by the person responsible for the autoclave. The inspection, service and repair records should be available upon request.
- Spore strips may be used to validate autoclave effectiveness.

## Personal Protective Equipment

Equipment to protect against scalds and burns include:

- Heat-insulating gloves that provide complete coverage of hands and forearm
- Lab coat
- Eye protection
- Closed-toe footwear

## Training

All personnel who use autoclaves must have successfully completed a training session from their supervisor on the safe operating procedures.

## Material Preparation

Ensure that the material is safe for autoclaving:

- Samples containing solvents or substances that may emit toxic fumes should not be autoclaved.
- Do not autoclave bleach!
- Glassware must be inspected for cracks prior to autoclaving.

Prepare and package material suitably:

- Loose dry materials must be wrapped or bagged in steam-penetrating paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing effectiveness of the process.
- Loosen all lids to prevent pressure buildup. All containers must be covered by a loosened lid or steam-penetrating bung.
- Containers of liquid must not exceed two-thirds (2/3) full, with lids loosened.
- Glassware must be heat-resistant borosilicate.
- Plastics must be heat-resistant, i.e., polycarbonate (PC), PTFE ("Teflon") and most polypropylene (PP) items.
- Discarded sharps must be in a designated 'Sharps' container.
- All items must be tagged with autoclave tape.
- Place items in **secondary containers** to secure and contain spills:
- Items should be placed in a stainless steel pan or other autoclavable container for their stability and ease of handling.
- Place containers of liquid, bags of agar plates, or other materials that may boil over or leak, into a secondary pan in the autoclave.
- The pan must be large enough to contain a total spill of the contents.
- Bags must not be tightly sealed as steam cannot penetrate.
- Biohazardous waste must be processed according to ASU guidelines.

## **Loading Autoclave**

- Wear lab coat, eye protection, heat-insulating gloves, and closed-toe shoes.
- Place material in autoclave. Do not mix incompatible materials.
- Do not overload; leave sufficient room for steam circulation. If necessary, place the container on its side to maximize steam penetration and avoid entrapment of air.
- Close and latch door firmly.

## **Operating Autoclave**

- Close and lock door.
- Choose appropriate cycle (e.g., gravity, liquid, or dry cycle) for the material. Consult the autoclave manual for assistance in choosing a cycle. The manuals for operation of the autoclave should be located near the autoclave.
- Set appropriate time and temperature if you are using a customized cycle.
- Start your cycle and fill out the autoclave user log with your contact information. A completed cycle usually takes between 1-1.5 hours, depending on type of cycle.
- Do not attempt to open the door while autoclave is operating.
- If problems with your autoclave are perceived, abort cycle and report it to your PI immediately.

## **Unloading Autoclave**

- Wear heat-insulating gloves, eye protection, lab coat, and closed-toe shoes.
- Ensure that the cycle has completed and both temperature and pressure have returned to a safe range.
- Wearing Personal Protective Equipment (PPE), stand back from the door as a precaution and carefully open door no more than 1 inch. This will release residual steam and allow pressure within liquids and containers to normalize.
- Allow the autoclaved load to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
- Do not agitate containers of super-heated liquids or remove caps before unloading.
- Wearing heat-insulated gloves remove items from the autoclave and place them in an area which clearly indicates the items are 'hot' until the items cool to room temperature.
- Shut autoclave door.

## **Autoclave Use Log**

- Entries must be placed in the log book each time the autoclave is used. These records are used for maintenance/service schedules and reporting of incidents, accidents and/or faults.
- Entries should include: operator's name, phone number, date, time and duration.
- The log book must be kept adjacent to the autoclave.
- An Autoclave Use Log example is provided in this document.

## **Maintenance and Repair**

- No person shall operate the autoclave unless the autoclave is in good repair.
- Only qualified professionals are permitted to make repairs.
- Report possible malfunctions to .
- Repairs are performed by your service contract or any other contractor you choose to hire.

## **Equipment Malfunction**

- If the autoclave does not operate exactly as expected, do not attempt to fix the problem. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected.
- Record the problem in the autoclave log book.
- Contact or your supervisor to report the problem.
- Only qualified professionals are permitted to make repairs.

## **Incident Response**

- All incidents, including a spill or release of biohazardous materials and recombinant or synthetic nucleic acid molecules, must be reported to your supervisor and Biosafety Officer.
- If any injury occurs seek first aid or, if necessary, seek medical assistance at ASU Health Services or by dialing 9191.
- If clothing is soaked in hot water/steam, remove clothing and place the injury in cool water.
- Place a notice on the autoclave indicating that it is not to be used until the cause of the incident is determined, procedures enacted to prevent future incidents, and the autoclave is deemed safe for operation.

## **Spill Clean-up**

- Spills may occur from a boil-over or breakage of containers.
- No operation of the autoclave is allowed until the spill is cleaned up.
- The operator is responsible for clean-up of spills. Contain the spilled material using paper towels. Use your laboratory's spill kit if necessary. Wait until the autoclave and materials have cooled to room temperature before attempting clean-up.
- Review the Safety Data Sheet if appropriate, to determine appropriate PPE, spill cleanup and disposal protocols that are necessary.
- Dispose of the waste following the protocol appropriate for the material (e.g., red biohazard bag). If

- materials have been intermingled, follow the clean-up and disposal protocol for the most hazardous component of the mixture.
- Cracked glassware must be disposed of properly.
- Record the spill and clean-up procedure in the autoclave log book.

## **Appendix A: Procedure for Spore Testing Sterilization**

The following procedure should be followed when testing to ensure that the autoclave effectively sterilized materials placed in the unit.

- Place vial in the center of the bag.
- Process the load using normal operating procedures.
- Follow any instructions provided by the testing supplier.
- If doing in-house testing, incubate at incubation temperature for 24 - 48 hours (see specific protocol included with ampules).
- Incubate a control vial that has not been autoclaved, media should turn yellow to indicate growth. If the control vial remains purple (colors may vary by test kit), there may be a problem with the batch of indicator vials and the test may not be valid. Repeat the run, and if the result is the same obtain a new set of test vials. Return the waste to the bin and re-run with a new batch of vials.
- If the test vial media is purple after incubating 72 hours, sterilization is successful, there was no growth.
- If the media turns yellow, the bacteria grew and sterilization failed.
  - Review the run chart to see if the physical conditions (time, temperature) were met. If they were, discontinue using the autoclave, return the waste to a biological waste bin, and contact the service provider to get the autoclave repaired.
  - Retest after the repairs are completed.
- Record all results and retain in the Autoclave logbook.