

## STEAM STERILIZATION

The objective of decontamination is to render contaminated material safe for subsequent handling. The autoclave that uses saturated steam under pressure is the most dependable method available in the laboratory for the destruction of all forms of microbial life. From both a safety and quality control perspective, all biohazardous material and all items (culture media, laboratory glassware, waste, etc.) contaminated with potentially infectious agents should be decontaminated prior to being washed or discarded. Steam sterilization is not recommended for water insoluble substances because it does not allow for steam penetration.

Procedures to be followed in operating steam sterilizers include:

1. Use EHS approved autoclave bags. Clear, polypropylene, autoclave bags depicting the universal biohazard symbol are available in the NCI-Frederick supply warehouse in various sizes for autoclaving laboratory waste. Alternative autoclave bags may be ordered with prior EHS approval. An autoclavable biomedical waste container lined with a polypropylene bag is also available from the warehouse (#66401506).
2. Ensure that the autoclave bags are not closed so tightly to impede steam penetration. Temperature indicator tape may be used to close the bag or placed on the bag as a visual indicator that the desired temperature was reached during the cycle.
3. Chemical temperature indicators shall be used for each load. At six-month intervals, EHS provides a biological indicator for biological validation of the autoclave. Labs are encouraged to validate autoclaves on a regular basis. Additionally several forms of chemical indicators are available such as autoclave tape and steam sterilization integrators.
4. Avoid overcrowding or stacking of items. To facilitate the displacement of air with steam in empty vessels, place empty flasks and containers on their sides.
5. Start timing the sterilization cycle when the thermometer in the exhaust line reaches the set temperature; do not rely on the pressure gauge as the temperature indicator. The autoclave should maintain 121°C (250°F) for a minimum of 60 minutes. Additional time is recommended for closed containers or densely packed material. If a shorter than recommended cycle time is desired then efficacy must be proven using biological indicators.
6. Remove and clean the strainer in the drain line at the front of the autoclave at weekly intervals or as needed.
7. When autoclaving liquids:

- a. Do not fill vessel beyond 75 percent capacity to allow for fluid expansion and prevent overflow. Be sure to use borosilicate glass such as Pyrex for containers. Thermal expansion is low for Pyrex, which decreases the risk of container breakage during the autoclave process.
  - b. Loosen caps on glassware to allow for venting and reduce the risk of breakage.
  - c. Slow-exhaust the autoclave for liquid loads. On this setting, the chamber pressure is reduced slowly, which provides sufficient time for liquids to cool sufficiently so the liquid does not boil as autoclave pressure is reduced.
  - d. Let the autoclave cool for at least 20 minutes after it reaches zero gauge pressure. Open door slowly and stand back until steam has cleared.
  - e. When liquids are being removed from a hot autoclave, wear heat-resistant gloves with gauntlet sleeves, safety eyewear or goggles, and a rubber apron.
8. Periodically check the door gasket for wear and the accumulation of debris. Clean the shelves and walls of the autoclave as necessary.

The most common autoclaves in use at NCI-Frederick are the gravity displacement type. In these units, supplied steam displaces air through a trap in the drain line. Once the air has been evacuated, the trap closes. Steam continues to fill the sterilizer until a pre-set temperature is reached (usually 120°C, 15 lbs./inch pressure) as indicated by the unit's temperature sensor.

Air in closed or upright containers (animal cages, Erlenmeyer flasks, Etc.) is not readily replaced with steam, and consequently, temperature in these containers may remain sublethal. If possible, containers should be placed on their side to enable the air to be replaced with steam. Also, the size and type of the materials being sterilized must be considered. A large container of liquid materials or materials with high insulating capacity such as animal bedding, clothing, or animal carcasses will require a greatly extended autoclaving time.

Modern autoclaves are usually equipped with a digital control system. These systems ensure that the autoclave will automatically move through a pre-set cycle. Large autoclaves are usually equipped with power-operated doors. The manufacturer's operating manual should be followed closely when operating autoclaves to obtain effective sterilization.

Autoclaved waste needs to be segregated into applicable waste carts (i.e., burnable, nonburnable, animal, special medical, etc.).

Call EHS at x1451 if you have questions about autoclaves or steam sterilization.