



ECKERD COLLEGE

Safe Operating Procedure

(2/09)

AUTOCLAVE OPERATION AND SAFETY

CAUTION!

Autoclaves are used in two rooms in Sheen Biology and one is used in GMSL. These devices are used to sterilize materials. Due to the high heat and pressure created in autoclaves during operation, proper loading, use, and unloading procedures must be followed to prevent burns and other accidents. Burns can result from physical contact with the structure of the autoclave and steam burns can occur from contact with steam leaving the apparatus. Explosive breakage of glass vessels during opening and unloading as a result of temperature stresses can lead to mechanical injury, cuts, and burns. Burns can also result from careless handling of vessels containing hot liquids.

- Report all malfunctions to Jennifer Gilkey, Biology Stockroom Supervisor and conspicuously tag the equipment as “Out-of-Service.”
- **Do not use an autoclave unless you have received specific operation instructions or are working under the direct supervision of an experienced autoclave worker.**
- Read and follow the recommendations made by the manufacturer in the owner’s manual.
- Firmly lock autoclave doors prior to starting the run to prevent sudden release of high-pressure steam.
- **When loading an autoclave, remember to loosen the caps of screw-topped vessels and bag closures.**
- Do not stack containers.
- Do not overload the autoclave.
- Do not overpack autoclave bags.
- Do not autoclave volatile chemicals.
- Avoid contact of hands, arms, and face with the walls of the autoclave or the emerging steam.
- **Report any malfunction of the apparatus, particularly if the steam valve fails to operate normally** to Jennifer Gilkey (8442) or Fawn Crotty (8447).
- Check autoclave operation periodically during the duration of a run.
- Do not use the pressure relief override valve to quickly achieve standard pressure so that the autoclave can be opened sooner. Sudden pressure

changes can cause bottles to burst and or plugs to be ejected from flasks. If the pressure is not being released without using the override valve (pressure), contact Jennifer Gilkey x8442 for assistance.

- **Most accidents occur during the final operations of opening and unloading the autoclave. When the pressure gauge reaches zero, wait one to two minutes before opening the autoclave. It is dangerous to begin opening the autoclave before the pressure gauge registers zero.**
- **Wear insulated gloves or mitts when unloading materials.**

There is a drawer of orange mitts next to the sink in SHB 100.

- When opening the door, stand so that the door shields your body from the contents of the autoclave, if possible. Watch your feet! Sometimes large volumes of condensate will accumulate in the autoclave (particularly when the steam has not been released) and run out as the door opens.
- After opening the autoclave, wait a few minutes before removing the contents. Remember glass containers may have cracked during autoclaving. Arrange a suitable table or trolley near the autoclave so that you do not walk around carrying flasks of hot liquid. While unloading, avoid striking the vessels against the walls of the autoclave.
- Vessels containing large volumes of liquid (5 - 20 liters) should be allowed to cool in the autoclave before being unloaded since they continue to boil for some time after the autoclave is opened. If a large vessel fractures or is otherwise broken while being handled, serious injuries could result.
- Remove debris (rubber bands, plugs, etc.) from the autoclave. If left, these will block the drain valves and may create a hazard for other users.
- Do not stack or store combustible materials next to an autoclave (cardboard, plastic, volatile or flammable liquids).
- **It is imperative to know that the autoclave has thoroughly sterilized its contents. Most autoclave tape is imprinted with a dye that changes color when the correct temperature is reached. The problem with this type of check is that the dye is on the surface of the load, and a positive reading does not ensure that the innermost parts of a large load are also sterile. However, an easy way to check this is to wrap something with autoclave tape (such as a disposable plastic test tube or pipette tip), attach string to it, and put it deep into the load. Tape the other end of the string to the outside of the bag so that you can easily pull the indicator out. (Do NOT open up a load of potentially infectious material to bury something inside.) Recover the indicator after the run and confirm that it too has changed color.**
- Liquids, whether autoclaved or not, are not to be placed in the trash without first absorbing them onto an inert solid medium (e.g., vermiculite, bentonite, etc.). In addition, all red-bag waste must have indicator tape that has changed color before disposal. To avoid confusion, autoclaved red-bag waste should be placed in a non-transparent outer bag or box that is not labeled with a biohazard symbol before being placed in the trash receptacle. Remember, hazardous waste regulations pertain to autoclaved waste as well, so it is imperative to consult with Jennifer Gilkey if your run

contained any agars or other materials that may contain a regulated substance (e.g., heavy metal such as Pb, Hg, Ag, Se, Ba, As, Cd, Cr, or other potentially toxic constituent).

Sterilization conditions (runs) differ with the type of materials to be sterilized. The general types of runs are "liquids" for any type of water-based solutions, "dry goods with vacuum," and "dry goods without vacuum." Autoclaves often have an additional "drying" cycle in which hot air is drawn through the chamber to dry materials before removal. Controls for different brands of autoclave vary, so always carefully follow the manufacturer's instructions about loading, load sizes, cycle types, and settings. General precautions based on run-type are listed below:

- The "liquids" run is longer than the other two but uses lower temperatures to minimize evaporation of the liquids being sterilized. Make sure seals on containers of liquids are loose so vapor expanding during heating will not cause rupture of their vessels. Do not autoclave flammable or volatile liquids.
- The "dry goods with vacuum" run moves steam and heat into the deepest parts of large bags or bundles of materials and produces the best conditions for killing persistent organisms. During this type of run, the chamber alternates between cycles of vacuum and high pressure. The chamber is pressurized with steam for a long period, followed by a short vacuum cycle. It is important that steam is able to reach the entire load, so carefully loosen autoclave bag closures prior to the start of the run.
- The "dry goods without vacuum" run simply pressurizes the chamber with steam for the duration of the cycle, and then returns to normal. This process is used primarily for items that have been pre-cleaned but need to be sterilized. Materials should be packed so that the steam can readily reach the whole load.

In addition to run conditions and settings, other factors can influence the efficacy of the autoclave. Other factors include the materials of construction of waste containers (i.e. polyethylene, polypropylene, metal, glass, etc.), volume of water added to waste containers, and size, density, and weight of the load.

For all autoclave uses, observe these precautions:

- Do not exceed the manufacturer's recommended pressures and temperatures.
- Arrange for regular inspection/testing of autoclaves and ancillary equipment in accordance with the manufacturer's specifications.