

Safe and Effective Use of Autoclaves

Autoclaves are easy to use but can pose a safety hazard if operated incorrectly. This fact sheet was developed to help prevent injuries and ensure that biological wastes are effectively decontaminated prior to disposal. Please refer to the EH&S Fact Sheet "Risk Group 1 Recombinant DNA Waste Disposal" and your Biological Use Authorization (BUA) for detailed information on the types of biological wastes that must be autoclaved, as well as packaging and other waste handling requirements.

What are autoclaves?

Autoclaves use water, pressure and heat to create superheated steam that kills microorganisms and spores. They are used to decontaminate certain biological waste and sterilize media, instruments and lab ware.

Factors for effective sterilization

Steam sterilization is mainly a function 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 is dependent upon the temperature and pressure. At 250°F (121°C) in a vessel pressurized to 20 psi, bags require at least 30 minutes to sterilize.

The autoclave's superheated steam must contact all areas of the load. Bottle caps should be loose during the autoclave cycle so vapor expanding during heating will not cause an explosion. Liquid materials such as media or saline in bottles should occupy no more than half of the autoclave chamber volume; this will permit steam to circulate freely around the load. Do not overfill autoclave bags and tie them loosely to allow steam to reach the center of bag contents. Larger loads and tightly packed materials will require more time.

Dry heat cycle - when to use

The autoclave may offer dry heat cycles. These can be utilized for sterilizing laboratory supplies, which can withstand high temperatures but would be affected by a steam cycle. The required exposure times for dry heat will depend on the material composition, load volume, and can require significantly more time than what would be required for steam sterilization at the same temperature. Because the required times for dry heat sterilization will vary on the material treated and the surface and content, it is best to vary initial loads to determine the most effective temperature and length of cycle.

Autoclave safety considerations

- Never autoclave flammable or volatile liquids, because these liquids could off gas and explode in the superheated chamber.
- Firmly lock autoclave doors and their gaskets in place before operating the autoclave to prevent a sudden release of high-pressure steam. Most, but not all, autoclaves have safety interlocks that prevent the autoclave from running if the door isn't closed properly.



Autoclave safety considerations

- Wear personal protection equipment:
 - Lab coat
 - Eye protection
 - Closed-toe shoes
 - Heat-resistant gloves to remove items, especially hot glassware
- Wait for the pressure gauge to drop to zero with zero time remaining before opening the door.
- Never open an autoclave set for “slow exhaust” until the cycle is complete. Superheated liquids can boil over and damage the autoclave and injure the operator. Never use the “fast exhaust” cycle for liquids as they will evaporate.
- Open the door cautiously. Stand behind the door and slowly open it. Allow 30 seconds to allow steam to escape before reaching inside.
- Let liquids stand another 10–20 minutes after the autoclave is opened to avoid any movement that could cause them to boil over, resulting in scalding. Remove all items carefully.
- Report any malfunctions or accidents immediately to your supervisor. Notify the facilities manager if PP-CS or the vendor may be needed to repair any autoclave.

Autoclave bags and equipment

- Autoclave trays made out of polypropylene or polycarbonate are used as a secondary container; waste bags touching the internal chamber may melt and cause costly repairs. Stainless steel tubs are an alternative to polypropylene. It is a safety hazard to use trays or tubs that are cracked, warped or compromised.
- Clear, autoclavable bags are used for recombinant DNA and/or BSL 1 waste. The bags cannot have biohazard symbols or wording. Once clear bags have gone through the cycle, they may be disposed of as regular trash.
- Red, autoclavable, “Biohazard” bags are used for BSL₃ waste. Autoclave all BSL₃ waste and place in the tubs provided by EH&S.
- Bottles - avoid autoclaving plastic bottles unless the plastic can withstand the autoclave cycle.
- Temperature indicators may be used to determine if the desired temperature was attained. Autoclave tape and temperature - indicating labels are attached to the outside of the bag and change color or appearance when the desired temperature is reached. These may be purchased from laboratory supply companies, although they are not required. To ensure the entire contents of bagged waste are decontaminated, a test of the autoclave must be conducted every month.

How to determine the effectiveness of an autoclave

Autoclave users are responsible for conducting a monthly test on the autoclaves and reporting any problems to the department’s facilities manager. Purchase a test kit from your laboratory supply company which uses *Geobacillus stearothermophilus*, one of the most heat-tolerant species of bacteria. If sterilization in an autoclave does not destroy the *Geobacillus* spores, the device is not working properly. (A good biological indicator for dry heat cycles



is *Bacillus atrophaeus*.)

Read the manufacturers' instructions prior to use. Check for expiration dates, storage, and other safety considerations.

Procedures:

1. Place the spore strip or ampoule in the center of the load. A string or wire is 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
2. Place the autoclaved test vial and a non-autoclaved control vial in an incubator, and incubate at 133 to 140°F (56 to 60°C). If you don't have an incubator, buy a spore test that you can ship to the manufacturer for incubation. Spore strips typically require incubation for up to seven days after the run (follow manufacturer instructions).
3. Any bacterial growth indicates that the autoclave is not working properly. Do not continue to use an autoclave if it fails the spore test – contact your facilities manager immediately.

Disposal and Other Considerations. Used indicators with spore growth should be autoclaved again and disposed of as regular trash. Do not conduct tests in bags containing chemical disinfectants as these may kill the strip agent.

Recordkeeping

It is the responsibility of the autoclave users to establish a schedule for testing. The following autoclave records must be maintained for three years in the autoclave room, preferably in a binder accessible by EH&S or PP-CS personnel:

- On-site maintenance records (remember that maintenance is based on hours of usage, rather than a chronological period)
- Autoclave use log (each load of material inactivated shall be logged as follows):
 - * Date, time, and operator's name
 - * Contact information: Laboratory, room number, phone number
 - * Type of material sterilized
 - * Temperature, pressure, and length of time the load is sterilized
 - * The autoclave print-out, if the autoclave has a working printer or the cycle wheel when filled.

The attached form "Monthly Autoclave Test Log" may be used to document maintenance and testing dates and results.



Monthly Autoclave Test Log

Building, Room # and Autoclave#

Date	Generator Name	Time at 121C	Max Temp	Max Press.	Wt. in Lbs.	Monthly sterilization Check Pass/Fail	Comments	Operator

Bacillus Ampoule Test Procedure, for Autoclave Monitoring

The following testing will be conducted on a monthly basis and results will be recorded in an autoclave log. Log records to be kept for a minimum of three years.

Initial Process:

1. Retrieve two spore ampoules (Bacillus Stearothermophilus).
2. Identify the indicators (spore ampoules) by labeling them with the proper information (date, autoclave number, control, test, etc.).
3. Place the spore ampoules in a horizontal position with representative materials to be sterilized. Both ampoules should be located in the part of the load that would be considered most questionable for sterilization. Remember to attach a piece of autoclave tape onto the load.
4. Select appropriate cycle to process the load (usually 30min for liquid or dry cycle).
5. Once the cycle has come to a finish allow autoclave to decompress and cool down (min. 10 min).
6. Remove load from autoclave and allow it to further cool down (10 - 15min).
7. Retrieve the spore test ampoules from the load.
8. It is common to see a color change from the spore ampoules from purple to black; usually this distinguishes indicators that have been exposed to steam to those that have not.

Incubation process:

Use any incubator that is adjusted to a temperature between 55⁰C and 60⁰C. Take both indicator ampoules from the load that was run and gently squeeze the plastic crusher to break the glass ampoule and place those in the incubator. Take a third spore ampoule (label control ampoule) that was not run in the load proceed to break the glass ampoule and also incubate in the same incubator, allow incubation and verify all three spore ampoules after 24 hours.

Interpretation:

1. Examine indicator ampoules for any color change after 24 hours. The presence of yellow color change indicates a fail test and positive bacterial growth. No color change indicates a passing test and proper sterilization of sample. The control ampoule should have a yellow color change.
2. If a fail test is confirmed run the test again and discontinue normal use of the autoclave for the time being. If the second test round fails again, inform all appropriate personnel as well as Environmental Health & Safety (Biosafety Officer ext. 3-6562). Discontinue full use of autoclave machine until approved for use.
3. If a passing test is confirmed from the first round or second round, you can proceed with regular use of the autoclave.
4. Record all results in your monthly autoclave log.
5. Ensure to dispose of all used Bacillus ampoules by autoclaving first before discarding.