KREC Autoclave

Standard Operating Procedures

Summary

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. An autoclave's function is to pressurize stem in an airtight chamber that increases the steam temperature inside the compartment significantly. The superheated steam kills all microorganisms and degrades most macromolecules rapidly. Autoclaves are generally easy to use but have the potential to be very hazardous and dangerous 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 standard operation procedures provides a review of the proper use of an autoclave to ensure these devices are used safely, effectively, and in compliance within 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, in addition to the conditions inside the autoclave chamber such as materials, containers, container placement, and total volume of the materials 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 material within a reasonable amount of 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. This allows steam to circulate completely around the material and into the load. Less dense materials such as bagged waste can occupy more space but should never come in contact with the autoclave chamber wall.
- ***Contact:*** In order to sterilize or decontaminate uniformly, superheated steam must contact all areas of the load.
- ***Time:*** Standard autoclave loads up to about 2.0 ft in volume require 30-60 minutes to sterilize at 15 psi and 121°C. Larger loads and tightly packed materials may require a longer time run with other factors to be considered before setting the time and temperature of the autoclave load.

Dry Heat

Some autoclaves offer dry heat cycles, which are useful for sterilizing laboratory supplies such as pipette tips that can withstand high temperatures but would be damaged by steam. The necessary exposure times for dry heat varies considerably depending on the materials composition, packaging, load volume, and possibly other factors. This may require more than triple the time needed for steam sterilization at the same temperature. In some cases, the required times for successful dry heat sterilization may 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 sometimes printed with the universal biohazard symbol. If the waste being autoclaved is not contaminated with insect infectious agents or certain high hazard plant pathogenic agents, the biohazard symbol is inappropriate and not be used for that material. 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.
- ***Bottles —*** Glass bottles with screw tops are used for autoclaving liquids such as water, some buffers, and culture media. Always loosen the screw top cap so that it barely engages the threads on the neck of the bottle. This allows steam pressure to be released from the bottle preventing it from being explosive or breaking the container. 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.
- ***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).
- ***Other –*** If you are unsure materials are autoclavable, please check product with vendor or with EH&S Specialist.

Secondary Containers

Autoclave bag contents may spill out or liquefy, block internal ports, and seriously damage the autoclave during an autoclave cycle. It is critically important to place autoclave bags in autoclavable secondary containers during a 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 with the exception of polypropylene. Non-polypropylene containers or other plastic containers will melt or distort permanently in the autoclave, cause a toxic/ noxious fume and could potentially long-term damage 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.

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 a specific cycle intended for liquids is not available on your autoclave that would include time for slow exhaust, do not attempt to treat liquids in the autoclave 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. Some recommendations are based on cumulative hours of use rather than specific calendar intervals. Autoclave gauges should be calibrated annually. If an autoclave fails to function correctly or a user finds a problem between scheduled inspections, the unit must red taped and professionally serviced before using. Do not resume operation of an autoclave until it has been inspected and repaired.

Training
All individuals who use autoclaves must be trained and must have continued annual training. 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 annually, and supervisors must maintain permanent training records. Online and in person training are required. Please schedule with Ryan or Julie.

Emergency Procedures
If an injury occurs during autoclave use, seek prompt medical attention.

- If injury needs medical attention contact 911.
- Contact front desk at 559-646-6011
    - Let them know your name and contact number.
    - Let them know the injury.
    - Let them know where you are located.
    - Contact your supervisor.
    - Fill out within 24 hours and incident report.
- If the injury does not require 911 assistance
    - Contact EH&S (209-573-3674)
    - Contact your supervisor.
    - Fill out within 24 hours and incident report.

Contact
UCANR Kearney Ag Research and Extension Center
Environmental Health and Safety Office
9240 S. Riverbend Ave
Parlier, Ca 93648
Julie Pedraza
559-646-7318
jjpedraza@ucanr.edu

Autoclave Failure

Discontinue use immediately if the autoclave is not working properly or fails the pre-inspection checklist. Do not attempt to fix the problem. Post a sign alerting others the autoclave is "Out of Service." Notify Ryan, Julie or Patrick.

General Autoclave Safety Practices

Caution: Do not autoclave items containing corrosives, solvents, volatiles or non-autoclavable materials.

- Prior to Loading
  - Fill out the user log.
  - Conduct a pre-inspection checklist.
  - Check inside the autoclave chamber for any items left by previous users that could pose a potential hazard.
  - Ensure the drain strainer is clean and free from obstruction before loading the autoclave.
  - Ensure the door gaskets have not deteriorated but are still intact and are pliable.
  - Note: If you are the first user of the day, use the touch screen to acknowledge that there has been a power interruption. Then proceed.
    - It will take about 30 minutes from the start up time for the generator to reach ~40 psi and the jacket to be at least 18 psi.

      Acknowledge when jacket is pressurized **Press this box when the jacket is about 18 psi or higher.**
- Loading the Autoclave
  - Do not overload the autoclave. Load autoclave according to the manufacturer's recommendation. Review the above training content.
  - Individual glassware pieces should be placed in an autoclavable secondary container on a shelf or rack and never placed directly on the autoclave chamber bottom or floor.
  - Make sure the door of the autoclave is fully closed, latched and secure.
    - **Close door, engaging pins by moving 1-handled wheel to the right until it stops. Tighten pins by gently rotating the 3-handled wheel clockwise until only as tight as you can make it with ONE FINGER.**
  - Verify the setting are on the correct cycle for the items being autoclaved before starting the cycle.
    - Select appropriate control cycle "Liquid" or "Gravity".
      - Liquid is for batches containing liquids.
      - Gravity is for dry items only.
  - Press "Run"
    - A) Conditioning evacuates air for a couple of minutes.
    - B) Waiting for Temp = when temperature is raping up in chamber.
    - C) Sterilizing= holding target temp for desired time.
    - D) Exhausting.
    - E) Cool Down.
- Opening the Autoclave
  - Wear the proper PPE, including…
    - Heat resistant gloves covering your arms.
    - Rubber apron for liquid materials.
    - Lab coat for dry materials.
    - Eye protection.
    - Closed-toe shoes if handling sharps (ex. broken glass)
    - Recommend using of tongs, cut-resistant gloves, or face-shield when handling hot liquids.
  - After Cool Down Cycle is complete the screen will read "**COMPLETE Open Door**".
  - **Verify the chamber pressure is at "0" psi.**
  - **Reverse the steps from how you closed the door.**
    - **Turn the 3-handeled wheel slightly clockwise, then fully counterclockwise. Don't force it. Disengage pins by moving the 1-handled wheel to the left.**
  - Open the door slowly, keep your head, face, and hands away from the opening.
  - 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.
  - Make sure to leave the autoclave clean for the next user.
- **Autoclave Failure**
  - Discontinue use immediately if the autoclave is not working properly or fails the pre-inspection checklist. Do not attempt to fix the problem. Post a sign alerting others the autoclave is **"Out of Service."** Notify Ryan, Julie, or Patrick.

Revised: 11/2023 JJP

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, leave an opening to allow heat to enter and exhaust out of the bag.
- Do not pack bags or other materials tightly into the autoclave chamber — leave some space between bags or bottles in the autoclave.
- When autoclaving liquid in bottles, place them in a secondary container with an inch of water to ensure content heats evenly.
- Bottled liquids should not be more than 2/3 full and screw cap lose on the bottle.
- Space bottles 2 inches between each other to allow circulation.
- 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. These incidents could lead to spill overs or broken glass.
- Do not autoclave solvents, volatile or corrosive chemicals such as phenol, trichloroacetic acid, ether, chloroform, bleach, or any radioactive materials. ***Call EH&S (559-646-7318/ 209-573-3674)*** 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 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, biologicals and plant pathogen waste can be disposed as solid waste in the dumpster outside.