UNBC UNIVERSITY OF NORTHERN BRITISH COLUMBIA

BSL-11 Autoclaving 101

1. **Scope:**
   This SOP applies to all those who will be using any autoclave on campus and will help you to load the autoclave and successfully decontaminate or sterilize your materials. This SOP will help you become familiar with how an autoclave works, introduce the various different cycles and how and when to use what cycle.

2. **Responsibilities:**
   Principal Investigators, Research Associates, students and anyone who is using an autoclave

Materials: -

- Autoclave (appropriate SOP on using the autoclave)
- Autoclavable tray
- Autoclavable indicator tape or biological indicator
- Required PPE: use of heat-insulating gloves, long pants, lab coat and closed-toe shoes when loading and unloading the autoclave

3. **Safety:**
   - Autoclaves work using high temperatures (> 121°C) and extreme pressure (> 15 PSI) to effectively kill microorganisms or render a bio hazardous material inactive.
   - The potential risks of using an autoclave are heat and steam burns, hot fluid scalds, injuries to hands and arms from the door, or bodily injury in the event of an explosion. Additional injury or exposure to biohazardous material may be incurred if the contents of the biohazardous waste are packaged improperly.
   - Before opening the door ensure that the cycle is completed and pressure in the autoclave is zero before opening the door at the end of a cycle.
   - Make sure that no personnel, including you, are directly in front of the door
   - Crack the door open slowly at first to release any residual pressure/steam that may be in the chamber to prevent burns
   - Turn off the autoclave to allow it to cool before cleaning up any spills. (Spills must be cleaned up immediately after the cycle has been run). You may need to contact facilities to have the autoclave shut off before cleaning.
   - Never put volatile chemicals or solvents (alcohols, chloroform), or corrosive chemicals (bleach, acetic acid, formalin, fixed tissues etc.), or radioactive materials in an autoclave
   - Use extreme caution when moving hot liquids and glassware as these could easily shatter
   - Do not autoclave sealed containers as they pose an explosion risk

4. **Procedures:**
   Steam sterilization relies on three (3) parameters to eliminate microbes and organisms: Time, temperature, and pressure. And these parameters can be manipulated into different cycle recipes to sterilize various types of loads. However, the cycle requirement for every load type—such as red bags, media, glassware, cages, animal bedding, and pipette tips—can vary significantly.

While the vast majority of common laboratory loads can be sterilized with one of the following three (3) basic cycles: Gravity, Vacuum (or Prevac), and Liquids; it is important to familiarize yourself with the various cycles to ensure you are properly sterilizing your materials.

Liquid cycles

Liquids must always be run under a liquid cycle to avoid "boil-over". Boil-over is simply a liquid boiling so violently that it spills over the top of its container. Boil-over will occur if the pressure in your autoclave chamber is released too quickly during the exhaust phase of the cycle. Significant liquid volume can be lost to boil-over, and this can result in unwanted spills on the bottom of your chamber and subsequent cleanup. A liquid cycle exhausts slowly to help prevent liquids from boiling over. To help prevent boil-over during the exhaust phase, the chamber pressure must be released slowly. Controlling the exhaust rate allows the liquid load to cool off as the surrounding chamber pressure is decreased. Liquid cycles have a slow period of exhaust where gravity or vacuum cycles chamber pressure is released quickly.

- Used for liquids

Gravity Cycle

This is the most common and simplest steam sterilization cycle. During a Gravity Cycle, steam is pumped into a chamber containing ambient air. Because steam has a lower density than air, it rises to the top of the chamber and eventually displaces all the air. As steam fills the chamber, the air is forced out through a drain vent. By pushing the air out, the steam is able to directly contact the load and begin to sterilize it. At the end of the cycle, the steam is discharged through the drain vent. However, the load can still be hot and possibly wet.

- Used for glassware, waste (including biohazardous waste), vented containers, and certain types of unwrapped instruments, etc.

Vacuum Cycle (also called a prevac cycle)

There are certain types of applications where air is not easily displaced from the chamber. Gravity air displacement (as described above) is not as effective on porous loads or partially vented containers. A sterilizer configured to run a Vacuum Cycle will be equipped with a vacuum system. A typical Vacuum Cycle will begin with a series of alternating steam pressure injections and vacuum draws to dynamically remove the air from the chamber. Drawing a vacuum to remove ambient air from the chamber allows steam to be sucked into areas where it would otherwise have difficulty penetrating. The absence of air within the chamber allows "steam to penetrate the load almost instantaneously" resulting in more reliable sterilization and shorter sterilization cycle times . A post-cycle vacuum can be programmed to enhance and quicken the drying process.

- Used for porous materials, cages with animal bedding, wrapped goods, surgical packs, etc.

1. **Autoclaving solid materials or waste:**

Autoclaving solid materials or waste is done using a gravity or vacuum cycle. Studies have shown that the processing time necessary to achieve decontamination of biological material depends on several loading factors. The following factors all impact decontamination time:

- load size – If the load is very large or packed too tight the steam may not be able to penetrate to the center of the load. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load, if at all. For a larger load it may be necessary to add 10-15 minutes to a cycle
- type of container
- moisture content

To ensure adequate steam penetration of solids:

- pack solid materials loosely
- bags/containers should be placed in a large, leak proof polypropylene or stainless steel tray to avoid or contain spills
- before processing, open bags/containers so that the steam can penetrate and effectively raise the temperature for adequate killing
- a small amount of water may be added to ensure heat transfer inside the bag/container. If a bag is closed during autoclaving, the temperature of the contents may not be raised sufficiently for decontamination.
- If you are processing more than one tray make sure that there is ample room between the trays so steam circulation is not impaired

The Right way: Proper loading of autoclave leaving plenty of space between items to allow steam to circulate around items.

The Wrong Way: Over crowded autoclave, all the packages are squished in together with little or no space between individual items.

2. Autoclaving liquids:

Liquids should always be run using a liquid cycle. However it is not just as simple as placing a load of liquids in an autoclave, and pressing the "start" button. The assumption that is made is that the product will be sterilized within the time period selected. But is it really an adequate exposure period?

It takes time for the autoclave to reach exposure temperature and additional time for the liquid load to reach and maintain temperature.

There are a number of variables that need to be taken into account before the exposure time is selected and before the button is pressed. There are also pre-cautions and suggestions to help you achieve proper sterility.

The key variables are:

1. What volume of liquid are you processing? The greater the volume of liquid, the longer it takes for the product to reach temperature.

2. What is the viscosity of the liquid you are processing? The greater the viscosity, the longer it will take for the product to reach temperature. Thicker, more viscous solutions absorb heat more slowly than products such as water.

3. What is the material that comprises the container? Different containers with the same volume of liquid will reach temperature at different rates. Metal containers conduct heat more rapidly than glass or polypropylene since materials of higher specific heat will conduct faster and give up that heat to the liquid contained within the container.

4. How large is the load and what is the effect of load size and density? The greater the physical size of the load, the longer it will take to reach exposure temperature. If the bottles in the load are jammed together, it essentially becomes one large mass we are trying to heat. If the bottles are separated enough to allow steam to envelope each bottle in the load, there will be some variation in come-up time from bottle to bottle but, for the most part, each bottle will come up at approximately the same time as long as the variables mentioned in 1-3 are consistent. Those bottles that are positioned nearest the heated jacket of the sterilizer chamber will tend to reach temperature faster than those in the center of the load. Total exposure time should be determined based on the slowest-to-reach bottle in the load.

To ensure adequate steam penetration for liquids:

- All packages must be positioned in the chamber to allow free circulation and penetration of steam, enhance air elimination, and prevent entrapment of air or water
- Do not overload. Leave space between items to allow for free circulation of steam

- When processing liquids in open containers, fill the containers to only 75% capacity (e.g., 750 ml for a 1 L container. This will limit the liquid loss to 6% of the volume.
- Ensure what you put in the autoclave is heat resistant and is manufacturered for autoclaving.
- Bottles, beaker, flasks, etc. should be covered with a cotton plug or stem penetrable bung and placed in a large, leak proof shallow pan
- Inspect glass containers for any cracks – these should not be autoclaved and properly discarded

5. Records:

None

6. List of attachments:

None

7. History:

Created by Lydia Troc on Oct 19, 2016

8. Approval: