

## SOP - AC Standard Operating Procedures for Safe Operation of Autoclaves

Date: February 15, 2012

### **Purpose:**

Sterilization refers to the complete killing of all living organisms, including spores. Common sterilization techniques include the application of wet heat, dry heat, chemicals, and radiation. The type of material, the container, and quantity of items to be sterilized determines which method to use. Various pieces of equipment are used for sterilization in laboratory animal facilities, but the autoclave is the primary means of sterilizing materials. Despite built-in safeguards, an autoclave presents the possibility of serious injury to technicians from hot surfaces and from the release of steam. It is important, therefore, that laboratory personnel understand the proper operation, limitations, and safeguards for sterilization by autoclaving.

### **Safety Precautions:**

- All operators must receive training on the safe operation of any autoclave that they will or may be required to use prior to using the equipment. Training may be delegated to a qualified individual, but it remains the responsibility of the Primary Investigator (PI) to ensure their personnel are adequately trained to understand proper packaging, loading, labeling, and operation procedures.
- All potentially infectious materials must be autoclaved before being washed and stored or disposed.
- Biohazardous materials must be labeled as such and must be sterilized by the end of each work day, or must be secured appropriately. Biohazardous materials should not be left in an autoclave overnight in anticipation of autoclaving the next day.
- For the autoclave process to be effective in achieving sterilization, sufficient temperature, time and direct steam contact are essential. Air must be completely removed from the sterilizer chamber and from the materials to allow proper steam penetration. Factors that affect air removal include type and quantity of material to be autoclaved, packaging, load density and configuration, and container type, size, and shape.

### **Risks:**

The potential safety risks for the autoclave operators are:

- 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.

### **Proper PPE:**

Personal protective clothing and equipment must be worn when loading and unloading the autoclave:

- Heat-insulating gloves protect hands and forearms.
- Face shields protect face and neck.
- Splash apron protect chest and legs.
- Closed-toed footwear protects feet.

## **Procedure:**

### **Preparation**

- Ensure that the material is autoclavable – oils, waxes, some plastics, flammable materials, radioactive materials, and samples containing solvents or substances that may emit toxic fumes should not be autoclaved.
- Glassware should be heat-resistant (Pyrex or Kimax) and inspected for cracks prior to autoclaving.
- Plastics should be heat-resistant (for example, polycarbonate (PC), PTFE ("Teflon") and most polypropylene (PP) items).
- Prepare and package material suitably:
  - Loose dry materials should be wrapped or bagged in steam-penetrable paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing efficiency of the process.
  - Containers of liquid should be a maximum volume of 2/3rds filled.
  - All containers should be covered by a loosened lid or steam-penetrable bung to prevent pressure buildup and avoid having bottles shatter during pressurization.
  - Use plain, unmarked containers for items that are not hazardous.
  - Items should be tagged with autoclave temperature tape to verify adequate thermal treatment.
  - Place items in containers to secure and contain spills. Place containers of liquid, bags of agarplates, or other materials that may boil over or leak inside a secondary pan (could be autoclavable plastic or stainless steel container) in the autoclave. The pan must be large enough to contain a total spill of the contents. Open, shallow metal pans are more effective in conducting heat and allowing air removal than tall, plastic tubs.
  - Adding some water to the secondary pan will help to heat items more evenly.

### **Use (Loading the Autoclave)**

- Ensure the drain strainer in the bottom of the autoclave is clean before loading the autoclave.
- Use a cart to transfer items to be autoclaved. To avoid back injuries, push the cart up to the autoclave door and gently slide the load into the autoclave.
- Never place autoclave bags or glassware in direct contact with the bottom of the autoclave. Place the secondary pan containing the items to be sterilized on the shelf or rack of the autoclave.
- Do not overload the autoclave. It is important to leave sufficient room for thorough steam circulation.
- Do not mix loads of liquids with solids.
- Firmly lock autoclave door prior to starting the run to prevent sudden release of high-pressure steam.
- Make sure that you have selected the correct cycle before starting the autoclave.

### **Use (Unloading the Autoclave)**

- After the run is completed, check the pressure gauge to ensure that the pressure in the chamber is "0". If pressure is not released, do not open the door. Inform your PI / supervisor about the malfunction.
- Before opening the door put on your eye protection and heat-resistant gloves or mitts. Be sure to wear closed-toed shoes (hot condensate may drip from the door). Rubber aprons in addition to rubber sleeve protectors are advisable.

- Carefully crack door open to release residual steam and allow pressure within liquids and containers to normalize. Wait a full five minutes if the autoclave load contains only dry glassware, and no less than ten minutes when you are autoclaving liquids before removing the items.
- Use caution when removing liquids, molten agar, etc. Liquids, especially large volumes, may continue boiling for some time after autoclaving. Do not agitate containers of super-heated liquid or remove caps before unloading to avoid getting splashed with scalding liquid.
- Slide a cart to the opening of the autoclave and pull the autoclave secondary container onto the cart for transport. Place the cart in a low traffic area while additional cooling occurs.
- Let the glassware cool for 15 minutes and liquid loads for a full hour before touching the items with ungloved hands.
- **NOTE:** If a faulty condition exists (e.g., sterilizer did not finish the cycle, or water leaks out when the door is unlocked), contact a service technician.