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Université des Acadiens

Information Sheet – Autoclaves

Autoclaves are sterilizers using high pressure and high temperature steam to sterilize media, glassware, instruments, waste, etc. To accomplish the desired end goal — and to protect the user and the environment from hazardous materials — the autoclave must be used correctly. Additionally, waste must be managed in compliance with state and local regulations.

Physical hazards involved with steam autoclaves are heat, steam and pressure. Biological hazards involve potential exposure to viable human pathogens.

Due to the high heat and pressure created in autoclaves during operation, you must follow proper loading, use and unloading procedures to prevent burns and other accidents. Burns can result from physical contact with the structure of the autoclave, and steam burns can occur from contact with steam leaving the apparatus. Burns can also result from careless handling of vessels containing hot liquids. Explosive breakage of glass vessels during opening and unloading and because of temperature stresses, can lead to mechanical injury, cuts and burns.

Autoclave performance for sterilization purposes is dependent on proper use.

General Precautions

If you are a principal investigator (PI), it is your responsibility to ensure that:

- staff and all students working in your lab are trained before operating any autoclave unit
- everyone understands and follows procedural and instructional documents

Personnel who use an autoclave must be trained to understand the proper operation procedures by the department.

Notre Dame University has posted a great video on "On-Site Biohazardous Waste Management: Autoclaving". Additionally, instruction sheets should be located in each autoclave room to assist with the operation of the equipment.

You must autoclave all potentially infectious materials before you wash, store or dispose of them.

You must label biohazardous materials as such, and you must sterilize them by the end of each workday, or you must secure them appropriately. Biohazardous materials should not be left in an autoclave overnight in anticipation of autoclaving the next day.

For the autoclave process to be effective in achieving sterilization, sufficient temperature, time and direct steam contact are essential. The air must be completely removed from the sterilizer chamber and from the materials to allow proper steam penetration.

Factors that affect air removal include:

- type and quantity of material to be autoclaved
- packaging load density and configuration
- container type, size, and shape

Associated risks

The potential safety risks for autoclave operators are:

- heat burns from hot materials and autoclave chamber walls and door
- steam burns from residual steam escaping the autoclave and from materials on completion of cycle
- hot fluid scalds from boiling liquids and spillage in autoclave
- hand and arm injuries when closing the door
- bodily injury if there is an explosion

Protection against scalds and burns

You must wear personal protective clothing and equipment when loading and unloading the autoclave, including:

- heat-insulating gloves to protect hands and forearms
- face shields to protect face and neck, if applicable
- lab coat to protect chest and legs
- closed-toed footwear to protect feet

Preparation for Autoclaving

Ensure that the material is able to be autoclaved. Items that should not be autoclaved include:

- oils and waxes
- some plastics
- flammable materials
- radioactive materials

- substances that may emit toxic fumes
- samples containing solvents

Use appropriate containers and packaging

Glassware should be heat-resistant borosilicate (Pyrex or Kimax), and you should inspect it for cracks prior to autoclaving.

Plastics should be heat resistant, for example:

- polycarbonate (PC)
- PTFE (“Teflon”)
- polypropylene (PP)

Prepare and package material suitably. You should wrap or bag loose dry materials in steam-penetrable paper or loosely cover them with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing the efficiency of the process.

Containers of liquid should be a maximum volume of 2/3 filled.

Cover all containers with a loosened lid or steam-penetrable bung to prevent pressure buildup and to prevent bottles from shattering during pressurization.

Use plain, unmarked containers for items that are not hazardous.

You should tag items or baskets with autoclave temperature tape.

Containing potential spills

Place items in containers to secure and contain spills. Place the following items inside a secondary pan (must be autoclavable plastic or a stainless-steel container) in the autoclave:

- containers of liquid
- bags of agar plates
- other materials that may boil over or leak

The pan must be large enough to contain a total spill of the contents. Open, shallow, metal pans are more effective in conducting heat and allowing air removal than tall plastic tubs. Adding some water to the secondary pan will help to heat items more evenly.

Loading the Autoclave

- Ensure the drain strainer in the bottom of the autoclave's sterilizer chamber is clean before loading the autoclave.
- Use a cart to transfer items to be autoclaved. To avoid back injuries, push the cart up to the autoclave door and gently slide the load into the autoclave.
- Never place autoclave bags or glassware in direct contact with the bottom of the autoclave.
- Place the secondary pan containing the items to be sterilized on the shelf or rack of the autoclave.
- You must use secondary containment pan under the bag to catch any leaks that may occur during autoclaving.
- Do not overload the autoclave. It is important to leave sufficient room for thorough steam circulation.
- Make sure that you have selected the correct cycle before starting the autoclave (see below).

Operating Parameters

The parameters for the sterilization cycle will depend upon the amount and type of material. Usually 121 degrees Celsius at 15 pounds-force per square inch (lbf/in2 or psi) for a minimum of 30 minutes. However, you can increase the temperature and cycle time depending on the size and type of load.

Autoclave manufacturers generally provide several pre-set cycle options that vary in the pre-set sterilization temperature, sterilization time and dry time.

- Gravity cycle: air removal from the autoclave chamber is achieved by gravity air purge; this is appropriate for loads where air removal from porous materials or penetration of steam into wrapped/packaged items is not required
- Vacuum cycle: air from the autoclave chamber is removed by pulsing between pressure and vacuum; this is suitable for wrapped or difficult-to-penetrate items
- Liquids cycle: gravity air purge removes air from the chamber as in the gravity cycle; deep vacuum is not used since liquids to be autoclaved would be expelled from their vessels

Unloading the Autoclave

After a run is complete, check the pressure gauge to ensure that the pressure in the chamber is "0." If pressure is not released, do not open the door. Inform your PI / supervisor.

Before opening the door, wear eye protection and heat-resistant gloves or mitts. Be sure to wear closed-toed shoes (hot condensate may drip from the door). Rubber aprons in addition to rubber sleeve protectors are advisable.

Use caution when removing liquids, molten agar, etc. Liquids, especially large volumes, may continue boiling for some time after autoclaving. To avoid being splashed with scalding liquid:

- do not agitate containers of super-heated liquid
- do not remove caps before unloading

Slide a cart to the opening of the autoclave and pull the autoclave secondary container onto the cart for transport. Place the cart in a low-traffic area while additional cooling occurs.

Before touching items with ungloved hands, allow for the following cooling times:

- glassware: 15 minutes

Please note: If a faulty condition exists (e.g., sterilizer did not finish the cycle, or water leaks out when the door is unlocked), inform the person responsible for contacting the autoclave service technician.

Using Heat-Resistant Autoclavable Bags

When you are autoclaving biohazardous waste materials inside heat-resistant autoclavable bags, be sure that you:

- use heat resistant autoclavable bags (usually clear) for waste materials that contain or may be contaminated with potentially infectious agents
- store biohazard waste bags in rigid leak-proof secondary containment pans
- store waste inside the autoclave room — not in hallways
- avoid compressing bags; this may create aerosols
- do not put sharp objects such as broken glassware into an autoclave bag
- use a secondary containment pan under the bag to catch any leaks that may occur during autoclaving

It is advisable to add some water to bags of solid waste (the water will vaporize into steam that will drive out residual air once sterilization temperature has been reached inside the bag).

Label the autoclavable bag with a “biohazard” symbol on it with commercially available autoclave temperature tape that changes color, e.g., visible black stripes or the word “autoclaved” appear once sterilizing temperature has been reached. Autoclave temperature tape only indicates that the desired temperature was reached — it does not indicate any information about time and pressure. Apply this tape across the “biohazard” symbol on the bag before autoclaving. Do not throw away any biohazard bags without covering all biohazard symbols.

Autoclave 45-60 minutes, at temperature and pressure of 121 degrees Celsius (250 degrees Fahrenheit) and 15 psi. When the cycle is finished, inspect autoclave temperature tape and visually check autoclaved bags. Bags should be left to cool for several minutes before removing them from autoclave.

Please note:

- parameter monitoring (pressure, time and temperature) is important to ensure efficacy of an autoclave
- biohazardous waste should not be left for "someone else" to autoclave

Repairs/Maintenance

No person shall operate an autoclave unless it has been inspected by a qualified inspector and a certificate of inspection has been issued. A current inspection certificate is posted near the autoclave.

Users are not to make repairs. Autoclaves shall be maintained and repaired by qualified people. Maintenance records of preventative maintenance records and repairs should be maintained by the department. This should include the date and company or employee name that performed any maintenance or repair to the unit. These verification records should be available upon request.

If the autoclave does not operate exactly as expected, the PI/LAB will place a notice on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected.

When maintenance work or repairs are needed, the PI/LAB must provide a safe work environment for the service technician. Please remove all items from the sterilizer chamber, clean any spills or leaks inside the chamber, remove untreated biohazardous materials from the vicinity, etc.

Incident Response

If any injury occurs, seek first aid or, if necessary, seek medical assistance by dialing:

Safety Office at 2-1840
University Police at 2-6447

If clothing is soaked in hot water/steam, remove clothing and place the injured area in cool water. Place a notice on the autoclave indicating that it is not to be used until:

- the cause of the incident is determined

- procedures are enacted to prevent future incidents
- the autoclave is deemed safe for operation

Report all other non-emergency incidents to the Safety Office at 2-1840 or by email to safety@louisiana.edu .

Spill Cleanup

Spills may occur from a boil-over or breakage of containers. You are not allowed to operate the autoclave until the spill is cleaned. If you are the operator, you are responsible for cleaning spills. The spilled material can be contained using materials from the spill kit to absorb or contain the spill.

Wait until the autoclave and materials have cooled to room temperature before starting the cleanup inside the autoclave.

Review the Safety Data Sheet(s) (SDS) of the spilled material(s), if appropriate, to determine which protective equipment, spill cleanup, and disposal protocols are necessary.

You must:

- clean the equipment and work area in order to collect and remove all spilled materials
- dispose of the waste following the protocol appropriate for the material

If materials have been intermingled, follow the cleanup and disposal protocol for the most hazardous component of the mixture.

Cracked clean glassware must be disposed of properly in a Sharps container, which may be obtained from Student Health Services.

Record the spill and cleanup procedure and inform Environment, Health and Safety so that the incident may be properly documented.