

# Autoclave Use & Safety Training

This one-time training provides an overview of autoclave use and safety measures for all personnel working in labs that utilize an autoclave.

Principal Investigators/Supervisors are responsible for ensuring lab-specific policies are trained and that training documented.



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# Regulatory Requirements

- [15A NCAC 13B.1200 North Carolina Medical Waste Management](#)
  - “Regulated medical waste” defined as blood and body fluids in individual containers in volumes greater than 20 ml, microbiological waste, and pathological waste that have not been treated ... must be treated prior to disposal
- [Uniform Boiler and Pressure Vessel Act of North Carolina § 95-69.8](#)  
requires certification by a commissioned inspection of all boilers-  
defined as “...a closed vessel in which water is heated, steam is  
generated, steam is superheated, or any combination thereof, under  
pressure or vacuum by the direct or indirect application of heat....”  
every two years

# Autoclave Principles

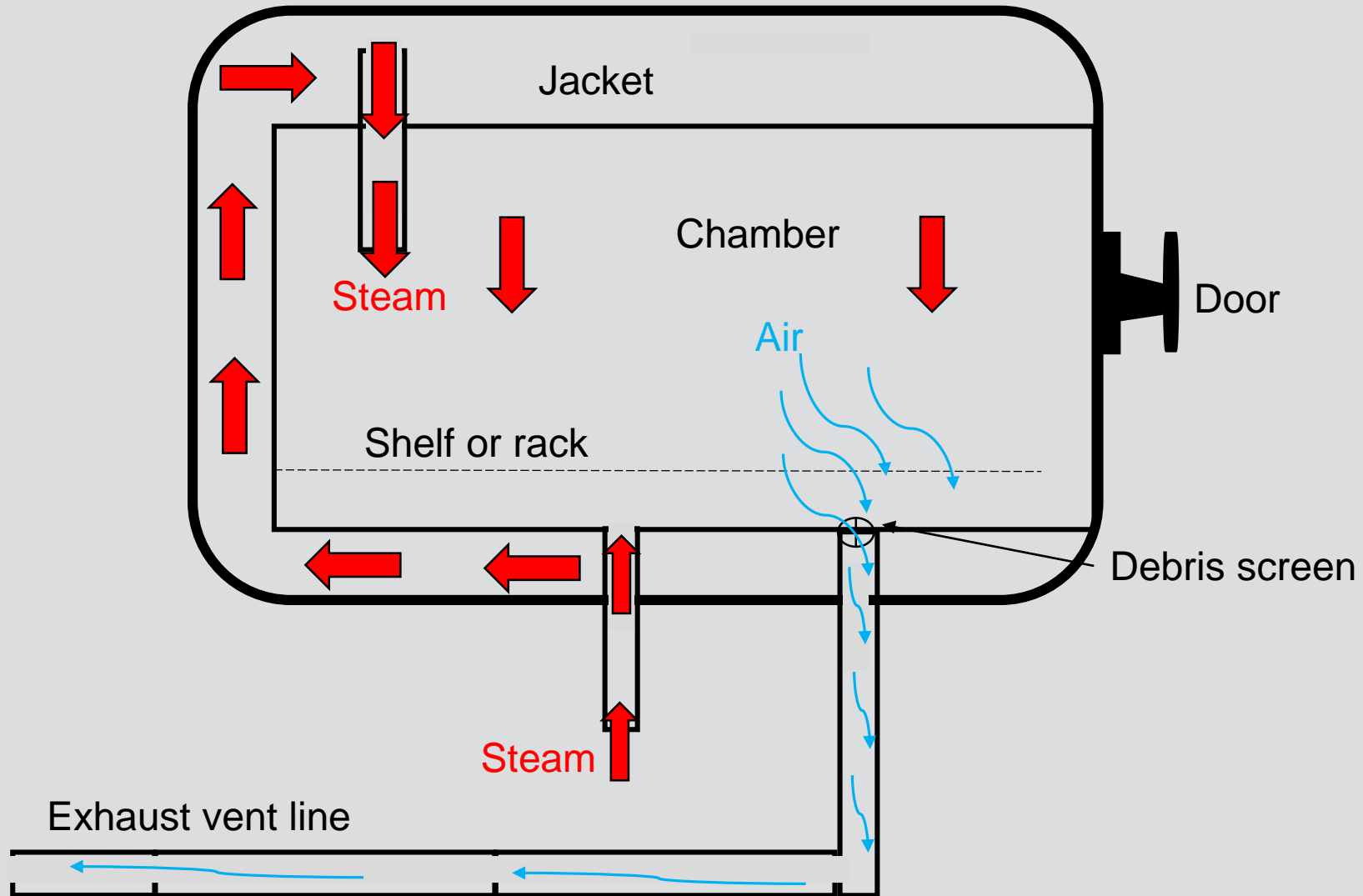
- Three critical parameters: time, temperature, and pressure
- Efficacious and efficient sterilization of bacteria, viruses, and even heat-resistant spores
- Sterilization of materials inside the chamber is occurs due to the high temperature achieved by placing steam under pressure



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# Anatomy of an Autoclave



# Autoclave Cycles

- Standard minimum sterilization parameters
  - 121 °C
  - 15 psi of pressure
  - 30 minutes
- Autoclave cycle parameters are determined by lab-specific risk assessment
- Common cycle types programmed for autoclaves
  - Gravity displacement
  - Vacuum
  - Liquid

# Gravity Displacement Cycle

- Steam enters from above and displaces the ambient air to the bottom of the chamber where it exhausts through drain
- By removing the air the steam directly contacts the contents and improves steam penetration and sterilization
- Can utilize fast or slow exhaust
- Best for non-porous materials - glassware, dry biohazard waste, vented containers and unwrapped instruments

# Vacuum Cycle

- All ambient air is mechanically evacuated from the chamber and chamber contents
- Vacuum can be used pre-cycle and/or post-cycle
  - Pre-vac removes all ambient air by alternating steam and vacuum to create a pressure/vacuum pulse
  - Post-vac cycle removes all steam in the chamber and “dries” the load
- Use for large or porous items including consumables (pipette tips, flasks, etc.), animal cages and bedding, wrapped instruments

# Autoclaving Solid Materials

- Never overfill biohazard waste bags – 75% full allows space to handle and close the bag and allows steam penetration
- Never completely seal waste bags
  - Leave 3 fingers wide opening at the top of all bags to let in steam
- All bags or containers must be placed in stainless steel or polypropylene trays – never set directly on shelf or rack
- Add 100 ml of water to each bag of waste to promote steam penetration



# Liquid Cycle

- Gravity cycle with slow exhaust rate
- Minimizes boil-over and evaporation
- Used for media, water, liquid biological waste, etc.



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# Autoclaving Liquids

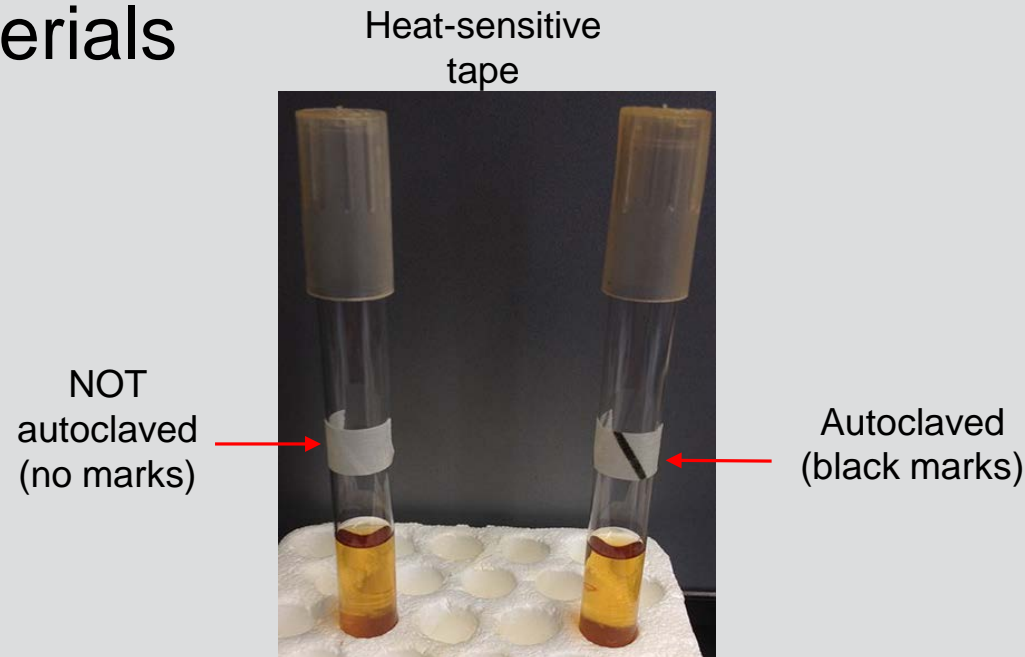
- Liquids should only be autoclaved in autoclaveable glass (Kimax or Pyrex) or polypropylene containers
- Never autoclave sealed containers; leave caps/stoppers loose
- Leave head space - fill to  $\leq 75\%$  capacity
- Consider increasing sterilization time to 25-30 minutes
- All liquid containers must be placed in stainless steel or polypropylene trays – never set directly on shelf or rack
  - Include  $\frac{1}{2}$  inch of water in the tray

# Standard Cycle Phases

- **Purge/Pre-vac** – steam displaces ambient air in chamber either by gravity or vacuum
- **Sterilization** – temperature and pressure are held constant at set points to sterilize chamber contents
- **Drying/cool down**
  - Gravity - Steam and condensate exit through chamber drain with no mechanical assistance. Exhaust can be fast or slow depending on contents.
  - Post-vac – Vacuum pulls steam and condensate through drain

# Heat Sensitive Tape

- Not an indicator of sterilization
- Marks become visible once critical temperature is reached
- Used as a process indicator to identify autoclaved vs. non-autoclaved materials



# Testing and Verification

- NC Medical Waste Management Rules require
  - Autoclaves be tested weekly under full load conditions for effectiveness in killing microorganisms
    - Use biological indicators with average spore count of  $10^4$ - $10^6$  organisms
  - A record of each test is required using the **Autoclave Test Log** (Laboratory Safety Manual Appendix E)
- Waste does not have to be held until the results of the testing confirm effectiveness
- Following autoclave repairs, a biological indicator should be included in the first run to insure proper functioning.

# Performance Indicators

- Biological

- Verifies that autoclave cycle parameters sterilize effectively
- *Geobacillus stearothermophilus* spores are commonly used



Purple = sterilized in cycle

Yellow = spore growth;  
not sterilized in cycle

- Chemical

- Demonstrate that one or more parameters have been met for appropriate time
- Used for instruments or tools



Autoclave strip inoculated with spores and culture for growth of any remaining spores

ABC Company

**Chemical Steam Indicator**

Indicator turns from Orange to Brown

Autoclave  
121 °C 15 min  
or  
134 °C 3.5 min

ABC Company

**Chemical Steam Indicator**

Indicator turns from Orange to Brown



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# Preventative Maintenance

- Keep debris screen clear of debris or sediment
  - Always use heat-resistant gloves
- Follow manufacturer's recommendations on cleaning residue from the chamber to prevent build up
- Perform a monthly check of gaskets for residue buildup and wear

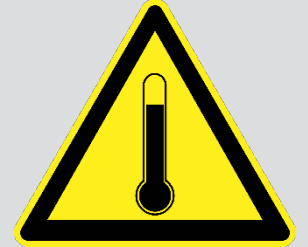


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# Autoclave Hazards

- High temperatures and hot water creates potential for burns and scalding.
- Inadequate decontamination of biohazards could cause personnel and environmental contamination
- Large heavy doors and shelving present ergonomic and pinch hazards
- Steam or pressure can escape from autoclave that is not functioning correctly burning or scalding the user



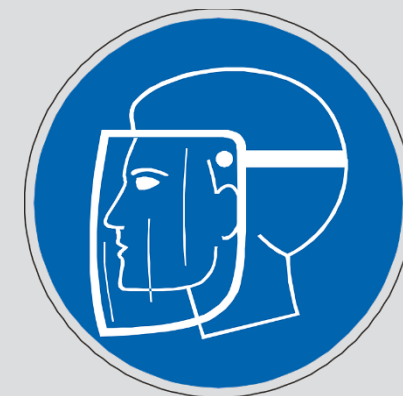
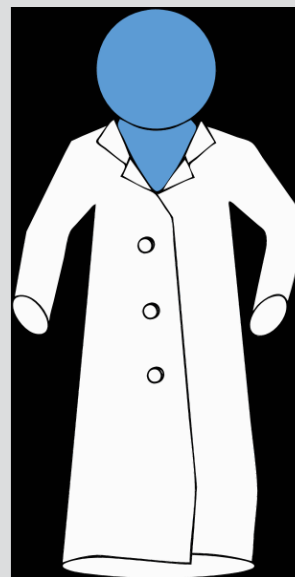


# Research Lab Best Practices

- Wear appropriate PPE
- Read owners manual to understand operation and safety mechanisms
- Do not store combustibles near autoclave
- Report malfunctions immediately
- Never use a malfunctioning autoclave

# Personal Protective Equipment (PPE)

- Eye/face protection
- Lab coat, buttoned
- Rubber apron if unloading liquids
- Heat resistant gloves



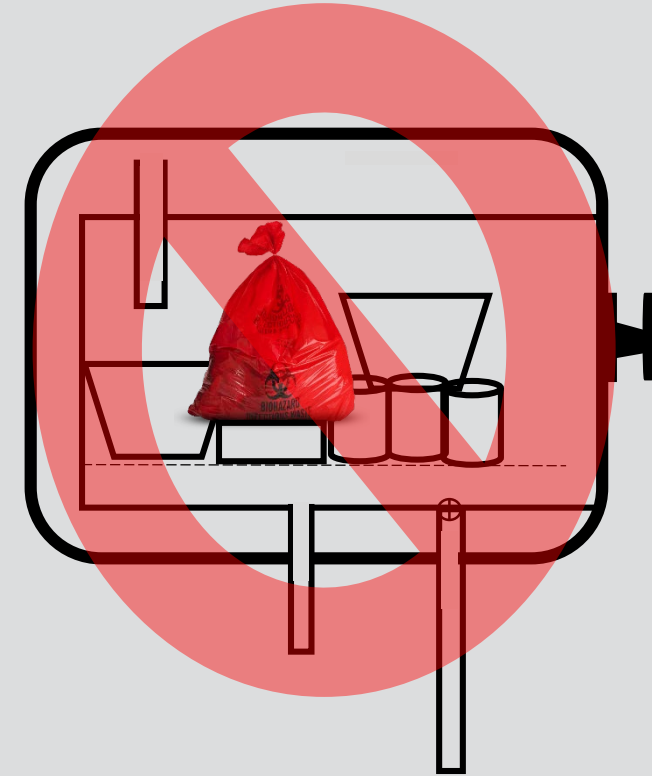
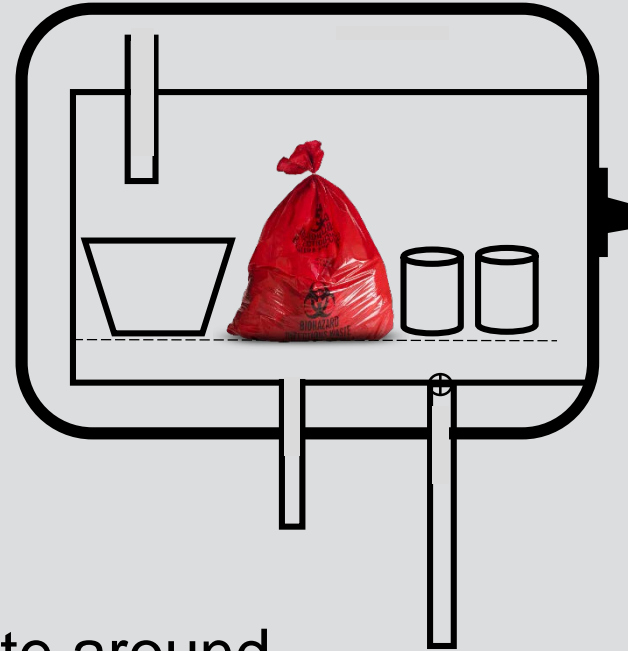
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# Never Autoclave.....

- Chemicals including bleach and alcohol
  - Do not autoclave empty chemical containers
- Batteries and aerosol cans
- Certain plastics
  - Polycarbonate (PC) may withstand only 30–50 cycles
  - HDPE, LDPE, PET, PETG should not be autoclaved

# Loading Autoclave



- Never over fill the chamber
  - Allow space for steam to penetrate around objects
- Do not allow items to touch interior surfaces of chamber
- Ensure door is sealed and locked prior to starting cycle



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# Unloading Autoclave

- Don appropriate PPE
- Verify cycle parameters were met
- Don't open the autoclave door if pressure is not 0 psi
- Crack door and wait 10 minutes to allow items to cool
- Step to the side when opening the autoclave; keep your face and body clear of the door and steam



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# Autoclave Failures

- Discontinue use immediately if autoclave is not working properly
  - Post a sign alerting others not to use the autoclave
  - Contact the service company responsible for the maintenance of your autoclave or EHS for further guidance
- If biological indicator indicates load was not sterilized
  - Evaluate autoclave readout tape to ensure all parameters were met
  - You do NOT have to hold waste – it may be discarded
  - Run another load with new biological indicator
    - If this also fails report to your autoclave service provider

# Autoclave Accidents



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# Autoclave Accident - 1992

- An employee had their back to the autoclave and another opened it while it was still under pressure
- The debris screen was missing from the drain allowing a stopper to plug the drain so that the autoclave remained pressurized
- A number of safety mechanisms failed
  - Locking ram, which prevents the door from being opened
  - Microswitch designed to shut the steam off when the ram does not engage
  - A drain temperature probe
  - The computer failed to abort the cycle when sterilization levels were not reached within a predetermined time
- One person was pronounced dead at the scene and the other sustained lower back injuries from being struck in the back by debris.





# Autoclave Accident - 2001

- Three employees were examining a failed 30 min gravity cycle containing 178 tightly capped glass bottles
- Several bottles exploded within the autoclave
- One employee began to don PPE to clean up the broken glass
- The remaining bottles in the autoclave exploded and the rack holding these bottles was ejected out of the autoclave causing first and second degree burns and injuries to arms and face
- Glass shards and steam were propelled across the room at high velocity
  - Shattered lights approximately 25 ft from the autoclave
  - The other two employees were at the door to the room and were not seriously injured but did receive burns and scalding injuries

[https://www.osha.gov/pls/imis/accidentsearch.accident\\_detail?id=200800530](https://www.osha.gov/pls/imis/accidentsearch.accident_detail?id=200800530)

# Quiz

- You must successfully complete a [post-training quiz](#) with a score of 80% or greater to receive credit for this training
- Please click the link above to complete and submit the quiz
- You must log in with your NCCU credentials

# Questions

[Department of Environmental Health and Safety](#)

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