

Recommendations for Autoclave Use: Materials Sterilization and Biohazardous Waste Decontamination

Objective

The following guidelines serve to help autoclave operators ensure a safe and effective process. For questions concerning autoclave guidelines and safety contact the Office of Biological Safety at 263-2037 or biosafety@fpm.wisc.edu.

Introduction

The autoclave is a common fixture in many laboratories, making it easy to overlook the hazardous nature of the apparatus. The autoclave's job is to render contents sterile, or free of any living organisms. Autoclaving is an effective and economical process of killing microbes through the application of moist heat (saturated steam) under pressure. Heat damages the cell's essential structures rendering the cell nonviable. Failure to completely sterilize materials unnecessarily raises the risk of serious health hazards.

The hot, pressurized steam generated by autoclaves presents a serious burn hazard. Conditions inside steam autoclaves can reach a temperature as high as 134°C (274°F) and a pressure as high as 30 psi. Stringent safety precautions and diligent maintenance must be observed in order to minimize the potential for injury to users.

Due to product variations between autoclave manufacturers it is important to always reference and follow specific unit manufacturer's operations manual.

Training for autoclave users is an essential component a successful autoclave program. Some benefits achieved through proper autoclave user training:

- ✓ Minimize the risk for injury to staff and damage to equipment or facilities
- ✓ Ensure sufficient sterilization of laboratory materials
- ✓ Ensure proper decontamination of biohazardous materials in accordance with regulatory requirements

Definitions

<u>Autoclave:</u>	An apparatus (as for sterilizing) using superheated steam under pressure. Hot, pressurized steam is generated at 134°C (274°F), 30 pounds per square inch (psi), for large autoclaves; and the 121°C (249°F), 15 psi for small table top autoclaves.
<u>Contaminated:</u>	Soiled, stained or infected by contact or association with a biological substance
<u>Decontamination:</u>	Reduction of biological contamination to a level which no longer poses a risk to persons or the environment.
<u>Sterilization:</u>	A preventative treatment method which destroys living microorganisms

Autoclave Safety

Autoclaves generate extreme heat and high pressure. Users should understand and respect the hazards autoclaves can create. Lack of proper maintenance or misuse will result in damage to the apparatus and facilities or injury to personnel.

Mechanical hazards include the large, heavy door, a loading carriage (if so equipped), and the common presence of glass in load materials. Autoclave doors and gaskets must be firmly locked into place before running 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 is improperly closed.

The exterior surfaces of autoclaves are typically very hot, particularly older models which have little or no heat shielding. Attach conspicuous warning signs to remind people of the heat hazard. Do not stack or store combustible materials next to an autoclave (e.g. cardboard, plastic, volatile or flammable liquids). Use heat-resistant gloves and appropriate Personal Protective Equipment (PPE) when removing materials from an autoclave. Take care not to touch the inner chamber surfaces and to avoid splashes of liquid materials.

If you are burned by contact with the autoclave or autoclaved materials, seek first aid and/or medical attention. You can treat minor burns yourself using standard first aid. Burns to the face, third-degree burns, or burns over large areas of the body are emergencies – seek immediate help and medical treatment (call 911). Regardless of the degree of severity, report the burn to your supervisor or principal investigator as an occupational injury. If a biological exposure or release occurs during an incident, a *First Report of Biological Exposure or Release Event* form must be completed and submitted to the Office of Biological Safety.

Each autoclave user must undergo operation and safety training prior to using the autoclave. Training must address the specific autoclave model(s) to be used. Familiarity with the manufacturer(s) operations manual is an essential element of use and maintenance training. If you cannot locate the manual, contact the manufacturer or search the manufacturer's web site to obtain a copy.

Training elements for autoclave use and safety should include:

- Proper use of Personal Protective Equipment (PPE)
- Minimization of aerosol formation
- Proper usage including loading and unloading recommendations
- Burn prevention
- Prevention of spills and accidents
- Procedure to manage spills and accidents

Autoclave Operation

A. User Precautions

- 1) All biohazardous materials, equipment, or apparatus should be autoclaved or otherwise disinfected before being washed for re-use or disposal in regular trash. Each individual working with biohazardous material is responsible for its disinfection before disposal.
- 2) It is useful to post or provide instrument operation instructions in close proximity to the autoclave.
- 3) Do not put sharp or pointed contaminated objects (such as serological pipettes or scalpel blades) directly into an autoclave bag. Place them in an appropriate rigid disposal container (such as a sharps container).

- 4) Use caution when handling an infectious waste autoclave bag, in case sharp objects were inadvertently placed in the bag. Never lift a bag from the bottom to load it into the chamber. Handle the bag from the top.
- 5) Do not overfill an autoclave bag. Steam and heat cannot penetrate as easily to the interior of a densely packed autoclave bag. Frequently the outer contents of the bag will be treated but the innermost part will be unaffected.
- 6) Do not overload an autoclave. An over-packed autoclave chamber does not allow efficient steam distribution. Considerably longer sterilization times may be required to achieve decontamination if an autoclave is tightly packed.
- 7) Do not autoclave items containing corrosives (e.g. acids, bases, phenol), solvents or volatiles (e.g. ethanol, methanol, chloroform), flammables, or radioactive materials.
- 8) Do not mix contaminated ("dirty") and clean items together during the same autoclave cycle. Clean items generally require shorter decontamination times (15-20 minutes) while a bag of infectious waste (approximately 24" x 36") typically requires 45 minutes to an hour to be effectively decontaminated throughout.
- 9) Before using the autoclave, check for any items left inside by previous users that could pose a hazard.
- 10) Be sure to clean the drain strainer before loading the autoclave in accordance with manufacturer's recommendations.

B. Autoclave Loading

- 1) Load the autoclave properly as per the manufacturer recommendations.
- 2) Loosen caps of containers of liquids prior to loading into the autoclave; sealed bottles may shatter or explode during pressurization. Never seal a liquid container with a cork or stopper.
- 3) Bags of autoclavables should be loosely closed to allow steam to penetrate to contents and maximize sterilization. Add water to a bag if the load is dry.
- 4) To contain liquid spills, place bottles/containers in a solid-bottom pan. To improve even heating of bottles, add 1-2 cm of water to the pan prior to autoclaving.
- 5) Place individual glassware pieces inside a heat resistant plastic or metal tray that sits on a shelf or rack; never place directly on the bottom or floor of the autoclave.
- 6) Use ONLY plastics known to be autoclavable. Non-autoclavable plastic materials will melt and plug drain line.
- 7) Make sure the door of the autoclave is fully closed and the correct cycle has been selected before starting the cycle.
- 8) Make a record of the run date, user's name, lab, cycle run, and nature of material/load.

C. Autoclave Unloading

- 1) After the completion of a run, slightly crack open the door. Wait a full five minutes if the autoclave load contains only dry glassware, and no less than ten minutes when you are autoclaving liquids before removing the items.
 - a) Never attempt to open the door while the machine is operating.

- b) Be sure steam pressure is down before opening the door.
- 2) Open the door slowly, keeping head, face, and hands away from the opening.
 - a) Be aware that evacuating the air from the chamber of the vacuum steam sterilizer prior to sterilization of contaminated material can create a potential hazard by releasing infectious material to the atmosphere.
- 3) When removing items from the autoclave, always wear appropriate PPE, including heat resistant gloves, lab coat and eye protection. A rubber apron, rubber sleeve protectors and a face shield are encouraged.
 - a) With liquid loads, be alert for a bottle still bubbling. Let liquid loads stand in an out-of-the-way place for a full hour before touching with ungloved hands. Scalding liquids can cause serious harm.
 - b) Remove solutions from the autoclave slowly and gently; some solutions can boil over when moved or when exposed to room temperature. Be aware that gels will melt when autoclaved.
 - c) Be on the alert for closed containers when emptying autoclaved contents. Superheated liquids may spurt from closed containers.
- 4) Clean up any spills immediately with appropriate precautions and cleaning materials
 - a) If there is a spill inside the autoclave chamber, allow the unit to cool before attempting to clean up the spill.
 - b) If glass breaks in the autoclave, use tongs, forceps or other mechanical means to recover fragments.
 - c) Do not use bare or gloved hands to pick up broken glassware.
- 5) For non-liquid loads allow items to cool for 15 min. before touching with ungloved hands.
- 6) For liquid loads, allow items to stand for a full hour before touching with ungloved hands. Post signage or notify others of the heat hazard present.
- 7) Do not to leave an autoclave operating unattended for a long period of time. Always be sure someone is in the vicinity while an autoclave is cycling in case there is a problem.
- 8) Waste material loads, once decontaminated and removed from the autoclave, should be marked for disposal by application of an "OK to Trash" sticker. Stickers can be obtained from the OBS.

Transporting Materials

The necessity for autoclave users to transport their materials to and from autoclaves increases potential for accidents in laboratory buildings; therefore, measures should be established to avoid spills of potentially infectious biological materials.

The following are guidelines for the transport of materials to or from autoclaves:

- 1) All materials being transported both to and from autoclaves are to be transported on carts, trays, or in bags.
- 2) All soiled material transported for decontamination must have secondary containment on the carts in order to minimize any spills. Secondary containers should be leak-proof.
- 3) All containers with biological/biohazardous material (liquid or solid) should be securely capped during transport. Caps can be loosened or removed just prior to autoclaving.

- 4) Containers of high-risk or highly pathogenic materials may require additional transport steps, including:
 - a) Surface decontamination with an appropriate disinfectant prior to transport to autoclave area
 - b) A sealed secondary container during transport
 - c) A transport cart equipped with spill containment materials (absorbent pads, gloves appropriate disinfectant)

Routine Maintenance and Efficacy Testing

The best way to ensure that an autoclave is performing properly is to conduct routine maintenance. Follow the manufacturer's recommendations for preventative maintenance and make sure all contractors are approved by the manufacturer. Users should perform the daily and weekly maintenance procedures described in the owner's manual.

Additionally, regular testing should include monthly efficacy tests with biological (i.e. *Bacillus stearothermophilus* spore testing) or chemical indicators (i.e. 3M™ Comply™ SteriGage™) that verify adequate temperatures and times have been reached inside the material/load to kill microorganisms. Sterility test results must be recorded and retained (a log book is useful for these records). Indicators (often called spore vials or strips) can be ordered through a laboratory supply company or UW MDS <http://www.bussvc.wisc.edu/mds/mds.html>.

Although many autoclave bags or tapes are imprinted with a dye that changes color when the correct temperature is reached, a positive reading does not ensure that the innermost parts of a large load are also sterile. The problem with this type of check is that the dye is on the surface of the load. To ensure full sterilization testing indicators should be buried in the center of a load to validate adequate steam penetration.

Examples of safe testing methods include:

- Place the fresh spore vial or strips inside a bag that has already been through a sterilization cycle for the test. Repeat the same sterilization procedure exactly. Then remove the strips for incubation.
- Place the fresh spore vial or strips inside of an autoclave safe screw cap tube. Tie a string around the neck of the tube. Bury the tube in the center of the load as the bag is filled. Thread the string out of the top of the bag before you tie it with autoclave tape. After the kill cycle is completed, open the bag and pull on the string to retrieve the spore strip for incubation.

The sterilization chamber should be cleaned and drained routinely according to the manufacturer's guidelines. In absence of manufacturer guidelines, the following procedure may be used:

- 1) Wash wetted portion of the chamber thoroughly using a mild diluted detergent (e.g. calgonite).
- 2) If a soft cloth or brush and detergent do not completely remove the surface film, a nylon soap pad may be used.
- 3) Never use a wire brush, abrasives, or steel wool on door and chamber assembly.
- 4) After washing, thoroughly rinse with clean, soft water.
- 5) Dry the chamber and leave open overnight.
- 6) Record the maintenance in the appropriate log.

Autoclave Failure

Autoclave failure is usually due to operator/user error or mechanical failure. Examples of operator/user error may include:

- Improper use of containers that block access of steam to the load
- Not properly venting bags or containers prior to autoclaving
- Not adding water to a dry load before autoclaving
- Autoclaving a bag of waste that is too large for the autoclave
- Over-filling an autoclave bag.

Mechanical failure of the autoclave must be addressed by a trained technician.

Contact the service company responsible for the maintenance of your autoclave or your department's safety representative for more information.

Recordkeeping

All autoclave records must be retained for a minimum of three years. Retention of records is the responsibility of the autoclave maintenance staff and/or autoclave users. All autoclave users should be aware of who retains records and/or where the records are stored.

Records to be retained are:

- Training records for all users
- Maintenance records
- Calibration records
- Autoclave logs
- Performance and efficacy testing records

Resources

- UW-Madison Environmental Health & Safety Department, Office of Biological Safety
First Report of Biological Exposure or Release Event, <http://www2.fpm.wisc.edu/biosafety/>
- Occupational Safety and Health Administration (OSHA), <http://www.osha.gov/index.html>
- Materials Distribution Services (MDS); University of Wisconsin - Madison
<http://www.bussvc.wisc.edu/mds/mds.html>
- Michigan State University
Office of Radiation, Chemical & Biological Safety
<http://www.orcbs.msu.edu/>
- University of Arkansas at Little Rock
Environmental Health and Safety
<http://ualr.edu/physicalplant/index.php/home/environmental-health-safety/>
- University of Missouri – Columbia
Environmental Health and Safety
<http://ehs.missouri.edu/>
- University of Ottawa
Office of Risk Management, Environmental Health and Safety Service
<http://www.uottawa.ca/services/ehss/>
- University of Pennsylvania
Office of Environmental Health & Radiation Safety
<http://www.ehrs.upenn.edu/>