**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 facilities, but the autoclave is the primary means of sterilizing materials. Despite built-in safeguards, an autoclave presents the possibility of serious injury to users 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. Chapter 10 of the [Biological Safety Manual](https://ehs.unc.edu/manuals/biological/) should be consulted for further information regarding safe procedures for disposal of biohazardous laboratory waste, appropriate autoclave settings, autoclave testing verification and maintenance.

**Safety Precautions:**

* All operators must receive hands-on 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 and that their personnel understand the risks associated with autoclave operation. Training must be documented, and records kept by the lab. Training should be specific to model and type of autoclave being used. An operations manual should be reviewed and available at all times to operators.
* All employees that use an autoclave must complete the [online autoclave training](https://ehs.unc.edu/training/self-study/autoclave-usage-and-safety/). To ensure that infrequent users do not neglect proper operating techniques, autoclave operating instructions should be posted in close proximity to the autoclave.
* All potentially infectious materials must be autoclaved before being washed 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 never be left in hallways, next to the autoclave on the floor or in other public spaced prior to autoclaving. Biohazard bags should remain in the laboratory until they are ready to be placed in the autoclave.
* 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 to every part of the waste load. If all the air is not allowed to escape from the waste during the autoclave cycle, then steam will not replace the air. 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.

* Make sure autoclave doors and gaskets are firmly locked into place before operating the autoclave
* The plug screen or drainer should be removed with heat-resistant gloves, checked, and cleaned frequently to ensure that it is free of dirt, dust, or sediment which may collect and cause a clog.
* Interior surfaces should be cleaned (according to manufacturer’s specifications) of any residues that collect over time.
* Gaskets, doors, shelves, and walls should be visually inspected on a regular basis for residue buildup and wear
* Do not autoclave toxic, volatile or radioactive material.

**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.
* Inadequate decontamination allows for the potential of biological hazards and personnel and environmental contamination
* Large heavy doors and loading carriages also present an ergonomic and pinch hazard

**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.
* Lab coat and splash apron protect chest, arms and legs.
* Long pants to protect legs per the minimum PPE policy.
* 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. DO NOT autoclave containers that are filled with liquid as this increases the likelihood of an overflow of hot liquids.
  + 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 must be tagged with autoclave temperature tape to verify adequate thermal treatment.
  + Place items in containers to secure and contain spills. Containers of liquid, bags of agar plates, or other materials that may boil over or leak MUST be placed 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. DO NOT overfill the secondary container as this poses a spill/splash risk when removed from the autoclave.
  + Biohazard bags should be closed and placed within secondary containers (bins, trays)
  + Do not seal the bags too tightly, as this will impede penetration of steam into the bag.
  + Indicator tape should be placed in an “X” pattern over the biohazard symbol.

**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, particularly if fragile/breakable (e.g. glass flasks and beakers) items are being transferred. 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)**

* Before opening the door, ensure that you have put on all required PPE (eye protection and heat-resistant gloves or mitts. Be sure to wear closed-toed shoes (hot condensate may drip from the door). Long pants protect legs in case of spills or steam. Lab coats and rubber aprons protect arms and torso.
* The pressure gauge must read zero before attempting to open the door. Carefully crack door open to release residual steam and allow pressure within liquids and containers to normalize. Be sure to stand away

from the door so as not to be exposed to steam escaping the autoclave.

* Wait a full five minutes if the autoclave load contains only dry glassware, and no less than

10 minutes when you are autoclaving liquids before removing the items.

* Use caution when removing liquids, molten agar, etc. Liquids, especially large volumes, may continue to

boil 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 Facilities Services. Place a sign on the autoclave that it is out of use. Do not continue to use the autoclave until it is operating correctly.