FACT SHEET

Berkeley UNIVERSITY OF CALIFORNIA EH&S OFFICE OF ENVIRONMENT, HEALTH AND SAFETY

General Autoclave Safety

This fact sheet was developed as a general guide to prevent injuries and promote proper autoclave sterilization of biological waste. Please refer to the EH&S Fact Sheet Management of BSL1 Recombinant DNA Waste and your research group's Biological Use Authorization (BUA) for detailed information on the types of biological waste that must be autoclaved.

Autoclaves

Autoclaves are used for steam sterilization – a process achieved by using superheated steam to kill microorganisms and spores. Autoclaves are used to decontaminate certain biological waste and sterilize lab materials.

Effective Sterilization

To work effectively, steam must be in contact with the materials directly (including within an autoclave bag). The parameters for an effective steam sterilization process are pressure, temperature, and time. In general, effective sterilization occurs when the steam temperature exceeds 250°F (121°C). Autoclave chamber pressurization should be about 16 pounds per square inch (psi) when at 250°F (though 12-22 psi is acceptable in most circumstances). The sterilization cycle should be at least 30 minutes for most BSL1 materials and at least 60 minutes for soil. Please reference your autoclave SOP and/or manufacturer instructions to select the correct settings for your autoclave and the items you intend to sterilize.

Dry Heat Cycles

The autoclave may offer dry heat cycles. These can be utilized for sterilizing laboratory supplies, which can withstand high temperatures but may be affected by a steam cycle. The required exposure times for dry heat will depend on the material composition and load volume, and the cycle can require significantly more time than what would be required for steam sterilization at the same temperature.

Liquid Cycles

When sterilizing liquid materials, to prevent "boil over" incidents – liquid boiling so rapidly that it spills over the top of its container – the autoclave pressure must be decreased slowly during the exhaust phase to allow the temperature of the liquid in the load to remain below its boiling point. Be sure that the autoclave has the correct program and is equipped for a "slow exhaust" during liquid cycles.

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BSL2, **red bag waste**, may not be autoclaved on campus. Please refer to the EH&S Fact Sheet Biohazardous Waste Management in Biosafety Level 2 Laboratories for additional details on disposal of BSL2 waste.

Before Using the Autoclave

The laboratory's supervisor or principal investigator is responsible for ensuring safety training is provided to personnel before operating an autoclave. This training must be documented and maintained with other safety training certificates.

General Safety Practices

Never autoclave flammable, volatile, reactive (e.g. bleach) or radioactive materials; contact EH&S for disposal of these hazardous materials.

Before loading the autoclave:

- Inspect the autoclave before loading; ensure the gaskets are not cracked or bulging and all drain screens are clear of debris.
- Check that the materials you intend to autoclave are compatible with the process; not all materials are heat safe.
- Use autoclave trays that are heat resistant – e.g. polypropylene (PP), polycarbonate (PC), or stainless steel trays. It is unsafe to use autoclave trays that are cracked, warped, or damaged. Polyethylene (PE) cannot be autoclaved.
- Do not autoclave broken or cracked glassware.
- Use only borosilicate glass bottles that can withstand the high autoclave temperatures.
- Bottles of liquid should not be more than ¾ full if clear liquids, down to ½ full if not (e.g. media +/- agar). Loosen caps to allow for steam to penetrate. Keep 1-2 inches of space between bottles.
- Bags for solid BSL1 or recombinant waste must be autoclavable clear or white bags. Biohazardous symbols or wording should not be present.
- Tape or tie bags loosely to allow steam to penetrate.
- Chemical indicator strips can be used to determine if the cycle reached at least 121°C at some point.

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Required Personal Protective Equipment (PPE):

- Lab coat
- Eye protection
- Closed-toe shoes
- Heat-resistant gloves to remove items, especially hot glassware

Additional PPE if hot liquids are involved:

- Rubber apron
- Face shield

Loading the autoclave:

- DO NOT overload the autoclave. Load the autoclave as per the manufacturer's recommendation.
- Always use a secondary container (e.g. PP tray) large enough to contain the contents and catch spills.
- Never place bags directly on the autoclave bottom or floor and do not allow material to touch the walls of the autoclave.
- Make sure that the door of the autoclave is fully closed and latched.
- Ensure the correct cycle for the items has been selected before starting the autoclave (see Liquid Cycles and Dry Heat Cycles).
- Do not start a malfunctioning autoclave.
- Remain by the autoclave until it reaches the expected pressure.
- Contact a lab supervisor or PI if problems arise.

Unloading the autoclave:

- 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.
- Never use the "fast exhaust" cycle for liquids as they will evaporate, boil over, or even explode.
- Wearing the appropriate PPE, open the door cautiously. Wait 30 seconds to allow steam to escape before opening more widely.
- Let liquids stand 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.
- Record the cycle run in the autoclave use log.
- Report any malfunctions or accidents immediately to your lab supervisor or PI. Notify the facilities manager if repairs to the autoclave may be needed.
- Autoclaved solid BSL1 waste may be disposed of as regular trash. Autoclaved liquid BSL1 waste may be drain disposed.

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Additional Resources at UC Berkeley EH&S

- Management of BSL1 Recombinant DNA Waste
- Biohazardous Waste Management in Biosafety Level 2 Laboratories
- Autoclave Testing Fact Sheet (forthcoming)

Contact bso@berkeley.edu with questions