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There is no response required.*

Autoclave Operations for Sterilization & Decontamination, Validation & Quality Assurance

1. General Autoclave Use Guidelines

An autoclave has broad applications in research and clinical settings. It is designed and operated to render its contents sterile, or free of any living organisms. If it fails to do so, research and clinical operations can be negatively impacted.

Autoclaves are frequently used in research for sterilization of tissue culture media, laboratory glassware and related items as well as for decontamination of certain waste materials from research laboratories¹.

In human and animal applications, serious health hazards can result if validation and quality assurance (QA) measures are not in place and carried out appropriately. In addition, the hot, pressurized steam (270° Fahrenheit, 30 pounds per square inch gauge) that autoclaves generate to do this job makes them serious burn hazards as well. And, because conditions created inside steam autoclaves are so extreme, autoclaves can easily malfunction if they are not carefully maintained.

Newer, modern autoclaves frequently rely on computer programming that has been pre-set for sea level operations. Adjustments may be required to operate properly at the Denver-Aurora altitude. Supervisors and PIs should assure that all research staff understand the instructions for autoclave use.

Before using any autoclave for the first time, read and thoroughly understand the owner's manual because many makes and models have unique characteristics. If you cannot locate the manual, contact the manufacturer and have a copy sent to you.

Training

Training on the safe and appropriate use of autoclaves should be developed, written and implemented as part of the On-the-Job training in each unit, so that all users are trained before operating an autoclave. Operator Manuals and any applicable written Standard Operating Procedures must be readily accessible for all users. Users should understand the time, temperature, pressure relationships required for proper materials

decontamination. Additional training on handling materials to be decontaminated should also be provided. Supervisors should maintain a permanent record of training provided to their staff.

Autoclave Settings and Applications

An autoclave uses different patterns of high heat, vacuum, and pressure to sterilize a load. The type of materials you sterilize will determine the type of sterilization "runs" you must use.

In general, categories of use are: "liquids" for any type of water-based solutions or "dry goods with vacuum," or "dry goods without vacuum."

You must follow manufacturers' instructions about loading, load sizes, and cycle types and settings carefully to achieve sterilization or decontamination. The "dry goods without vacuum" run simply pressurizes the chamber with steam for the duration of the cycle, and then returns to normal. This process is used primarily for items that have been cleaned but need to be sterilized. Materials should be packed so that the heat and pressure can readily reach the whole load.

You must assure seals on containers of liquids are loose so vapor expanding during heating will not cause an explosion. Never autoclave any flammable or volatile liquids because they could explode. Never autoclave liquids which contain bleach or other hazardous chemicals.

When an autoclave is used for decontamination procedures, i.e. with biomedical wastes, the "dry goods with vacuum" run is the most appropriate for this application. This "run" assures steam and heat will penetrate into the deepest parts of large bags or bundles of materials and produces the best conditions for killing persistent or infectious organisms.

It is important that steam and pressure be able reach the entire load, so carefully loosen autoclave bag closures once they are in the autoclave.

2. Quality Assurance

It is imperative to know that the autoclave has thoroughly sterilized its contents, particularly for those items or materials (such as biomedical wastes) which **must have a documented record of decontamination.**²

While most autoclave bags or autoclave tapes are imprinted with a dye that changes color when the correct temperature is reached, this type of indicator is not sufficient to document sterilization or decontamination for most regulatory requirements. The dye is on the surface of the load, and does not serve to indicate that the innermost parts of a

large load are also sterilized. A biological monitoring SOP (as described below) must be implemented.

Biological Indicator Monitoring

Biological indicator systems are designed to demonstrate that an autoclave is capable of killing microorganisms. Typical biological indicator systems consist of a vial with spore strips or a small glass ampule of growth medium with spores and indicator dye.

For those autoclaves which are used for routine sterilization or decontamination of research materials, where it is imperative to demonstrate and document the loads are rendered sterile, the standard-of-care requires the use of a biological indicator³ **at least monthly** to confirm that the autoclave is working properly. If the biological indicator fails, you must examine the autoclave to identify and correct the problem. You will also re-autoclave the load in a properly working autoclave, to ensure sterility.⁴

Users must use a biological indicator (e.g. *Bacillus stearothermophilus*) placed at the center of a load processed under standard operating conditions to confirm the attainment of adequate sterilization conditions. The biological indicator is removed from a load after it has been autoclaved. Then, the biological indicator is incubated at 56°C for up to three days.

If the autoclaved biological indicator is turbid (cloudy, indicating growth) the autoclave did not function properly. The supervisor or PI should be notified if this occurs, and a maintenance call should be made to address the problems. A sign should be posted indicating the autoclave is not in working order until such time as repairs are made.

Users are required to prepare written **Standard Operating Procedures** (SOP) for each autoclave used for decontamination processes for research materials or wastes treated on-site. The SOP must address each of the following, as appropriate for each type of load (e.g. biomedical waste, animal cages with bedding, tissue culture wastes, etc):

- Time & Temperature
- Pressure
- Type of waste
- Type of container(s)
- Closure on container(s)
- Pattern of loading
- Water content
- Maximum load quantity

Recordkeeping

It is the responsibility of each unit to ensure that training, recordkeeping, and testing is conducted for each autoclave assigned or belonging to their unit. Recordkeeping is best maintained by the individual users of the autoclave. Each unit should designate a "point-of- contact" who will routinely:

1. Test and assure proper operation of the unit;
2. Notify users when an autoclave is not functioning properly;
3. Complete all necessary Quality Assurance/Quality Control documents.

A durable note book should be used as a permanent record of autoclave use. The autoclave log book should be located in an easily accessed location near the autoclave. The autoclave log book for each autoclave should have at least the following information entered:

Autoclave Manufacturer	Autoclave Serial Number
Department	Room Location
Date Log Book Started	PM/Maintenance/Repair Work Done

The main section of the autoclave record should be set up to including the following information:

- Autoclave User
- Date Used
- Materials Decontaminated/Sterilized
- Process Type
- Run Duration (Cycle Time)
- Biological or other Indicator Used
- Indicator Results, and

A means of collecting “Wheel Graphs” or “Data Strips” for documentation.

For those units required to decontaminate wastes on-site, users must maintain records and procedures specified for temperature monitoring, quality assurance/quality control, and biological indicator monitoring for a period of not less than two (2) years for the decontamination of potentially infectious materials and wastes.

Disposal of Spores

Live incubated or other live biological spores should be autoclaved again before disposal or incinerated. Please refer to the [Biomedical Waste Disposal Plan](#) for additional disposal information.

Temperature Monitoring

Users must check and document recorded temperatures of each complete cycle to ensure the attainment of a minimum temperature of two hundred fifty degrees Fahrenheit (250°F) or (121°C) for at least one-half (1/2) hour or longer, depending on quantity and compaction of the load, in order to achieve sterilization of the entire load.

Note: Depending on the nature of the materials, greater time and / or temperatures may be necessary to effectively sterilize a load.

Tape or Bag Indicators

Users must use a heat-sensitive tape or other device **for each load that is processed** to indicate the load has undergone the steam sterilization process.

Tape indicators are adhesive backed paper tape with heat sensitive, chemical indicator markings. Autoclave bags are made with similarly heat sensitive, chemical indicator markings.

These are suitable for and should be used on every load, for verifying “runs” of individual loads, only to indicate a temperature of at least 250° F (121° C) was reached during the sterilization process and to show that the material has been processed.

A three to four inch strip of autoclave tape placed on the outside of the autoclave pan, bag, or individual container is sufficient. If the temperature sensitive tape does not change color, the materials or biomedical waste is not considered decontaminated.

Note: These indicators can only be used to verify that the autoclave is reaching normal operating temperatures for decontamination, 121°C (250°F). Tape indicators alone are not designed nor intended to prove that organisms have actually been killed nor do they indicate a load was heated for the proper time.

3. Preventive and Routine Maintenance and Repairs⁵

The best way to ensure an autoclave is working properly to sterilize and decontaminate materials and wastes, is to have regular preventive maintenance and maintenance activities performed at least semi-annually, and every 3 months is preferable and may be required by the Institutional Biosafety Committee or the Biosafety Office for high use/high impact areas.⁶

Arrangements can be made with the manufacturer representative, under a service contract, with other qualified vendors, and if staffing and resources permit, the Building Maintenance & Operations unit of the Dept. of Facilities Operations will provide fee-for-service preventive maintenance and repair services to University departments when a contract is established. The current policy on available autoclave services by BM&O is on the webpage at <http://www.ucdhsc.edu/admin/facilities/services.htm>

In addition, users should perform the daily and weekly maintenance procedures described in the owner's manual. Users should assure the drain strainer is clean before each run. It is worthwhile to document these procedures in the QA documents kept on each autoclave.

Malfunctioning Autoclaves

If an autoclave is not working properly, discontinue use immediately. Post a sign alerting others not to use the autoclave and notify the PI or Lab Manager to arrange for repair.

4. Autoclave Safety

Autoclaves generate extreme heat and high pressure. Users should understand and respect the hazards these can create.

Autoclave doors and their gaskets must be firmly locked into place before running the autoclave to prevent a sudden release of high pressure steam.

At the end of a decontamination cycle make sure that the pressure in the autoclave chamber is near zero before opening the door. Slowly crack open the autoclave door and allow the steam to gradually escape from within the autoclave.

CAUTION: Opening the autoclave door too quickly may result in glassware breakage and/or steam burns on your skin. The decontaminated materials should be allowed to cool for 10 minutes before they are taken out of the autoclave.

Most, but not all, autoclaves have safety interlocks that prevent the autoclave from running if the door isn't closed properly. Determine if yours has an interlock--you'll need to use extra caution if it doesn't.

Do not stack or store combustible materials next to an autoclave (cardboard, plastic, volatile or flammable liquids).

Use heat-resistant gloves when removing materials after sterilization and avoid touching the inner chamber surfaces.

If you are burned, treat it as an emergency.

You can treat minor burns yourself using standard first aid. If it is a more severe burn, call 911 from any campus phone to get help.

Regardless of the degree of severity, report the burn to your supervisor or Principal Investigator as an occupational injury and follow up with appropriate medical care.

5. Considerations for Your SOPs

Primary Containers

Polypropylene bags are typically used to contain biomedical waste materials during decontamination cycles in autoclaves. Also known as "biohazard bags" or "autoclavable bags" they come in a wide variety of sizes, shapes and colors.

Autoclave bags are usually placed in polypropylene or stainless steel pans during decontamination cycles to catch liquids that may drain out of the bag.

Autoclave bags must be left loosely opened during decontamination “runs” to allow steam to penetrate into the bag.

Things to Avoid:

Avoid dead air pockets where steam cannot penetrate (ie., closed screw cap tubes) because temperature within the air pocket is much lower than the saturated steam.

Avoid dry packages, add some water to the load. To avoid creation of infectious aerosols while adding water, trickle water down the sides of the container instead of pouring water directly onto the material in the container.

When recycling media bottles or preparing sterile media

The borosilicate glass bottles (that we receive media in) are often recycled to make more media. These bottles need to be autoclaved on a repeated basis. Putting just enough water in the bottom of the secondary container or tray in which the bottles are placed, to cover the bottoms of the bottles, will reduce thermal shock on the bottom of the bottles and minimize breakage. It won't eliminate breakage entirely.

Secondary Containers

Use a tray or secondary container with a solid bottom and walls to contain any spills. Add a quarter to a half - inch of water when recycling/sterilizing media bottles so they will heat more evenly.

Processing Times

After loading and starting the autoclave, **processing time starts after the autoclave reaches normal operating conditions:** Temperature = 121°C (250°F); Pressure = 15 psi.

Decontamination conditions will vary, based upon the type of load, load volume (loose packed or tightly packed), container type (polypropylene, glass, stainless steel), and type of material to be decontaminated. Processing times will vary according to the conditions of each decontamination cycle and should be documented in the SOP. In general, the larger the load, the longer it will take to decontaminate.

The processing time to decontaminate lab and medical waste is at least 60 minutes (unless a shorter interval has proven effective when tested with biological indicators). Add additional time if polypropylene containers are used.

A minimum of at least 90 minutes is recommended for decontaminating bagged biomedical wastes, in low sided polypropylene containers with bags half filled and loosely gathered. If bags are tightly closed, a processing time of 120 minutes is

recommended. If your autoclave is equipped to operate at 132°C (270°F), you may be able to reduce processing time if the *Bacillus stearothermophilus* spores are killed at the shortened cycle time.

Suggested Autoclaving Times

A minimum decontamination processing time of 60 minutes is recommended for materials in metal pans with the lid removed. The US Environmental Protection Agency (EPA) has reported that "Infectious wastes from departments of health care facilities may be rendered noninfectious by subjecting the waste to autoclave temperatures of 121°C (250°F) and 15 minutes of prevacuum of 15 psi for the following dwell times when proper containers are used:"

EPA Recommended Decontamination Processing (Dwell) Time

ITEM	DWELL TIME
Trash	60 Minutes
Glassware	60 Minutes
Liquids	60 Minutes / Gallon
Animal Carcasses	Do Not Autoclave
Animal Bedding	120 Minutes

Other considerations

- Load the autoclave properly per manufacturer recommendations.
- Before loading containers of liquids into the autoclave, the caps must be loosened to avoid having the bottles shatter during pressurization.
- Don't load non-autoclavable plastic materials.
- Place individual glassware pieces inside a heat resistant plastic tray that sits on a shelf or rack. Never place glassware directly on the bottom or floor of the autoclave.
- Make sure the door of the autoclave is fully closed and the correct cycle has been selected before starting the cycle.
- Wear heat-resistant gloves when first opening the door after a run.
- 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.
- When removing items from the autoclave, always wear a rubber apron in addition to rubber sleeve protectors, heat resistant mitts and a face shield.
- Remove the load and let the glassware cool for 15 minutes before touching it with ungloved hands.
- 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.

¹ Certain laboratories and facilities of the University may have on-site treatment plans for biomedical wastes or research related wastes in accordance with federal, state & local regulations , as directed by the Institutional Biosafety Committee or Biosafety Office, or to comply with infection control measures otherwise required.

² Under current federal laws, all biomedical wastes generated in work with **Category A agents** (per U. S. DOT regulations) must be treated on campus, by an appropriate method prior to co-mingling with biomedical wastes that are transported off-campus for further treatment and disposal. Contact the Biosafety Office, Dept. of Environmental, Health and Safety, 303-724-0235 or **biosafety.program@uchsc.edu**

³ Various kits are available from science supply companies. The UCDHSC School of Dental Medicine also provides a service for **Sterilization Monitoring** as well.

⁴ One means to verify sterilization or decontamination for an individual load, which does not need a documented record of treatment, is to wrap something with autoclave tape (a disposable plastic test tube or pipette tip are possibilities), and attach string to it as its being put deep into the load. Tape the other end of the string to the outside of the bag so that you can easily pull the indicator out (Do NOT open up a load of potentially infected material to bury something inside). You can then recover the indicator after the run and confirm that it too has changed color.

⁵ UCDHSC Facilities Operations has specific information on the services they provide and any charges that may apply to set up a Preventive Maintenance program for autoclaves and/or provide repairs. Please consult Facilities Operations, Building Maintenance and Operations Section

⁶ Any UCDHSC research laboratory required to autoclave biomedical wastes to comply with federal and state infectious/regulated medical waste regulations must have a quarterly PM program in place. Questions about this should be directed to the Biosafety Office of the Department of Environmental Health and Safety, 303-724-0235 or by email to **Biosafety.Program@UCHSC.edu**