



## AUTOCLAVE OPERATION AND USE

---

### Background

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 due to temperature stresses can lead to mechanical injury, cuts, and burns. Autoclave performance for sterilization is dependent on proper use. This SOP provides guidance related to the prevention of injuries as well as effective sterilization.

### Autoclave 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 gravity cycles 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.

## Things to Do When Operating an Autoclave

- **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.
- **Record each load run in an Autoclave Log** like the one found at <https://ehs.unl.edu/forms>.
- This is a good practice to keep track of autoclave users and it can also aid in troubleshooting when performance issues arise.
- **Understand the temperature and pressure readings** on the printed autoclave tape reflect the chamber temperatures and may not reflect the temperatures achieved in the material that is autoclaved.
  - Most autoclaves are equipped to provide a printed tape documenting the conditions of the run. 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.).
- Use only those types of containers, bags, and lids that are designed for autoclaving. Inspect vessels for cracks or chips. Only use unblemished containers. **Note: not all biohazard bags are rated for autoclave use. Also, some are rated only to a certain temperature; make sure the bags you use are compatible with your waste cycle.**
- Autoclave sharps (e.g., razors, slides, scalpels, needles, etc.) **only** when contained within a rigid, leak-proof, puncture-resistant container.
- Place soiled glassware and lab ware in secondary containers and autoclave them in the solids cycle.
- Loosen lids on containers of liquids and closures on autoclave bags.
- 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 such a way to allow for condensed steam to drain (i.e., on their sides).
- Report all malfunctions to your supervisor and clearly tag the equipment as "**Out-of-Service.**" Wastes that were in a failed run must be re-autoclaved before disposal.
- **ALWAYS use secondary containment.** Steel autoclave containers achieve appropriate temperatures quicker than polypropylene containers and thus are recommended. If you use plastic containers, ensure that they are certified for use at the temperatures that will be achieved in the autoclave run.
- **Use chemical indicator tape or a chemical integrator strip with every load; it is very important to use lead-free tape to avoid potential implications under the hazardous waste regulations.** Consult EHS if you use an autoclave tape containing lead or if you are unsure if your autoclave tape contains lead.
- **Periodically conduct verification testing with a biological indicator to ensure the autoclave is achieving sufficient temperature and pressure to sterilize the load in the**

**autoclave.** Guidance for autoclave testing and recommended frequency can be found in the EHS SOP, **Autoclave Performance Testing**.

### Things NOT to do when Operating an Autoclave

- **Do not** autoclave flammable or corrosive liquids. Never autoclave bleach solutions; this can damage the plumbing of the autoclave.
- **Do not** place loose sharps (e.g., glass pipettes, broken glass, razors, slides, etc.) in autoclave bags.
- **Do not** stack items and “stuff” the chamber. There must be sufficient clearance on all sides of the load to allow steam to circulate.
- **Do not** stack or store combustible materials next to an autoclave (cardboard, plastic, volatile or flammable liquids).
- **Do not** autoclave materials containing hazardous wastes or radioactive materials.
  - Hazardous waste and ionizing radiation regulations pertain to autoclaved waste as well, so it is imperative to consult with EHS if your run contains 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.



**DO NOT use an autoclave unless you have received specific operation instructions or are working under the direct supervision of an experienced autoclave worker.**

### Loading the Autoclave

- Plan the load in accordance with the autoclave settings and load configurations that will ensure proper autoclave performance.
- Use a chemical indicator (e.g., autoclave tape) in every load to demonstrate that the chamber temperature reached 121°C.
- Use a cart to transfer items to the autoclave. To avoid overexertion injuries, push the cart up to the autoclave door and gently slide the load into the autoclave.
- Prior to loading the autoclave, visually inspect the drain strainer to ensure it is clean.
- Use the appropriate autoclave setting as described above.
- Firmly lock autoclave doors prior to starting the run to prevent sudden release of steam.

## 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.

## Autoclaving for Decontamination of Biohazardous Waste

Biohazardous waste includes microorganisms, cells, tissues, body fluids, animal carcasses and animal waste as well as recombinant or synthetic nucleic acid-containing materials.

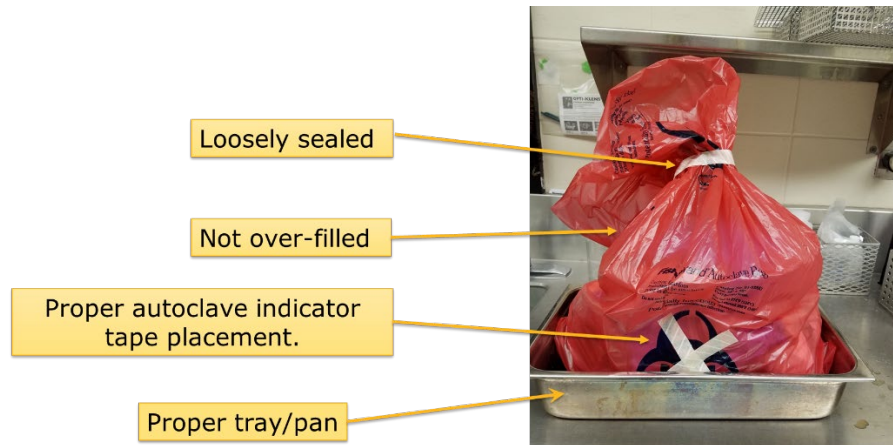
### Staging waste

- Inspect glassware for cracks.
- Ensure corrosive, flammable, combustible, toxic or radioactive materials are **NOT PRESENT** in the load.
- Ensure there are no uncontained sharps, glass, or sealed liquid containers (e.g., culture flasks) in the load.
- Place autoclave tape in an “X” pattern over the biohazard symbol on waste bags or sharps containers (Figure 1).



**Figure 1** Autoclave tape utilizes a chemical reaction to indicate a temperature of greater than 121°C was achieved. This color change is usually displayed with hash marks or words that appear on the tape when the proper temperature is reached.

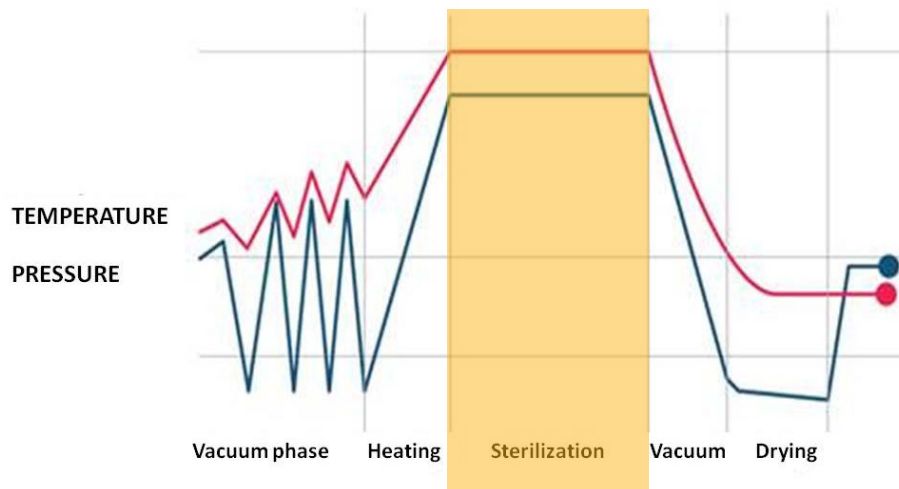
- Properly staged biohazardous waste bags should be loosely sealed, not filled over ¾ full, have autoclave tape placed in an “X” pattern on the outside of the bag (covering the biohazard symbol if one is printed on the bag), and placed in a heat-resistant tray or pan.



Biohazardous waste waiting to be autoclaved **must** be staged in the laboratory where it was generated. **DO NOT** place un-autoclaved waste in an autoclave room if the autoclave is in use.

### Waste Cycle Parameters

Recommended cycle settings for biohazardous waste are at least 121°C and 15-17 psi for at least 30 minutes sterilization time. Keep in mind that the sterilization time is not the full cycle length; the entire run will include additional time to reach temperature and then cooling/drying time.



Temperature	Sterilization Time (min)	Pre-Vac Pulses (psi – in/Hg)	Dry/Cool Time (min)
≥ 250°F (121°C)	30-90	15-17 psi, 30-35 in/Hg (0-6 pulses)	0-30

Autoclave waste cycles must meet certain requirements to ensure that the cycle is effective in decontaminating the biohazards in the material being treated. The effective sterilization time for biohazardous waste can vary from 30 minutes to 90 minutes at 121°C. Waste loads with spore-forming microbes, biological toxins, prions or materials with greater density (e.g., carcasses or soil) usually require longer sterilization times and/or higher temperatures (up to 132°C) to achieve effective decontamination and inactivation.



**IMPORTANT:** Be cautious when using higher temperatures as some materials are not compatible with this elevated temperature and will melt or be damaged. Verify the temperature rating of the autoclave waste bags you are using to ensure compatibility with the cycle parameters.

Generally, autoclave waste cycles for solid waste are of the pre-vacuum pulse/post-vacuum type as illustrated in the graph above. This ensures that steam reaches all parts of the load.

Autoclave cycles for typical mixed solid biohazardous waste should achieve a temperature of at least **121°C (250°F) and pressure of 15-17psi for a minimum of 30 minutes** during the “Sterilization” phase of the cycle.



Contact EHS for guidance on cycle parameters if you need to deviate from the typical cycle parameters indicated above or are unsure if autoclaving will be effective for the material you want to sterilize.

Even if the autoclave you use does not have a dedicated “waste” cycle, you **must** make sure the cycle you do use for biohazardous waste is within the ranges discussed above.

If you are unsure if the autoclave cycle you use meets these requirements, most autoclaves allow you to print the cycle parameters out via the autoclave tape or you can reference the operator’s manual for instructions on how to verify the cycle parameters.

## Autoclaving Contaminated Plant Material and Soil

Autoclaving plant material and soil requires special consideration to the parameters used as the organic nature of the materials can sometimes affect the effectiveness of the sterilization cycle. Soil is often autoclaved prior to use to ensure that it is sterile, and no undesired pathogens or pests are present. The parameters discussed below are specifically for treatment of plant and soil materials that contain recombinant or synthetic nucleic acid molecules, pathogens or pests, or plants and soil that may be contaminated by these materials.

- **Recombinant nucleic acid-containing plant material**
  - Autoclave cycle parameters should include a sterilization time of 60 minutes or more at 121°C and 15-17psi.



- Do not overfill bags.
- **Contaminated soil (pathogens and/or genetically modified plants)**
  - Soil is much denser than mixed soil and plant material so containers must be filled much less than their capacity.
  - EHS has determined through a validation study that the following parameters result in successful decontamination of soil.
    - A maximum depth of 9" in a trash can or similar container.
    - 121°C and 16 psi for 180 minutes or two (2) 90-minute cycles.
  - Soil autoclaved in plastic or metal trays and less than 6" deep, may only require a single cycle of 90 minutes at 121°C to achieve sterilization.
  - Smaller quantities of contaminated soil may, by extrapolation, require shorter autoclave times.
  - Biological indicators should be used to verify effective sterilization of these materials. Indicators should be placed in the middle of the load and submerged within the soil at least to half the total depth. Attaching a string to the vial can facilitate easy retrieval after the run.
- Deviation from these parameters should be done in consultation with Environmental Health and Safety (EHS) to ensure that effective decontamination/sterilization is achieved, particularly for waste materials.

## Disposal of Autoclaved Waste

- Liquids that have been autoclaved may be poured down the sink if all chemical components are listed on the EHS 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 (including lead-free autoclave tape) that do not contain any chemical constituents regulated under the hazardous waste laws or radioactive material may be disposed in the regular trash following autoclaving and demarcation.
  - Seal the bag by taping or tying the bag shut.
  - Securely seal sharps container lids with tape.
  - Place the autoclaved waste bag in a black (opaque) trash bag. (Figure 2)
  - If any free liquids (i.e., condensate) are present, add sufficient absorbent to the bag.
- Tag autoclaved toxic and/or potentially regulated solids (including autoclave tape containing lead) for collection by EHS.



**Figure 2** Proper autoclaved waste disposal



**IMPORTANT: Autoclave users are responsible to provide black/dark trash bags/liners for final disposal of autoclaved waste. Custodial services does not provide these bags.**

## **Routine Maintenance**

The best way to ensure effective autoclave operation is regular maintenance. Consult the owner's manual for autoclave-specific maintenance recommendations.

## **Malfunction**

Malfunctioning autoclaves should be taken out of service until repairs can be made. A sign should be placed on the autoclave indicating that it is out of service. The sign should include contact information for the person coordinating repairs and list an alternate autoclave to use, if one is available, while repairs are completed.

If an autoclave malfunctions with a biohazardous waste bag inside, the load must be autoclaved again in a functional autoclave prior to disposal. If there is another autoclave available, then take the load to the other autoclave and run the waste cycle on that autoclave. If an autoclave is not available, then hold the load/waste until the autoclave is repaired and re-run the load or contact EHS for assistance with disposal of the waste.

After repairs are completed, evaluate performance through verification testing performed by the service provider or by including a biological indicator test ampoule in a test run prior to resuming normal use operations. The result of the biological indicator test should indicate no growth after exposure to the cycle parameters.