

Autoclave Use

Steam sterilization of materials is a dependable procedure for the destruction of all forms of microbial life. They are common laboratory tools that must be properly used to be effective. This establishes guidelines for the effective use of steam sterilizers (autoclaves) for the decontamination of cultures and other potentially biohazardous materials.

See CDC/NIH Classification of Human Etiologic Agents On The Basis Of Hazard for a complete list of agents capable of infecting humans.

Successful Components of Sterilization

This should include validation of decontamination effectiveness. Validation of effectiveness includes monitoring temperature, pressure and cycle duration time for each cycle and providing periodic decontamination challenges (quality assurance), i.e. use of biological indicators. A logbook should be maintained to record autoclave use and be available for inspection.

- **Temperature:** This denotes heating in an autoclave employing saturated steam under a pressure of approximately 15 psi to achieve a chamber temperature of a least 121°C (250°F) for a minimum of 30 minutes.
- **Time:** The time is measured after the temperature of the material being sterilized reaches 121°C (250°F).
- **Contact:** Steam must contact all areas of the load. Autoclave bags should be left partially open during decontamination to allow steam to penetrate into the bag. Air pockets or inadequate steam supply will cause sterilization failure.
- **Containers:** Materials that are to be decontaminated should be carried to the autoclave in leak proof containers. Containers used to hold material while being autoclaved are described below.
 - Primary Containers – Clear autoclave bags come in a wide variety of sizes. They are usually placed in a secondary container during decontamination cycles to catch liquids that may drain out of the bag.
 - Secondary Containers – Plastic or stainless steel containers are commonly used to contain material during autoclaving. Polypropylene plastic pans with 6-12 inch sides are favored over polyethylene and polystyrene because it can withstand autoclaving without melting. Stainless steel containers are durable and a good conductor of heat.
- **Indicators:** These are tools used to validate the decontamination process.
 - Chemical indicators change color after being exposed to 121°C (250°F), but they have no time factor.
 - Tape indicators can only be used to verify that the autoclave has reached normal operating temperatures for decontamination.
 - Biological indicators are designed to demonstrate that an autoclave is capable of killing microorganisms.
 - **A load test using *Bacillus stearothermophilus* monthly.**
- **Recordkeeping:** Records of maintenance, logs, chart recorders, calibration results and *Bacillus stearothermophilus* load tests should be kept for a minimum of three years.

Autoclave Training and Operation

Principal Investigators, or supervisors must train and qualify their staff for operation of steam autoclaves for decontamination of materials. Qualified autoclave users should understand the time, temperature, pressure relationships required for proper materials decontamination.

Additional training on handling materials to be decontaminated should also be provided.

Supervisors should maintain a permanent record of training provided to their staff.

- Personnel should wear proper personal protective equipment, i.e. heat resistant gloves, eye protection and a lab coat, particularly when unloading the autoclave.
- Regularly inspect your autoclave components for proper operation. Follow the autoclave manufacturer's recommendations for inspections and service. Autoclaves should be inspected on a regular basis and temperature gauges should be calibrated at least annually. If a problem is found, promptly notify your area supervisor who will call for maintenance. **Do not operate an autoclave until it has been properly repaired.**
- **Never place sealed containers in an autoclave.** Large bottles with narrow necks can simulate sealed containers if filled with too much liquid.
- **Do not** autoclave items containing solvents, volatile or corrosive chemicals (phenol, trichloroacetic acid, ether, chloroform, etc.) or any radioactive materials. Call the Office of Research Safety at (330) 672-1977 or (330) 672-4347 if you have questions regarding waste disposal.
- After loading and starting the autoclave, processing time starts after the autoclave reaches normal operating conditions of 121°C (250°F) and 15 psi pressure.
- Decontamination conditions vary with type of load therefore processing times will vary according to the conditions. A minimum of 30 minutes is needed to decontaminate biological waste.
- At the end of a decontamination cycle make sure that the pressure in the autoclave chamber is near zero before opening the door. Slowly crack open the autoclave door and allow the steam to gradually escape from within the autoclave.
- Take care when opening autoclave doors. Opening the autoclave door too quickly may result in glassware breakage and/or steam burns on your skin.
- Allow materials inside the autoclave to cool for 10 minutes before removing them from the autoclave.
- After autoclaving, waste can be disposed of as solid waste except if it is a human pathogen then it must be placed in a red bag with biohazard symbol and held for disposal via incineration by a biohazardous waste handler.