

AUTOCLAVE OPERATION AND PERFORMANCE TESTING

(For assistance, please contact EHS at (402) 472-4925, or visit our web site at <http://ehs.unl.edu/>)

Autoclaves are used in many areas 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. Burns can also result from careless handling of vessels containing hot liquids. Explosive breakage of glass vessels during opening and unloading as a result of temperature stresses can lead to mechanical injury, cuts, and burns. Autoclave performance for the purpose of sterilization is dependent on proper use. This SOP provides guidance related to prevention of injuries as well as effective sterilization.

Run Settings

Autoclave manufacturers generally provide several pre-set cycle options.

- Gravity – In this mode, evacuation of air from the autoclave chamber prior to the sterilization portion of the run is accomplished by gravity air purge. A gravity cycle is appropriate for loads where air removal from porous materials or penetration of steam into wrapped or packaged items is not required.
- Vacuum cycle – In this mode, evacuation of air from the autoclave chamber prior to the sterilization portion of the run is accomplished by pulsing between pressure and vacuum. As the number of pulses (prevacs) increases, so does the efficiency of the air removal and subsequent steam penetration. A vacuum mode is suitable for hard goods, with a minimum of 3 prevacs for wrapped or difficult to penetrate hard goods.
- Liquids cycle – This mode is similar to a gravity mode in that air is evacuated from the autoclave chamber by gravity air purge. Pulling a deep vacuum is not conducted since liquids to be autoclaved would be drawn out of their vessels.

Often, the autoclave manufacturer will provide two pre-set cycles for each type of run options presented above (e.g., gravity, vacuum, and liquids). The pre-set cycles for each type of run will vary in the pre-set sterilization temperature, sterilization time, and dry time.

General Precautions

- Read and follow the recommendations made by the manufacturer in the owner's manual. Ensure regular maintenance of autoclaves and ancillary equipment in accordance with the manufacturer's specifications. Autoclaves that generate their own steam (e.g., not fed by central or building steam units) are considered "boilers" under State Law and must have an annual boiler inspection/certification. The

current certificate must be readily available. Facilities Management and Planning coordinates all boiler inspections

- Most autoclaves are equipped to provide a printed tape documenting the conditions of the run. Users should understand that the temperature readings on the tape reflect the chamber temperatures, and may not reflect the temperatures achieved in the material that is autoclaved. The chamber temperature and material temperature will correlate only when the ideal run parameters (i.e., pressure, prevac, etc.) have been established for the load conditions (i.e., load size, load distribution and configuration, depth of the autoclave pan, wetness of the load, etc.). External thermocouple reading devices measuring the conditions at various points in test loads can be used to qualify the performance of the autoclave for particular settings and load conditions/parameters.
- Use only those types of containers, bags, and lids that are designed for autoclaving. Inspect vessels for cracks or chips. Only use unblemished containers.
- Loosen lids on containers of liquids and closures on autoclave bag.
- Do not autoclave flammable liquids.
- Arrange loads to allow free circulation of steam. If items that can hold water are in the load (e.g., trays, pans), place them in a position to allow for condensed steam to drain (i.e., on their sides). Do not stack the load.
- Report all malfunctions to your supervisor and conspicuously tag the equipment as "Out-of-Service."
- Do not stack or store combustible materials next to an autoclave (cardboard, plastic, volatile or flammable liquids).
- ALWAYS use secondary containment when sterilizing or decontaminating. Steel autoclave containers achieve appropriate temperatures quicker than polypropylene containers and as a result are recommended.
- Do not use an autoclave unless you have received specific operation instructions or are working under the direct supervision of an experienced autoclave worker.
- Remember, hazardous waste and ionizing radiation regulations pertain to autoclaved waste as well, so it is imperative to consult with EHS 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). Consult with EHS prior to autoclaving radioactive materials.

Loading the Autoclave

- Plan the load in accordance with the autoclave settings and load configurations that will ensure proper autoclave performance.
- Use a cart to transfer items to the autoclave. To avoid back injuries, push the cart up to the autoclave door and gently slide the load into the autoclave.
- Use the appropriate autoclave setting as described above.
- Firmly lock autoclave doors prior to starting the run to prevent sudden release of high-pressure steam.
- Use an autoclave indicator (e.g., autoclave tape) to demonstrate sterilization of wastes.

Unloading the Autoclave

- After the run is completed, check the pressure gauge. If pressure is not released, do not open the door. Contact the Building Maintenance Reporter for malfunctions; do not use the pressure relief override valve.
- While wearing eye protection and insulated gloves or mitts, carefully open the autoclave using the door to shield your body from the contents of the autoclave. Hot condensate may drip from the door so ensure your feet are protected.
- Use caution when removing liquids. Liquids, especially large volumes, may continue boiling for a time after autoclaving.
- Slide a cart to the opening of the autoclave and pull the autoclave secondary container onto the cart. Place the cart in a low traffic area while additional cooling occurs.

Disposal of Autoclaved Waste

- Liquids that have been autoclaved may be poured down the sink if all chemical components are listed on the sewer disposal list. If the liquid contains chemicals that are not approved for sewer disposal, the vessel must be tagged for pick-up by EHS.
- Non-toxic solids that do not contain any chemical constituents that are regulated under the hazardous waste laws or radioactive material may be disposed in the regular trash following autoclaving and demarcation. Ensure the biohazard symbol has been completely obliterated or secondarily contain the autoclaved bag in a black trash bag. If any free liquids (i.e., condensate) are present, add sufficient absorbent to the bag. Tag autoclaved toxic and/or potentially regulated solids for collection by EHS.

Autoclave Performance Checks

All laboratories designated as biosafety level 2 or higher must participate in the EHS autoclave performance check program. Each month, EHS distributes test kits that contain the following: test organism ampoule (*Geobacillus stearothermophilus*), shipping tube, shipping label, documentation form, and instructions.

1. Open envelope and remove falcon tube, SOP, and documentation form. If the packing material appears wet, contact EHS for a replacement ampoule.
2. Verify that the appropriate PI name is listed on tube.
3. Open falcon tube and remove packing material.
4. Remove ampoule and inspect for cracks. If you will be autoclaving the same day as receipt, hold the ampoule at room temperature until use. If you will be autoclaving a different day, store the ampoule in a refrigerator. When you are ready to autoclave, allow the ampoule to set at room temperature for at least one hour.
5. To perform the test, use autoclave tape to secure the test ampoule to the outside of autoclave bag.
6. Autoclave using the appropriate cycle/settings

7. Allow time for the autoclave to cool down and for pressure to return to atmospheric.
8. Using insulated gloves or mitts, remove autoclave tray and bag from autoclave.
9. Remove test ampoule from side of bag.
10. Return ampoule to Falcon tube and secure with packing material. Record parameters on documentation form (included in test packet) and return form and test ampoule(s) to EHS by the 15th of each month. If assigned to test multiple autoclaves, but are unable to perform multiple tests on the same day, store tested ampoule(s) in a refrigerator and return all ampoules in the same Falcon tube when all tests have been completed
11. Place the return address label on envelope.
12. Place in campus mail.

EHS will incubate test ampoules and check for growth. If the autoclave is functioning properly, no growth will be observed and EHS will ship a new ampoule to the lab at the beginning of the following month. If the ampoule shows signs of growth, the test has failed and EHS will arrange for a confirmatory test. If the second test also fails, place an "Out of Order, Do Not Use!" sign conspicuously on the autoclave and submit repair order.