ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Revised 9/25)

AUTOCLAVE OPERATION AND USE

Purpose

This SOP provides guidance related to effective use of an autoclave for material sterilization and prevention of injuries during autoclave use.

Autoclaves are used to sterilize materials, including biohazardous waste, by using steam under pressure for a given amount of time. The moisture in the steam efficiently transfers heat to the items to destroy the protein structures of microorganisms and cells. Autoclave performance for sterilization is dependent on proper use.

Each group using the autoclave will need to identify what they will be autoclaving, the volume to be autoclaved and identify the proper sterilization temperature and time to penetrate all the material/ waste to sterilize. Make sure to adjust the sterilization time of the run and/ or temperature of the run as needed for the mass/ density to penetrate the complete load (all the way to the center of the waste bag). Larger loads will take longer to heat up and cool down than smaller loads.

Autoclave manufacturers generally provide several pre-set cycle options, and these preset options are typically for sterilizing instruments or liquid materials use. You will want to adjust or add cycles specific for your biohazardous waste load needs. This is essential for public health and environmental protection.

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 stress can lead to severe cuts and burns.

Autoclave Run Settings

Commonly recommended temperatures for steam sterilization are 121ºC (250ºF), 130-132ºC (266-270ºF), or 134ºC (273ºF). The exposure time is the time necessary to sterilize the material in the load and does NOT include the entire cycle run time. The exposure phase is dependent on the size, shape, weight, density and material composition to be sterilized.

- **Gravity Displacement Cycles (121-134ºC)**

  In this mode, evacuation of air from the autoclave chamber prior to the sterilization portion of the run is accomplished by gravity air purge. Gravity cycles are primarily used to process glassware, biohazardous waste, unwrapped metal equipment and nonporous surgical equipment whose surfaces have direct steam contact. The penetration time is longer because of incomplete air elimination. This is the case when autoclave bags are used. Biological waste requires at least 60 minutes (for 10lbs of waste) at 121ºC because the entrapped air remaining in the load of waste impedes steam permeation and heating efficiency.

- **Pre-Vacuum Cycles (121-134ºC)**

  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. The advantage of using a vacuum pump is that there is nearly instantaneous steam penetration. A vacuum mode is suitable for non-liquid material, biohazardous waste loads, animal bedding and caging, packs and porous material, with a minimum of 3 prevacs for wrapped or autoclave bagged waste.

- **Liquid Cycles (121-125ºC)**

  This mode operates on a modified gravity cycle, tailored to control the cooling phase and extend the duration of the heating phase so that the liquid stays in the containers and does not boil out. The chamber pressure is released slowly, controlling the exhaust rate. Liquid cycles are suitable for media, agar, water, and other liquid solutions that you want to keep.

Guidelines for Cycle Types:

Cycle Type	Application/Load	Temperature	Sterilization Time (Minutes)	Pre-Vac Pulses psi – in/Hg	Dry Time (minutes)
Gravity	Glassware, unwrapped goods, waste, red bags	250°F (121°C)	30-90 [60 for normal waste load]	None	0-30
Pre-vacuum and/or post-vacuum	Wrapped goods, packs, animal bedding, cages, waste, red bags, porous materials	250°F (121°C)	20-90 [60 for normal waste load]	18 psi, 20in/Hg 0-6 pulses	0-30
Liquids	Media, LB broth, water	250°F (121°C)	20-90	–	0.5 psi/minute

https://consteril.com/resources/sterilization-cycles/

Things to Do When Operating an Autoclave

- **Be trained to operate the autoclave**
- **Wear appropriate attire to protect yourself from heat and steam:** Lab coat (long sleeves), pants, close-toed shoes, eye protection, long autoclave/ heat resistant gloves
- **Read and follow the recommendations made by the manufacturer** in the owner's manual.
- **Ensure regular preventative maintenance** of autoclaves and ancillary equipment in accordance with the manufacturer's specifications (typically annual).
- **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 containers, bags, and lids that are designed for autoclave temperatures and pressures**.
    - 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 and temperature (121ºC, 130ºC, 131ºC, 134ºC).
- **Use chemical indicator tape or a chemical integrator strip with every load**
    - Chemical indicators are used to monitor the presence or attainment of one or more of the parameters required for a satisfactory sterilization process and are used for quality control.
    - If indicator tape is used, 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**.
    - Autoclave temperature and length of time need to be increased depending on the mass of the load, the liquid volume and/ or the temperature of the material placed in the autoclave run (room temperature or refrigerator temperature, size of carcasses, size of autoclave bags, liquid volume and mass, etc.)

  - Using a biological indicator and placing it in the middle of your load (under your autoclave waste or in a mockup waste bag) can help to establish load conditions (i.e., load size, load distribution and configuration, depth of the autoclave pan, wetness of the load, etc.).

- **Wait for the autoclave to reach the correct temperature and pressure** before leaving it.

- **Record each load run in an Autoclave Log** like the one found at https://ehs.unl.edu/forms.
  - Keeps track of decontamination of biohazardous waste before disposed in the environment
  - Aids in troubleshooting when performance issues arise

- **Understand the temperature and pressure readings**. The temperature reading reflects the chamber temperatures and **not the temperatures achieved in the material that is autoclaved.**

- **Inspect vessels**
  - Check for cracks or chips and only autoclave and use unblemished containers.
  - Loosen lids on containers of liquids and use a liquid cycle.
  - As needed, loosen or empty lids on containers of liquid waste and place them in an autoclavable pipet boat/sterilization pan, then place into an autoclave bag, then into the secondary autoclave proof bins/containers. Autoclave them using the gravity or liquid cycle.
  - Make sure sharps (e.g., razors, slides, scalpels, needles, etc.) are contained within a rigid, leak-proof, puncture-resistant container before autoclaving.

- **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** of the autoclave 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.

Things NOT to do when Operating an Autoclave

- Do not leave materials in the autoclave overnight.
- Do not place sharps (e.g., glass pipettes, broken glass, razors, slides, etc.) directly into autoclave bags.
- Do not overfill/ overload the autoclave bags/pans/bins/rack(s)/autoclave.
    - There must be sufficient clearance on all sides of the load to allow steam to circulate.
- Do not stack one biohazard bag on top of another biohazard bag.
- Plastic and glass containers cannot be sealed airtight. The lids must be opened loosely to prevent explosions in the autoclave.
- Do not stack or store combustible materials next to an autoclave (cardboard, plastic, volatile or flammable liquids).
- Do not autoclave materials containing chemical hazardous waste or radioactive materials.
    - Chemical 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 autoclave flammable or corrosive liquids.
    - Bleach solutions can pit and damage the autoclave. Do not autoclave bleach solutions.

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

Loading the Autoclave

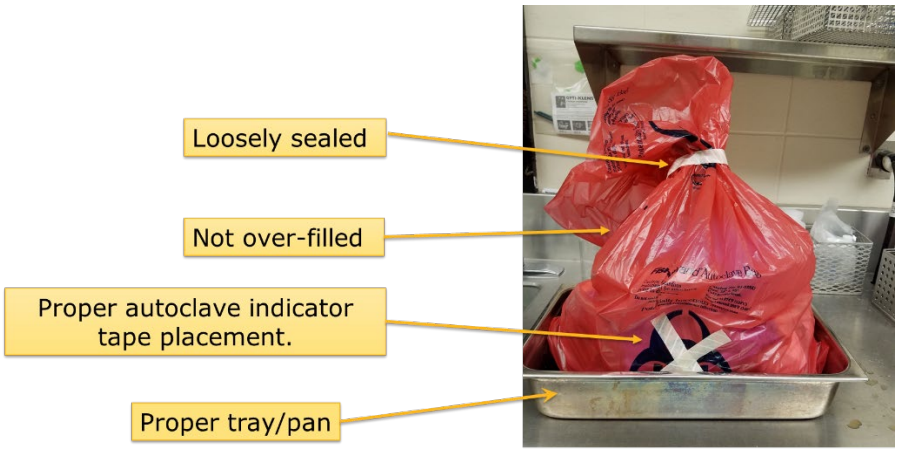
- Plan the load in accordance with the autoclave settings and load configurations that will ensure proper autoclave performance and steam sterilization penetration.
- Use a chemical indicator (e.g., autoclave tape; chemical indicator strip) in every biohazardous waste load to demonstrate that the chamber temperature reached the proper temperature (at least 121°C) and time (at least 60 minutes) for sterilization of the material in the autoclave chamber.
- Prior to loading the autoclave, visually inspect the drain strainer and clean it if there is debris.
- Use a cart to transfer items to and from the autoclave. Push the cart up to the autoclave door and gently slide the load into the autoclave.
- Use the appropriate autoclave settings for temperature, sterilization time and pressure (see above).
- Firmly lock autoclave doors prior to starting the run to prevent sudden release of steam.
- Remain in the autoclave room until the autoclave reaches the correct temperature and pressure.

Autoclaving Biohazardous Waste

To protect public health and the environment from biological material used in the lab, it is important to **sterilize** the biological waste before it is discarded and disposed of in the waste stream. This process is achieved by using the autoclave before biohazardous waste disposal.

Biohazardous waste includes but is not limited to: microorganisms in liquid culture, cells, tissues, body fluids, recombinant or synthetic nucleic acid-containing molecules and materials, animal waste such as carcasses, bedding, caging, bottles, husbandry, plant material, soil and other potentially infectious material that came into direct contact with the above.

- Biohazardous waste bags should be loosely sealed with autoclave tape or a twist tie, not filled over ¾ full, have autoclave tape placed in an “X” pattern on the outside of the bag (covering the biohazard symbol), and placed in a heat-resistant autoclave tray or pan (See figure below)
- Sharps containers should be taped closed with autoclave tape, not filled past the fill line and have autoclave tape placed in an “X” pattern on the outside of the bag (covering the biohazard symbol) and placed in a heat-resistant autoclave tray or pan (See figures below).

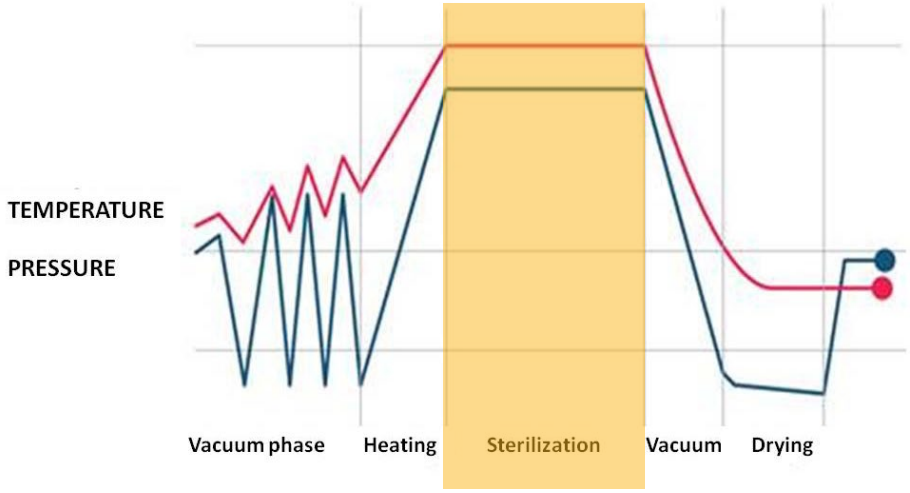


STOP

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 already in use.

Biohazardous Waste Cycle Parameters

Recommended cycle settings **for biohazardous waste** are 121°C- 130°C and 17-28 psi for at least **60** 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)	60-90	17-29 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 60 minutes to 90 minutes at 121°C. Waste loads with higher volume of material, spore-forming microbes, biological toxins, prions or materials with greater density (e.g., plant material, carcasses, soil) usually require longer sterilization times (90- 120 minutes) and/or higher temperatures (130- 134°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 material are of the pre-vacuum pulse/post-vacuum type. This ensures that steam reaches all parts of the loaded material.

Autoclave cycles for typical mixed biohazardous waste should achieve a temperature of at least **121°C (250°F) and pressure of 15-17psi for a minimum of 60 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 same needs to be performed AFTER the soil/plant material is used with a plant pathogen, pest or genetically modified material for the same reason. The parameters discussed below are specifically for the 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.

- **Recombinant nucleic acid-containing plant material**

  - Autoclave cycle parameters should include a sterilization time of 60- 90 minutes or more at 121°C and 15-17psi. You may also be able to increase the temperature to 130°C and use a sterilization time of 60 minutes.
  - 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 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.

Autoclaving Contaminated Animal Waste

Autoclaving animal waste and carcass material requires special consideration to the parameters used as the organic nature of the materials can sometimes affect the effectiveness of the sterilization cycle and cleanliness of the autoclave. The parameters discussed below are specifically for the treatment of animal caging and carcass materials that may be contaminated.

NOTE: Autoclave temperature and length of time need to be increased depending on the load mass/ volume and temperature (room temperature or refrigerator temperature, etc.) of the load.

- Animal carcasses and other animal material, like eggs, need to be contained in two biohazard bags (double bagged) to prevent liquid leakage/mess and need to be completely thawed (at least 48 hours) and need to be at (or close to) room temperature before they are autoclaved.

- Animal carcasses/ animal material needs to lie flat and be a single layer in the biohazard bag.
- For eggs: euthanize embryos, then place the egg(s), if frozen, in the refrigerator for at least 48hrs before staging the eggs for autoclaving to get to room temperature before autoclaving. The eggs need to be at room temperature before they are autoclaved.
- After unloading the autoclave, the bags containing the carcasses are placed into another autoclave bag, placed in secondary containment and transported via a cart to the incinerator, where they are incinerated to provide an extra level of confidence for inactivation.

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 Service Desk for malfunctions; do not use the pressure relief override valve.
- While wearing eye protection, lab coat and insulated gloves or mitts, carefully open the autoclave using the door to shield your body from the steam and contents of the autoclave. Hot condensates 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.
- Document the autoclave run in the autoclave log, including that the chemical indicator passed for biohazardous waste autoclave runs.
    - Record the biological indicator results once the results are read, as applicable.

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 hazardous waste team.
- 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 of 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 if you haven't already done so.
- Place the autoclaved waste in a black (opaque) trash bag (Figure 2).
- If any free liquids are present, add sufficient absorbent to the or drain in the sink.

Figure 2 bag

- Tag autoclaved toxic and/or potentially regulated solids (including autoclave tape containing lead) for collection by EHS.

IMPORTANT: Autoclave users are responsible for providing chemical indicators, biological indicators and black/dark trash bags/liners for final disposal of autoclaved waste. Custodial services do not provide these.

Routine Maintenance

The best way to ensure effective autoclave operation is regular annual 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
- 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 in your lab until the autoclave is repaired and re-run the load or contact EHS for assistance with proper 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 mockup load to the cycle parameters.