

Overview

Autoclaves are widely used in biomedical laboratories at Weill Cornell Medicine (WCM) but pose many hazards, including potential physical harm (e.g., heat, steam, and pressure) and biological risks. This Update provides guidelines on the usage of autoclaves on campus and off-site locations.

Each autoclave has unique characteristics. **Review and understand the owner's manual before using any autoclave for the first time and as needed, and contact the manufacturer to request on-site training if required.** Ensure the owner's manual is readily available in case questions arise during operation. For general autoclave safety training and guidance, contact EHS.

Maintenance is vital to the effective and safe use of an autoclave. **Follow the manufacturer's recommendations for preventative maintenance and ensure all contractors are approved by the manufacturer.** Maintenance should include periodic validation tests (e.g., Bacillus stearothermophilus spore testing) to ensure the autoclave is functioning properly.

Applicability

This update applies to all supervisors and personnel using autoclaves.

Responsibilities

Environmental Health and Safety (EHS) provides assistance and training for autoclave safety as requested, and updates informational resources as necessary.

Principal Investigators and Laboratory Managers establish and update policies and procedures for their personnel. They also ensure that laboratory staff receives proper training and complies with safety requirements, and is familiar with this Update.

Laboratory Personnel working with autoclaves must follow these guidelines and contact Environmental Health and Safety for any needed assistance and training.

Procedure

Utilize the following autoclave safety practices.

1. GENERAL PRECAUTIONS

- **LABORATORY SUPERVISION:** Autoclaving is considered a laboratory operation and must be supervised by an FDNY C14 Laboratory Certificate of Fitness holder. Please consult the [EHS website](#) for more information.
- **TRAINING:** All autoclave operators must receive training on safety practices prior to using the equipment. Training may be delegated to a qualified individual, but the supervisor must ensure their personnel is adequately trained. Training requirements may include annual completion of Laboratory Safety or Annual Hospital Training (AHT) programs, depending on the department. This Update should be reviewed as well.
 - **Know the location of the nearest safety shower and eyewash stations** in the event of accidental exposure to steam or hot water.
 - **Review the owner's manual** and understand the proper operating instructions for the specific autoclave unit and safety procedures. Do not use an autoclave for anything other than its intended purpose.
- **PERSONAL PROTECTIVE EQUIPMENT (PPE):** Wear suitable personal protective equipment when loading, operating or retrieving materials from an autoclave:
 - **Standard PPE:** Closed-toed shoes, lab coat, safety glasses, and heat-resistant insulated gloves.
 - **When Autoclaving Liquids:** Standard PPE plus face shields and liquid-resistant apron.
 - **When Autoclaving Sharps:** Standard PPE plus cut-resistant gloves when removing items from the autoclave.
- **REQUIRED SIGNAGE:** Autoclave locations must display the EHS Autoclave Use and Safety sign (contact EHS to request sign).





■ IN THE EVENT OF INJURIES:

- **Immediate First Aid:** If accidental exposure to steam or hot water occurs, immediately use a safety shower, eyewash, or nearest sink to cool the affected area.
- **Seek Medical Assistance:** Based on the severity of injuries, contact **NYP EMS at 212-472-2222** to have emergency medical services brought to the autoclave room. For less severe injuries, go to the NYP Emergency Department (24/7) or Workforce Health and Safety / Student Health Services (during business hours).
- **Inform:** Report any injuries to the appropriate supervisor and Environmental Health and Safety.

■ PROHIBITED ITEMS: Autoclaves must not be used with the following:

- Corrosives (e.g., acids, bases, phenol, etc.), solvents (e.g., ethanol, methanol, chloroform), or radioactive materials: Contact EHS or review the [EHS Program Manual, Section 5.2 - Waste Disposal Procedures](#) for proper decontamination and disposal procedures.
- Vertebrate animal carcasses: should be disposed of via the Research Animal Resource Center (RARC).

2. BEFORE USING AUTOCLAVE

■ AUTOCLAVE PRE-CHECK BEFORE LOADING:

- Check gaskets and seals to ensure they are undamaged and free of dirt and debris. Wipe with a soft cloth to clean as needed.
- Check inside for any items left by the previous user that could pose a hazard (e.g., sharps).
- Clean the drain strainer.
- Refer to owner's manual for other autoclave pre-checks for the specific unit.

■ AUTOCLAVE-SAFE CONTAINERS: Only use autoclave-compatible plastics. Certain plastics cannot be autoclaved. Plastic types are identified by the manufacturer's initials imprinted on the container bottom.

- **Use:** Polypropylene (PP, recycle #5); Polycarbonate (PC). Nalgene Labware/Thermo Scientific's Autoclaving web page provides additional plastic considerations.
- **Do Not Use:** Polyethylene (PE, recycle #1); High-density polyethylene (HDPE, recycle #2).

3. AUTOCLAVE CYCLES

■ CHOOSE THE CORRECT AUTOCLAVE CYCLE: there are two basic types of autoclave cycles.

Cycle	Description	Typical Application or Load Type
Gravity or "fast exhaust"	During this cycle, steam displaces the air in the chamber, and the force of gravity causes the heavier air to exit the chamber via the sterilizer drain. Once the chamber fills with steam, it holds it at a set pressure and temperature for a fixed time. At the end of the cycle, a valve opens, and the chamber rapidly returns to atmospheric pressure. Drying time may also be added to the cycle.	Dry goods, glassware, solid waste, etc.
Liquid or "slow exhaust"	This cycle prevents sterilized liquids from boiling over by releasing the steam slowly at the end of the cycle. This allows the pressure in the containers to reach equilibrium without causing the liquid to overflow.	Liquids

- Effective sterilization is the product of temperature, pressure, and time.
 - **Temperature:** Effective sterilization occurs when the steam temperature exceeds 250°F (121°C).
 - **Pressure:** Autoclave pressurization should be at least 20 pounds per square inch (psi).
 - **Time:** The amount of time needed to sterilize most organisms depends on the temperature and pressure. At 250°F (121°C) in a vessel pressurized to 20 psi, bags require at least 30 minutes to sterilize.

Steam must contact all areas of the load to sterilize. Larger loads and tightly packed materials will require more time to ensure steam reaches denser areas.

4. LOADING THE AUTOCLAVE

- Always follow the manufacturer's recommendations for loading the autoclave.
- **SECONDARY CONTAINMENT:** Place all items in a heat-resistant tray on a shelf or rack. Use autoclave trays made out of polypropylene, polycarbonate, or stainless steel. The trays should have a solid bottom and walls to hold the contents and catch spills. Do not use trays or tubs that are cracked, warped, or compromised.



- **GLASSWARE AND LIQUIDS:** Never put individual glassware pieces directly on the autoclave bottom or floor. All items must be placed in a heat-resistant tray then on the autoclave shelf or rack.
 - Liquid materials such as media in bottles should occupy no more than half of the autoclave chamber volume. This will allow steam to circulate freely around the load.
 - Loosen container caps before loading to prevent bottles from shattering during pressurization; including empty and liquid-filled bottles. Expanding vapor in bottles during heating can also lead to rupturing.
 - Containers should only be ½ - ¾ full to allow for vapor expansion.
- **PACKING AUTOCLAVE BAGS INTO THE AUTOCLAVE:** never place red bags directly into the autoclave bottom or floor. All items must be placed in a heat-resistant tray then on autoclave the shelf or rack.
 - **Use only autoclave-rated bags with an autoclave.** Standard red bags provided by Housekeeping are not approved for autoclaves and will melt during autoclave operations.
 - Avoid packing bags tightly within the autoclave to ensure that heat & steam can circulate.
 - Seal bags loosely to allow for effective steam penetration.
 - Do not allow bags to touch the interior walls of the autoclave.
 - Ensure sufficient liquid is packed with contents of autoclave bags if dry (e.g., 10 ml of water; a zip lock bag containing water).
- **AUTOCLAVE INDICATOR TAPE:** Autoclave tape is a heat-sensitive chemical indicator that changes color when exposed to temperatures of 250°F (121°C). Chemical indicators do not indicate that the autoclave load has been sterilized; they only note that a temperature of 250°F (121°C) has been reached within the autoclave. If the tape does not change color, the chamber's temperature was not reached and the cycle should be rerun. If the tape fails to change color on two consecutive loads, contact your laboratory manager and autoclave contractor immediately. To validate the autoclave load has been sterilized, see Section 6-Validation Testing of Autoclave.
 - Place autoclave tape on the material being autoclaved (e.g., bag, bottle, tray, etc.) as an initial verification of a successful autoclave cycle.
 - **Use only lead-free autoclave tape.** Replace lead-containing autoclave tape with lead-free alternatives. Typically, tapes with stripes (////) contain lead. Tapes which display the word "Autoclaved" as an indication of steam sterilization are lead-free. Lead-free options are available from WMC Preferred Vendors, such as VWR and Fisher-Scientific. Unused rolls of lead-containing autoclave tape should be disposed of via EHS as [chemical waste](#).
- **CONFIRM THAT DOOR IS PROPERLY CLOSED:** Make sure the door of the autoclave is fully closed (latched) and sealed before starting the cycle. Follow manufacturer's instructions on how to complete this process correctly.

5. OPENING THE AUTOCLAVE DOOR AND UNLOADING ITEMS

- **ZERO PRESSURE BEFORE OPENING:** Wait until the pressure gauge reads zero before opening the autoclave door.
- **PUT ON PPE BEFORE OPENING THE AUTOCLAVE.**
- **OPEN DOOR CAUTIOUSLY:** Stand behind the door (if possible) or to the side and slowly open it. Allow 30 seconds to let steam escape before reaching inside.
- **ALLOW LIQUIDS TO COOL PRIOR TO REMOVAL:** Let liquids sit for 10-20 minutes prior to removal from the autoclave. This allows the liquid to cool enough to avoid any boil-over while removing.
- **ALERT** others in the area that a heat hazard is present.

6. VALIDATION TESTING OF AUTOCLAVE

Regular testing must be conducted on the autoclave to validate it is working properly. Using biological indicators (BI) that contain *Geobacillus stearothermophilus* spores is an effective and easy way to test the autoclave cycle is reaching microbial "kill." *Geobacillus* spores are one of the most heat-tolerant species of bacteria, and if the sterilization cycle in an autoclave does not destroy them, the device is not working properly. Read the BI's manufacturers' instructions prior to use. Check for expiration dates, storage, and other safety considerations.

TESTING PROCEDURE

- Place the spore strip or ampoule in the center of the load. It is important to place the BI in a typical load and in the load's densest area. Running a BI in an empty incubator will only result in a false negative.
 - A string or wire can be used to retrieve the spore indicator after the run. Secure the strip or ampoule with autoclave-tape and a length of cotton string with the string extending out of the bag opening (some indicators are manufactured with an attached string). Remove the ampoule after the cycle is completed.



- Follow BI's manufacturer instructions for processing. Typically, this involves incubation in a media solution anywhere from two to seven days after the run (follow manufacturer instructions). Ensure a negative and positive control is also incubated.
- Any bacterial growth of the test BI indicates that the autoclave is not working properly. Do not continue to use an autoclave if it fails the spore test – contact your laboratory manager and autoclave contractor immediately.

References

- EHS Program Manual, Section 5.2 - [Waste Disposal Procedures](#)
- Nalgene Labware - Technical Data. [“Autoclaving”](#)
- University of California at San Diego - [Autoclaving Guidelines for Sterilization of Lab Equipment](#)
- University of California at Berkley – [“Safe and Effective Use of Autoclaves”](#)

Consumable Supplier List

Biological Indicators:

- Mesa Labs: <https://biologicalindicators.mesalabs.com/all-biological-indicators/>
- 3M Biological Indicators: https://www.3m.com/3M/en_US/company-us/all-3m-products/~/All-3M-Products/Health-Care/Medical/Sterilization-Monitoring/Biological-Indicators/?N=5002385+8707795+8707798+8711099+8753017&rt=r3
- Steris: <https://www.steris.com/healthcare/products/sterility-assurance-and-monitoring/biological-indicators>

Lead-Free Autoclave Tape:

- Fisher Scientific: <https://www.fishersci.com/shop/products/fisherbrand-lead-free-autoclave-tape-4/p-4006732>
- VWR: <https://us.vwr.com/store/product/15341459/vwr-autoclave-tape-lead-free>
- 3M: https://www.3m.com/3M/en_US/company-us/all-3m-products/~/autoclave-tape-Lead-Free-Autoclave-Steam-Indicator-Tape/?N=5002385+3294768922&rt=rud