Effective Use of Autoclaves

SafetyNet #: 26

Autoclave Use

Autoclaves (steam sterilizers) are metal pressure vessels that are used for steam sterilization of media, instruments, and lab ware, and for decontamination of biological waste. Autoclaves function by pressurizing steam in an airtight chamber, which increases the steam temperature significantly. The superheated steam kills all microorganisms and degrades most macromolecules rapidly. Autoclaves are generally easy to use but they are ineffective and potentially very hazardous if operated incorrectly, especially if the door is opened too soon or too rapidly after a run is completed. Autoclave waste packaging, treatment, and handling are also subject to regulations which carry substantial penalties if waste is handled incorrectly. This SafetyNet provides a review of proper use of autoclaves to ensure that these devices are used safely, effectively, and in compliance with applicable regulations.

Factors in autoclave function

Steam

The energetics of steam makes it far more efficient for sterilization and decontamination than dry heat at the same temperature. Effective steam sterilization depends on the interaction of temperature, pressure, and time, but additional conditions inside the autoclave chamber such as materials, containers, container placement, and total volume of the materials also influence sterilization success. Each of these factors must be controlled within a narrow range of values or condition

- **Pressure/temperature relationship:** Pressurization to 15 psi typically "superheats" steam to about 121°C (250°F), which is adequate to kill all microorganisms and to decontaminate or sterilize in reasonable time.

- **Volume:** "Dense" materials such as media in bottles to be treated in the autoclave should occupy no more than half of the autoclave chamber volume, so that steam can circulate completely around and into the load. Less dense materials such as bagged waste can occupy somewhat more space but should never contact the autoclave chamber wall.

- **Contact:** To sterilize or decontaminate uniformly, superheated steam must contact all areas of the load.

- **Time:** Other factors being equal, autoclave loads up to about 2.0 ft³ in volume require 30-60 minutes to sterilize at 15 psi and 121°C. Larger loads and tightly packed materials may require much more time.
Dry heat
Some autoclaves offer dry heat cycles, which are useful for sterilizing laboratory supplies such as KimWipes that can withstand high temperatures but would be damaged by steam. The necessary exposure times for dry heat vary considerably depending on materials composition, packaging, load volume, and possibly other factors, and may be more than triple the time needed for steam sterilization at the same temperature. Because the required times for successful dry heat sterilization vary so much the user may need to experiment extensively with appropriate times and temperatures to develop a consistently successful dry heat sterilization protocol.

Containers:
Primary Containers

- **Autoclave waste bags** — Autoclave waste bags are used for bulk dry disposable materials such as paper towels, plastic centrifuge tubes, bundled serological pipettes, and plastic petri dishes (with or without agar-based culture media). Autoclave bags are made of translucent, colorless, steam resistant plastic film, usually with a pre-applied "sterilization indicator" and often printed with the universal biohazard symbol. If the waste being autoclaved is not contaminated with animal infectious agents or certain high hazard plant pathogenic agents the biohazard symbol is inappropriate and should be defaced before use. **Autoclave bags should be loosely taped or tied during decontamination to allow steam to penetrate into the bag. Air pockets in tightly sealed bags may cause localized sterilization failure.**

- **Medical waste bags** — In California bags for medical waste (human cells, cell lines, tissues, or body fluids, or microbial agents pathogenic to humans) are red with a preprinted universal biohazard symbol, manufactured to California medical waste management regulation specifications. Medical waste can only be treated terminally by inactivation processes that are monitored and inspected by the California Department of Public Health (CDPH), such as in a CDPH-registered autoclave. However, no CDPH registered autoclave is available for general use at UC Davis. Instead, most UC Davis medical waste is taken from the laboratories to accumulation sites, and is transported by vendors to approved medical waste treatment facilities without being autoclaved first. **Exception:** medical waste generated in Biosafety Level 3 laboratories is generally autoclaved within the laboratory and then disposed through the medical waste stream.

- **Bottles** — Glass bottles with screw tops are used for autoclaving liquids such as water, some buffers, and culture media. Always loosen the screw top so that it barely engages the threads on the neck of the bottle. Do not attempt to autoclave liquids in plastic bottles. Always verify that the plastic caps and seals used on your media bottles are heat-resistant before using them in an autoclave.

- **Hard-walled plastic sharps** containers should **not** be autoclaved (except sharps from BSL3 laboratories), but should instead be disposed through the sharps waste stream. For any type of sharps you can request a sharps container pickup on the EH&S website: [http://safetyapps.ucdavis.edu/EHS/wasterequest/sharps.cfm](http://safetyapps.ucdavis.edu/EHS/wasterequest/sharps.cfm) [1]. Alternatively, filled and closed red medical waste sharps containers can be transported to a medical waste accumulation site.

- **Miscellaneous** — Steam and heat-resistant pouches are commonly used to sterilize surgical
instruments. Metal cans with snug-fitting metal lids may be used to sterilize glass serological pipettes (although this is less common nowadays because disposable pipettes are widely used).

Secondary Containers
Autoclave bag contents may spill out or liquefy, block internal ports, and seriously damage the autoclave during the autoclave cycle, so it is critically important to place autoclave bags in secondary containers during decontamination cycles. Likewise, liquid media bottles must always be autoclaved in leakproof tubs. Polypropylene plastic or stainless steel tubs with 6-12 inch sides are commonly used to contain material in the autoclave. Do not use polyethylene or any other type of plastic tub except polypropylene because many other plastics will melt or distort permanently in the autoclave. Repeated autoclaving will eventually crack polypropylene—watch for this and replace these tubs at the first sign of "crazing" or other deterioration. Stainless steel containers with leakproof seams are durable and good conductors of heat, and may be good alternatives to polypropylene tubs.

Indicators:
Used to validate the sterilization/decontamination process.

- **Chemical indicators** change color after being exposed to 121°C (250°F), but most provide no information on how long they remained at that temperature.

- **Tape indicators** can only be used to verify that the autoclave reached normal operating temperatures for decontamination. Like most chemical indicators they provide no information on time at that temperature.

- **Biological indicators** including spore strips or ampoules are the "gold standard" — they verify that the autoclave is performing correctly and is capable of killing microorganisms. A load test using Geobacillus stearothermophilus for steam cycles should be performed at least monthly, and more often for autoclaves that are routinely used to inactivate infectious agents. Biological indicators for dry heat cycles usually use Bacillus atrophaeus (=Bacillus subtilis DSM 675 or 2277). Spore strips typically require incubation for up to seven days after the run, but many ampoule tests only require 48 hours. Place the spore strip or ampoule in the center of the load and retrieve it for completion of the test after the load is processed in the autoclave. If the load is a single bag, the strip should be tied or autoclave-taped to a length of cotton string or a wire and placed in the center of the bag contents with the string or wire extending out of the bag opening (some indicators are manufactured with an attached string). The string or wire is used to retrieve the spore strip after the run. When using biological indicators be sure to follow all instructions meticulously for use and post-run treatment. Avoid using spore strips in bags with disinfectant-soaked materials, because contact with the disinfectant may kill the strip agents and yield a potentially false negative test.

**Exhaust:**
Always use slow exhaust when autoclaving liquids. If you use fast exhaust the media will boil out of the bottles as the steam exits the chamber. Fast exhaust may be used for bagged or other dry material. If slow exhaust or a specific cycle intended for liquids is not available on
your autoclave, do not attempt to treat liquids in that unit.

**Recordkeeping:**
Records of maintenance, logs, chart recorders, calibration results and biological indicator load tests should be kept for at least three years.

**Service:**
A professionally trained service provider should inspect the autoclave according to the autoclave manufacturer’s recommendations for inspection intervals and service. Most such recommendations are based on cumulative hours of use rather than specific calendar intervals. Autoclave gauges should be calibrated at least annually. If an autoclave fails to function correctly or a user finds a problem between scheduled inspections, the unit must be professionally serviced. **Do not resume operation of an autoclave until it has been inspected and repaired.**

**Training:**
All individuals who use autoclaves must be trained. Autoclave users should understand the time, temperature, and pressure requirements and should also understand the correct loading procedure, the appropriate type of exhaust for the materials, and the correct method for opening the autoclave after a run. Users and facility managers must also be able to verify that the autoclave is functioning correctly, by using indicators or by monitoring the run parameters. Training must be renewed at least annually, and supervisors must maintain permanent training records.

**Best Practices:**
- Wear proper personal protective equipment, including heat resistant gloves, eye protection and a lab coat, when opening or unloading the autoclave.
- Do not pack dry material tightly in autoclave bags.
- Do not pack bags or other materials tightly into the autoclave chamber — leave some space between bags or bottles in the autoclave.
- **Never place tightly sealed containers such as screw-capped glass bottles in an autoclave — they will very likely explode during the autoclave cycle.** Bottles with narrow necks can also explode if filled with too much liquid.
- Do not autoclave solvents, volatile or corrosive chemicals such as phenol, trichloroacetic acid, ether, chloroform, bleach, or any radioactive materials. Call EH&S (530-752-1493) if you have questions regarding chemical waste disposal.
- After loading and starting the autoclave, processing time starts after the autoclave reaches normal operating conditions of 121°C (250°F) and 15 psi pressure.
- Sterilization/decontamination conditions vary with type of load, and therefore processing times will vary. A minimum of 30 minutes is needed to decontaminate an average load of biological waste. For best results use biological indicators to validate overall autoclave
performance and to validate specific runs when necessary.

- At the end of a cycle verify that the chamber pressure is less than 1.0 psi before opening the door. Open a hinged autoclave door just 2-3 inches and allow the steam to escape from within the autoclave before opening the door all the way. Stand well back from the autoclave door. Wear long-cuffed heat resistant gloves (or autoclave gloves) to protect your hands and forearms. Sliding type autoclave doors usually cannot be opened at all until the steam is evacuated from the chamber. Always open the door as slowly as possible.

- Allow materials inside the autoclave to cool for at least 10 minutes with the door open before unloading the autoclave. Removing contents too soon may heat stress the component material.

- After treatment in the autoclave, biotechnology and plant pathogen waste can be disposed as solid waste in the lab waste stream. At UC Davis animal pathogen waste (from the School of Veterinary Medicine) is usually disposed through the medical waste stream without prior autoclaving.

**Regulated medical waste must be treated in accordance with the Medical Waste Management Act requirements:**


**Contact**

**Biological Safety Office**

biosafety@ucdavis.edu 530-752-1493
FAX: 530-752-4527

**More information**