

Appendix H - Autoclaves

Autoclaves are safe and highly effective when used properly. They sterilize equipment and supplies and

they kill biologicals and denature proteins. Autoclaves do not remove chemical contamination and should not be used in conjunction with chemical disinfectants.

Autoclave Cycles

There are three basic autoclave cycles:

1) Gravity Cycle (aka Fast Exhaust): This cycle is used for dry items such as glassware or metal instruments. This cycle cannot be used for liquids because the rapid pressure release will cause any liquids to boil.

2) Liquid Cycle (aka Slow Exhaust): This cycle is used to sterilize liquids. The slow pressure release at the end of the cycle keeps liquids (which have been heated to 121C) from boiling. A table of autoclave times based on liquid volume is given below.

3) Pre-Vacuum Cycle: This cycle is used for porous materials, animal bedding, etc. Some of the air in the chamber is evacuated to create a negative pressure space. This allows the steam to penetrate deeper into the materials placed in the autoclave to help ensure that even the deepest pockets within these materials become sterile.

When an autoclave is used to kill biologicals prior to disposal, follow the procedures set forth in the appropriate chapter of the Biosafety Manual.

Safety Rules for Autoclaves

- ☐ Hot glassware being removed from the autoclave must be handled with dry, heat-resistant gloves.
- ☐ Use the liquid (slow-exhaust) cycle for liquids and let them stand for 10 minutes before removal from the autoclave.
- ☐ For media bottles (glassware with a sealable cap), the cap must remain loose while the bottle is in the autoclave. This will prevent it from building pressure and exploding.
- ☐ Secondary containment must be used for items being placed in the autoclave. Items should never be placed directly on the floor of the autoclave.
- ☐ Not all plastics can be autoclaved. Polypropylene and polycarbonate may survive the autoclave; polyethylene, even high-density polyethylene will not. Be sure your item is autoclave safe before placing in the autoclave. If there is any doubt, use secondary containment.
- ☐ To ensure adequate steam penetration, add 1 cup of extra water for dry loads and leave bags partially open.
- ☐ Red biohazard bags must never be used in an autoclave, even if they are autoclave-safe. Clear autoclave bags must be used.
- ☐ Never open an autoclave until the chamber pressure is zero.

- ☐ Stand back from the door upon opening to allow excess steam to be released safely.
- ☐ An indicator, such as autoclave tape, must be used with each load to ensure the chamber has reached the appropriate temperature.

Other Autoclave Operating Suggestions

- ☐ Placing 1-2 inches of water in the bottom of the secondary container with bottles will help

prevent bottle bottoms from breaking.

☐ Autoclaving new glassware for 90 minute will partially temper is, making it stronger.

☐ If there is doubt about whether or not a piece of glassware is autoclave safe, it should be wrapped in foil. This will capture the pieces of glass if the glassware were to crack or shatter.

Autoclave times based on liquid volume

These autoclave times are only given as guidance. More time may be required for certain organisms or

other biologicals. Allow 10-20 minutes extra for crowded items.

<500 mL 500mL - 1L 1L - 2L 2L-4L 4L

30 minutes 40 minutes 45-55 minutes 55-60 minutes 60 minutes

For any additional questions regarding autoclave use and safety, contact EHS at

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Revision #1

Created 2025-10-23 18:40:59 UTC by Melissa Dooley

Updated 2025-10-23 18:41:45 UTC by Melissa Dooley