

## Guidelines for the Safe Use of Autoclave

### Autoclave Safety

The biological hazards involve potential exposure to viable human pathogens. Autoclaves are used to sterilize laboratory equipment or materials such as glassware, media, reagents, or waste. Autoclaves are commonly used because they are a dependable means of achieving the necessary level of killing efficacy (or sterility assurance level) for most biological materials. In addition, autoclaves do not generate other chemical antimicrobial waste or sources of contamination.

Standard autoclave conditions for the types of materials that need sterilization should be established. Autoclave treatment conditions to achieve sterility will vary in relation to the volume of material treated, volume of the autoclave, the contamination level, the moisture content, and other factors. Treatment conditions for typical materials are listed below:

- Laundry: 121°C (250°F) for a minimum of 30 minutes.
- Trash: 121°C (250°F) for at least 45 minutes per bag. Size of the autoclave and size of the bags greatly affect sterilization time. Large bags in a small autoclave may require 90 minutes or more.
- Glassware: 121°C (250°F) for a minimum of 25 minutes.
- Liquids: 121°C (250°F) for 25 minutes for each gallon.
- Animals and bedding: Steam autoclaving is not recommended (sterilization time required would be at least 8 hours). Incineration in an approved facility is the recommended treatment of these wastes

### Autoclave safety considerations

Autoclaves typically use different combinations and patterns of high heat, vacuum, and pressure to sterilize the load. These combinations and patterns are used in autoclave run cycles or *runs* and are based on the type of material to be sterilized. General types of runs include *liquids* for any type of water-based solutions, *dry goods with vacuum*, and *dry goods without vacuum*.

First, review the operational and safety instructions found in the manufacturer's operating manual before start using the autoclave

- Wear personal protection equipment:
  - Lab coat
  - Eye protection
  - Closed-toe shoes
  - Heat-resistant gloves to remove items, especially hot glassware
- Load the autoclave properly per the manufacturer recommendations.
- Choose the right bags, pans, and other containers
  - Polypropylene Autoclave Bags: can be punctured or burst in the autoclave. These bags should therefore be placed in a rigid container during autoclaving

- Polypropylene Containers and Pans: nonconductor of heat and take longer to autoclave than the same materials in a stainless steel pan
- Stainless Steel Containers and Pans. Good conductor of heat and hence short sterilizing time, but costly
- Be sure to clean the drain strainer before loading the autoclave.
- Before loading containers of liquids into the autoclave, the caps must be loosened to avoid having the bottles shatter during pressurization.
- Use a tray with a solid bottom and walls to contain bottles and catch spills.
- Add 1/4 to 1/2 inch of water so the bottles will heat evenly.
- Don't load plastic materials that are not compatible with the autoclave.
- Do not autoclave items containing materials such as corrosives, solvents, volatiles, or radioactive materials that may contaminate the autoclave, create an inhalation hazard, or explode
- Individual glassware pieces should be within a heat resistant plastic tray on a shelf or rack, and never placed directly on the autoclave bottom or floor.
- Make sure the door of the autoclave is fully closed and the correct cycle has been selected before starting.
- Before removing autoclaved items, wait 5 minutes for loads containing only dry glassware, and 10 minutes for autoclaved liquid loads.
- When removing items from the autoclave, wear a rubber apron, rubber sleeve protectors, heat resistant mitts and a face shield. Remove the load and let the glassware cool for 15 minutes before touching it with ungloved hands.
- Be alert for autoclaved liquid bottles which are still bubbling. Let liquid loads stand in an out-of-the-way place for a full hour before touching with ungloved hands. Hot glassware and scalding liquids will cause burns and serious harm.
- Report any malfunctions or accidents immediately to your supervisor
- Clear, autoclavable bags are used for recombinant DNA and/or BSL 1 waste. The bags cannot have biohazard symbols or wording. Once clear bags have gone through the cycle, they may be disposed of as regular trash.
- Red, autoclavable, "Biohazard" bags are used for BSL2 waste.

### Testing Autoclaves for Effectiveness

Autoclaves used for pathogen kill-loads or clean glassware sterilizing cycles, should be routinely tested once per month for killing effectiveness. Before placing new autoclaves into service, killing effectiveness testing must be completed. The most common method of testing is using commercially available test indicator kits with spore strips (usually *Bacillus stearothermophilus*). The spore strips are placed in the center of a typical load and run through a sterilization cycle. The spore strips are incubated with the non-autoclaved strips. To remove the spore strips from the biohazard bag without exposure to the contents, place the fresh spore strips inside of a glass screw cap tube. Tie a string around the neck of the tube. Bury the tube in the center of the load as you build it. Thread the string out of the top of the bag before you tie it with autoclave tape. After the kill cycle is completed, open the bag and pull on the string to retrieve the spore strip for incubation. If growth is noted on the autoclaved spore strips, try increasing the run time. If growth still occurs with run times of 45 minute or more, the autoclave may need maintenance and repair.

Autoclave performance information:

Autoclaves shall be tested before being placed into service, and then retested periodically for effectiveness.

### **Testing Periodicity or Schedule**

- Every 40 hours of use or monthly (whichever is shorter). Required for autoclaves that are used to inactivate human or non-human primate blood, tissues, clinical samples, or human pathogens.
- Every 6 months. Required for autoclaves that are used to inactivate other material.

### **Method of Testing**

- A commercially available test indicator kit that uses bacterial spores (*Bacillus stearothermophilus*) is the approved method of testing autoclave efficiency. Most spore vial test kits require 56 to 60 ° C incubation of the autoclaved test vial along with a non-autoclaved control vial. Incubation causes surviving spores to grow.

### ***New autoclave***

- Before placing an autoclave into service, a test load approximating the weight and density of the type of waste generated shall be autoclaved with test spore vials. The spore vials should be placed at the bottom, top, front, rear, and center of the autoclave chamber. This can be achieved by either:
  - placing vials at those positions within one large test load, OR
  - making several smaller test packs with 1 vial at the center of each and placing the packs at those locations within the chamber.

The appropriate parameters for sterilization including temperature, pressure, and treatment time shall be determined in this way.

- ***Autoclaves already in use***  
Needs periodic testing, place a spore vial in the very center of a test load prior to autoclaving.

### **Recordkeeping**

The following records regarding autoclave use must be kept:

- On-site maintenance records
- Autoclave use log (Each load of material inactivated shall be logged as follows:)
- Date, time, and operator's name
- Type and approximate amount of waste

- Confirmation of sterilization
- Record the temperature, pressure, and length of time the load is sterilized. Please note that temperature sensitive autoclave tape **is not** sufficient to indicate that the load reached sterilization conditions because the tape will change color at lower temperatures, **OR**
- Save the autoclave print-out, if the autoclave has a working printer.

### **Autoclave Operating Procedures**

A written sterilization procedure shall be in place for each workplace. This shall include the following:

- Parameters
- Appropriate parameters for sterilization shall be determined from the testing with spore vials.
- The time it takes to sterilize a load will change, depending upon the load density and the sterilization cycle one chooses. Therefore, tests should be performed which imitate these various situations.
- Protocol
- Identification of standard treatment containers and proper load placement shall be made.
- Cleaning
- The autoclave and work areas shall be cleaned after every use and the work area shall be disinfected as needed.