Onsite Biohazardous Waste Management:
Autoclaving

REFERENCE GUIDE
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Environmental Health & Safety Office (EH&S)

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Biological Waste / Biohazardous Waste

Biological Waste
Includes any material that once contained or now contains living organisms, or that is a product, portion, or waste of a living or once-living organism.

Biological waste that is infectious to humans (biohazardous waste) must be managed and disposed of as medical waste.
Biohazardous Waste

• Infectious biological agents:
  Bacterial
  Fungal
  Parasitic
  Rickettsial
  Viral Agents
  Prions
  and/or toxins

• Also includes human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown (hepatitis B virus (HBV), human immunodeficiency virus (HIV), etc.)
Biohazardous Waste Management

- Biohazardous waste must be managed in compliance with state and local regulations. One way to sterilize potentially infectious materials is autoclaving before waste is disposed.
- Biohazardous materials must be labeled as such and must be sterilized by the end of each workday, or must be secured appropriately.
- Biohazardous materials should not be left in an autoclave overnight in anticipation of autoclaving the next day.
Biohazardous Waste Can Be Treated Onsite Using Autoclaves

- Autoclaving provides an economical process of killing pathogenic microorganisms through saturation with moist heat (steam) under pressure.
- This process makes the microorganisms nonviable by destroying (denaturing) essential proteins and structures.
Effective Autoclave Process

• For an autoclave process to be effective in achieving sterilization, sufficient temperature, time and direct steam contact are essential.

• Air must be completely removed from the sterilizer chamber and the materials placed within to allow steam penetration.

• The user must also ensure that the materials being autoclaved will be at the necessary temperature for the sufficient amount of time to achieve sterilization.

• Factors that affect sterilization efficiency include type and quantity of material to be autoclaved, packaging, load density and configuration, and container type, size, and shape.
Autoclave Operation

• The most common steam sterilizer found in laboratories is the gravity displacement autoclave.

• Saturated steam enters the top of the chamber by a steam pressure control valve. As the steam enters, it pushes the air out through a trap in the drain line.

• Once all the air is evacuated, the trap closes. Steam continues to fill the autoclave chamber until a preset temperature and pressure is reached.

• The sterilization temperature has to be reached in all parts of the autoclave and its load, and must be maintained for the specified period of time. Once the cycle is complete, the pressure is vented and the steam is allowed to cool.
Autoclave Operation
Automatic Autoclaves Operate in Three Basic Autoclave Cycles:

1. **Gravity or "Fast Exhaust" Cycle**—Used to sterilize dry goods, glassware, etc. This cycle charges the chamber with steam and holds it at a set temperature for a set period of time. At the end of the cycle a valve opens and the chamber rapidly returns to atmospheric pressure.

2. **Liquid or "Slow Exhaust" Cycle**—Used to prevent sterilized liquids from boiling. Steam is exhausted slowly at the end of the cycle, allowing the liquids (which will be super-heated) to cool.

3. **Pre-Vacuum Cycle**—Used for porous materials. This cycle partially evacuates the chamber prior to introducing steam for greater steam penetration. Pre-vacuum cycles are not available on all machines.
Autoclave Cycle to Treat Biowaste:

Liquid or "Slow Exhaust" Cycle

• Biowaste in autoclavable biohazard bags
  – Any of the following contaminated with biohazardous materials:
    plastic pipettes, pipette tips, syringes without needles, Petri dishes with media and
    microbial growth, used culture flasks, paper towels, bench paper, biohazardous animal
    bedding, and gloves.

• Liquid biowaste (use with type I borosilicate glass containers with vented closures; 2/3 full only)
  – Liquid biohazardous cultures or specimens, including human and animal blood, body fluids, tissue culture, infectious microorganisms, and viruses.
Autoclave Safety

- Due to the high heat and pressure created in an autoclave during operation, proper maintenance, 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, from steam venting from the unit, or 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**.

- Operator needs to know what to do in an emergency. E.g.:
  
  1) How to override a non-responsive control system
  2) What to do if the autoclave will not release pressure
Autoclave explosions!
Burn Emergency

• If you are burned, you should seek medical treatment as soon as possible. Burns to the face / eyes, third-degree burns, or burns over large areas of the body should be treated as emergencies.

• Dial UT Arlington Police Dispatch at 817-272-3003 to get medical assistance!

• Minor burns can be treated by using first aid procedures:
  • immerse the burn in cool water immediately
  • remove clothing from the burn area and keep the injured area covered with a non-adhesive, sterile gauze
  • the burn site can be rubbed with lotion when the skin has cooled
  • regardless of the degree of severity, report the burn to your lab supervisor or Principal Investigator
Autoclave Maintenance

• Autoclave maintenance is an important aspect of a properly functioning autoclave. These units are serviced by a qualified contractor.

• Notify the appropriate autoclave trainer/technician if there are any problems/mechanical failure with the unit.

• Discontinue use immediately if an autoclave is not working properly. Also post an 'Out of Service'' note on the unit if you find a problem alerting others not to use the autoclave.
Autoclave Maintenance

Report malfunctioning of an autoclave!
• When maintenance work or repairs are needed, the user must provide a safe work environment for the service technician.

  – remove all items from the sterilizer chamber
  – clean any spills or leaks inside the chamber
  – remove untreated biohazardous materials from the vicinity
Preventative Maintenance

In order to maintain the autoclave’s effectiveness:

• The plug screen/drainer should be removed with heat-resistant gloves, checked, and cleaned frequently to ensure that it is free of dirt or sediment which may collect and cause a clog. Look out for any sharps that may have become loose and gotten caught in the plug screen/drainer.
Preventative Maintenance

• Surfaces:
  – The interior surfaces should be cleaned (according to manufacturer’s specifications) of any residues that collect over time.
Preventative Maintenance

• Surfaces:
  – On a regular basis, gaskets, doors, shelves and walls should be visually inspected for residue buildup and wear.

Melted plastic on an autoclave shelf from an un-autoclavable container
Gasket Around Door
Gasket Around Autoclave Jacket
Prevent Injuries When Using The Autoclave by Observing These Precautions:

• **Do not** exceed the manufacturer’s recommended pressures and temperatures.

• **Do not** use an autoclave unless you have received specific operation instructions or are working under the direct supervision of an experienced autoclave user.

• **Read** and **follow** the recommendations made by the manufacturer in the owner’s manual before use.

• **Do not** stack or store combustible materials such as cardboard or plastic containers, or flammable liquids next to the autoclave.

• **Use** Personal Protective Equipment (PPE)!
PPE When Autoclaving:

Eye / Face protection
Goggles or safety glasses with side shields (a full face shield is a good idea)

Clothing
A rubber apron and a lab coat (long pants only, no open toed shoes)

Gloves
Always wear heat resistant gloves

The correct PPE is VERY important!
Autoclaving Biohazardous Waste - Precautions

- Use bags or other containers properly labeled with word “Biohazard” and biohazard sign to gather items that contain or may be contaminated with potentially infectious agents.
- Used bags for disposal must be heat resistant (autoclavable) biohazard bags (usually red or orange).
- Biohazardous waste should be stored in rigid, leak proof, closable containers before it is autoclaved!

- Biohazard sign:
Autoclaving Biohazardous Waste - Precautions

• Autoclave rooms are BSL-2 rooms! Biohazardous waste should be stored inside these rooms and not in the hallways!

• NO FOOD / DRINKS or cosmetics are allowed in the room since biohazard(s) present!
How to Dispose of Biohazardous Liquids?

• Liquid infectious waste may be autoclaved or chemically disinfected and then disposed via the laboratory drainage system.

• Do not pour melted agar into sink or floor drains. Allow it to cool and solidify for disposal as a solid waste.
Disposal of Bleached Liquid

• Add bleach to liquid to create 10% solution
  - Allow to set 24 hrs or overnight
• Dispose of contents via sink and flush with water
  WEAR PPE!
Biological Waste Box (Stericycle Box)

- Biohazardous materials not autoclaved (that either cannot be autoclaved or when the laboratory chooses not to autoclave themselves) must be placed in a Stericycle waste box lined with a red biohazard bag.
Biological Waste Box
(Stericycle Box) Contents

**Solids Only in the Box:**
- Pipette tips
- Paper towels
- Solidified liquids
- Petri dishes
- Plastic ware
- Non-sharps
- Gloves

**Not Allowed in the Box:**
- Liquids, Chemicals,
- Radioactive Material,
- Trash
- Sharps
PROPER DISPOSAL OF PIPETTE TIPS

- Collect ALL pipette tips at the point of generation in 8 ½ x 11 biohazard bags
- When ¾ full, place biohazard bags in the Stericycle box for offsite disposal
- Do not place any pipette tips in the trash
Decontamination - Definition

• Decontamination is a process of making an item free of living microorganisms.

• Autoclaving is a physical means to inactivate/destroy pathogenic microorganisms in/on an item so that they are no longer capable of transmitting infectious particles, and the item is rendered safe for handling/disposal.
Autoclaving Biohazardous Waste - Preparations

• Do not double bag waste or tightly seal containers (loosen the caps of screw-topped vessels and bag closures), as this will impede steam penetration
• Avoid compressing waste bags since this may create aerosols!
• Do not put sharp objects such as broken glassware into an autoclave bag
• It is advisable to add some water (50-100 ml = 1/4-1/2 cup) to bags of solid wastes (the water will vaporize into steam that will drive out residual air once sterilization temperature has been reached inside the bag)
• Do not mix loads of liquids with solids
• To prevent the bottoms of bottles from breaking, place them in a tub with 1 to 2 inches of water
Autoclaving Biohazardous Waste - Preparations

• **Steam Chemical Integrators:**

• 3M™ Comply™ SteriGage™ Steam Chemical Integrators must be used inside each load to be autoclaved to monitor time, temperature, and steam exposure conditions and can provide the necessary sterilization assurance needed when decontaminating biohazardous waste.

• Use autoclave tape to attach the steam chemical integrator to the inside of autoclave bag.
Autoclaving Biohazardous Waste - Preparations

- Before autoclaving waste bags with “biohazard” symbols on them, label these bags with commercially available autoclave temperature tape that changes appearance, e.g., visible black stripes appear upon adequate thermal treatment. Autoclave temperature tape only tells that desired temperature was reached not time or pressure. **Apply this tape across the “biohazard” symbols on the bag before autoclaving!**
- Do not throw away any biohazard bags without covering all biohazard symbols
Autoclaving Biohazardous Waste - Preparations

• Place containers of liquid, bags of agar plates, or other items that may boil over or leak inside secondary containment in the autoclave. Plastic or stainless steel containers/ pans are appropriate secondary containers. Never place autoclave bags or glassware in direct contact with the bottom of the autoclave!
Autoclaving Biohazardous Waste - Preparations

- Make sure plastic bags and pans are autoclavable. Not all plastics can be autoclaved. Polypropylene and polycarbonate will survive, but polyethylene and high density polyethylene will not. The different types of plastic can be identified by looking for initials imprinted on the bottom of containers (PP = polypropylene, PC = polycarbonate, PE = polyethylene, HDPE = high density polyethylene).
Autoclaving Biohazardous Waste - Preparations

• Do not autoclave items containing:

• corrosives (e.g., acids, bases, phenol)
• solvents or volatiles (e.g., ethanol, methanol, chloroform)
• radioactive materials.
Autoclaving Biohazardous Waste - Preparations

When getting ready to load the autoclave:

- Before opening the door make sure that the pressure in the autoclave chamber is near zero. This will prevent possible steam burns and shattered glassware!

- Always stand behind the autoclave door to shield you from steam that may escape the chamber. Slowly open the autoclave door and allow the steam to escape gradually. Allow all steam to escape before looking directly into chamber.

- Make sure the chamber is empty, there is direct access to it, and that the plug screen in the bottom of the autoclave is clean.
Autoclaving Biohazardous Waste

- Wearing heat-resistant gloves, place tub(s) with materials to be autoclaved into chamber. The walls of the autoclave chamber are very hot. Avoid contact. Do not wear gloves that are wet.
- Do not stack containers in the autoclave nor overload the autoclave! Leave sufficient room for thorough steam circulation!
- Make sure that the secondary tubs fit into autoclave chamber without obstructing the door.
- Close door and turn wheel until the door is locked. Most autoclaves have a safety interlocking system, which prevents the instrument from working if the door is not properly closed; however, some older models may not have this built in safety mechanism. If not, special precautions should be observed to insure that the door is properly sealed before the process begins. If steam is leaking around the door during the sterilization process, the door has not been sealed properly. In this event, shut down the system as safely as possible. Let the unit cool, and reset the door. Give special attention to making sure that the door is sealed tight, and restart the run. If the problem persists, the unit needs to be serviced by a qualified technician.
Autoclaving Biohazardous Waste

• For automatic autoclaves, choose the appropriate program for your particular load (liquid, dry goods, etc.).
• Start autoclave cycle.
• Observe initial autoclave operation.
• If anything appears unusual (e.g., water leaking past gasket of door) or if the autoclave fails to complete the cycle, report this to your lab supervisor or Principal Investigator.
Decontamination Procedure

• Process the load according to established procedures (refer to [SOP for Steam Autoclaves](#)): Autoclave items contained in autoclavable bags for 50 to 60 minutes, at temperature and pressure of 121°C (250°F) and 15 pound-force per square inch (psi).

• Some biohazardous waste may be required to be autoclaved for a different amount of time.
After Decontamination Procedure

- Always make sure that the autoclave cycle has been completed before attempting to open the autoclave door.

- Check that pressure in the chamber has reached normal atmospheric pressure.
After Decontamination Procedure

- Decide on a place to safely put autoclaved load before removing anything from the autoclave. There should not be anything that will obstruct removal and placement of the autoclaved load.
- Retract the door gasket (if necessary) on the autoclave.
- Opening the door may be done manually or automatically through the control panel, depending on the autoclave.
After Decontamination Procedure

• Always stand behind door, using door as a shield. Open the door partially. It should open easily, **do not force door open.**

• While standing behind autoclave door, allow steam to escape from autoclave chamber. Never look into the chamber immediately after opening door.

• When all the steam has escaped, open door completely.
After Decontamination Procedure

• From a safe distance, observe the autoclave load. Do not remove anything from the autoclave that is still boiling. Wait until the load has cooled further.
• Be very careful of liquids, molten agar, etc. to avoid getting splashed with scalding liquid.
• Do not agitate containers of super-heated liquid or remove caps before unloading.
• If the heat resistant gloves become soaked with very hot water, scalding of the skin can result.
After Decontamination Procedure

• Wearing heat-resistant gloves, remove secondary tub from autoclave chamber. Avoid contact with walls of autoclave chamber.

• Unload hot items onto a cart for transport.

• After autoclaving, waste can be disposed of as normal solid waste after it has been properly labelled and bagged.

• Close autoclave door to allow safe passage for others.
Handling of Autoclaved Waste

• Bags should be left to cool for several minutes before removing from autoclave
• When cycle is finished, inspect autoclave temperature tape, and visually check bags to ensure autoclave was working correctly
• After processing remove the integrator from the autoclave bag and interpret the results (see next two slides).

• Attach a label to autoclaved waste bag that states: “Treated in accordance with the provisions of 25 TAC § 1.136(a)”
Steam Chemical Integrators

Instructions how to interpret the results of Steam Chemical Integrators:

• After processing, the dark color should have entered anywhere into the ACCEPT area window of the 3M™ Comply™ SteriGage™ Steam Chemical Integrator. This means that all the critical parameters of steam sterilization have been met.

• If the dark color is in the REJECT area window (has not entered the ACCEPT area window), this indicates a REJECT result which means that the items in the load were not exposed to sufficient steam sterilization conditions. The load should be returned for reprocessing and the cause of the sterilization process failure should be investigated.

• Record the steam chemical integrator test result in the autoclave waste treatment log (ACCEPT or REJECT).
Interpretation The Results of Steam Chemical Integrators:

REJECT THE LOAD!

ACCEPT THE LOAD!
Handling of Autoclaved Waste

- Once cool, securely close autoclaved biohazard bag and place into opaque bag to be placed in the regular trash!
Autoclave Use Log Needs to Be Filled Out!

Record every load in autoclave use log!

New form includes Steam Chemical Integrator results!
Documentation

Document the autoclaving of each biohazardous waste load in a log that lists:

• the date of treatment
• the person autoclaving the waste
• the conditions of treatment (length, pressure, temperature)
• the weight of autoclaved waste (lbs)
• program # used
• the name and extension of the person performing the treatment
• the initials of the person
• the Steam Chemical Integrator Test results
Monitoring Autoclave Performance

• In order to comply with quality assurance procedures, autoclaves must be tested periodically to ensure effectiveness.

• Testing parameters include:
  – physical parameters
  – chemical integrators
  – biological indicators
to monitor the conditions inside the load during the sterilization cycle.
Monitoring Autoclave Performance

Chemical indicators (i.e., autoclave tape)

– Autoclave tape and indicators printed on bags do not show that the load has been sterilized. These are used only to indicate that a load has been processed since the stripes appear very quickly even at low temperatures. Use of these would not be regarded as an adequate means of monitoring autoclave performance.
Monitoring Autoclave Performance

Physical Parameters

• The physical parameters (i.e., pressure and temperature readings) provide instantaneous feedback to confirm that the physical conditions were adequate for sterilization; however, they must not be used as the sole indicator of sterility.
Monitoring Autoclave Performance

Chemical Integrators

• 3M™ Comply™ SteriGage™ Steam Chemical Integrators are used inside each load that is autoclaved to monitor time, temperature, and steam exposure conditions and can provide the necessary sterilization assurance needed when decontaminating biohazardous waste.
Monitoring Autoclave Performance

Biological Indicators

• Environmental Health and Safety Office checks the autoclave performance for waste treatment with commercially available biological indicators (ampoules of *Geobacillus stearothermophilus* spores) at an appropriate frequency to ensure that the sterilization parameters are effective in treating biohazardous waste.
Monitoring Autoclave Performance

- *Geobacillus stearothermophilus* spores are rendered unviable at 121 degrees C.

- For the test, ampoules with spore suspensions of *G. stearothermophilus* are autoclaved along with a load.

- Upon completion of the cycle, the spore ampoules are incubated for 48 hours at 55 °C and then observed for signs of growth. If growth of the autoclaved spores occurs after incubation at 55° C, then sterilization was inadequate.
Biological Indicator Test Results

The purple indicator color confirms that the autoclave treatment has been successful!

The yellow indicator color confirms that the autoclave treatment has not been adequate!
The date, process information, and results of each verification test using biological indicators are documented.

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<tr>
<th>Autoclave Make:</th>
<th>Model:</th>
<th>Serial #:</th>
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<tr>
<td>Biological Indicator:</td>
<td>Type:</td>
<td>Building/Ro om</td>
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<tr>
<th>Date of Autoclaving &amp; Incubation</th>
<th>Operator</th>
<th>Cycle Selected &amp; Temperature (°C)</th>
<th>Cycle Time</th>
<th>Number of test ampoules</th>
<th>Biological Indicator Expiry Date &amp; Lot #</th>
<th>Positive Control (non-sterilized biological indicator) shows growth (+)</th>
<th>Results Pass (P) Fail (F)</th>
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In Summary
Autoclave Use Guidelines to Follow
When Treating Biohazardous waste:

• Biological waste should not be left for “someone else” to autoclave!
• Place autoclave temperature tape on the autoclave bag over the biohazard symbols. Make sure bags designated for autoclaving are used.
• Place waste inside autoclave using secondary containment (pan) under the bag/containers. If waste does not contain any moisture, add water inside bag for steam generation.
• Autoclave waste in biohazard waste bags 50 to 60 minutes, at temperature and pressure of 121° C (250° F) and 15 psi.
• When cycle is finished, inspect autoclave temperature tape, visually check bag(s) to ensure autoclave was working correctly, and confirm Steam Chemical Integrator Test result(s).
• Attach “Treated in accordance…”-label to autoclaved waste bag(s), place waste in black opaque bags, and seal them with tape or tie wrap.
• Place waste in regular trash cans.
• Log activities in the Autoclave Waste Treatment Log.
Refresher: Autoclave Safety Animation

• Please follow this link to refresh your knowledge of the proper use of an autoclave to decontaminate biohazardous waste!

https://www.youtube.com/watch?v=T901F2W7wks

• The presentation (9:25 min) includes the following topics:
  – Autoclave overview
  – Energy flow in an autoclave
  – Personal protective equipment
  – Proper use of an autoclave to decontaminate biohazardous waste

• This Autoclave Safety Animation is a Laboratory Safety Project (2010) by
  – The National Biosafety and Biocontainment training program,
  – The National Institutes of Health, and
  – Dartmouth Collage
Thank You!
Safe Autoclaving!