## USING AN AUTOCLAVE FOR STERILZATION

These notes are for reference only and are not intended to replace training by your lab supervisor. Ensure you familiarize yourself with your lab protocols and the appropriate safety data sheets (SDS).

## TRAINING IS IMPORTANT TO HELP YOU UNDERSTAND;

- what the potential risks are to you or others
- how to ensure proper sterilization of your run
- how to use and care for the equipment

## BASIC PRINCIPLES OF AN AUTOCLAVE:

- steam is produced from the boiler or steam generator inside the autoclave
- steam enters the chamber of the autoclave and contacts cooler surfaces
- the steam condenses and causes a decrease in volume, creating negative pressure which draws in more steam
- condensation occurs as long as there is a temperature differential
- steam penetrates the contents of autoclave
- the steam (moist heat) kills organisms by coagulating proteins
- typical temp of an autoclave is 121$^{o}$C
- some autoclaves operate at 134$^{o}$C to inactivate prions (e.g. chronic wasting disease)
- most contents require autoclave cycles with a minimum of 30 minutes @121$^{o}$C to achieve sterilization

## PRIMARY RISKS WHEN USING AN AUTOCLAVE:

- high temperature may cause burns, scalds (e.g. chamber and shelving is hot, steam when opening autoclave door, autoclaved liquids)
- high pressure –autoclaved liquids could boil over, contents could explode if not vented (e.g. container with tightened lid would over pressurize)
- improper sterilization –may lead to biological hazards and risk to personnel and the environment

## PERSONAL PROTECTIVE EQUIPMENT (PPE) SHOULD INCLUDE;

- closed-toed shoes (no sandals, flip flops etc.)
- heat resistant gloves
- eye protection
- lab coat

## CHOOSING YOUR CYCLE

- you need a minimum of 30 minutes sterilization @ 121$^{o}$C
- verify the sterilization time of the cycle on the digital display screen
- depending on the volume of product or waste, the cycle length may need extended e.g. densely packed bag of biohazardous waste or 2L solution
- Do **NOT** mix different waste streams in an autoclave run e.g. do not autoclave liquids with biohazardous solids

| TYPES OF CYCLES | |
|---|---|
| **Gravity displacement** | • air inside the autoclave chamber is displaced by incoming steam - may create pockets of trapped air (ineffective sterilization of dense loads)<br>• use for empty glassware, dry goods,<br>**We typically use cycle 7 for Getinge models** |
| **Pre-vacuum** | • a vacuum is pulsed multiple times and removes the autoclave chamber air before steam is introduced<br>• this forces steam to penetrate the load<br>• most effective for dense loads<br>• use for pipette tips, wrapped items, glassware that must be kept upright, gloves, paper towels, **bags packed with biohazardous contents**<br>**We typically use cycle 1 for Getinge models** |
| **Liquid** | • use only for liquids + biowaste bags with agar in petri dishes<br>• the cycle has a slow exhaust to help prevent liquid boil over<br>• the program does not have a drying time<br>**We typically use cycle 13 for Getinge models** |

## GUIDELINES FOR USE OF AUTOCLAVE

**Do not use the autoclave unless you have received training from your lab supervisor, lab manager or Science Facilities personnel**

**Always use a secondary container to place your autoclave load into.**

- the container will help to capture anything in the event of a spill or boil over
- check to ensure the secondary container is in good condition e.g. no cracks
- never autoclave a sealed container or bag—items can over pressurize and explode
- do not overload the autoclave
- when loading the autoclave, make sure that nothing is touching the sides of the chamber
- always allow for air circulation inside the chamber. This includes allowing space between the walls of the autoclave and the load, and positioning the load to allow for steam penetration (e.g. adjust a biohazard bag so the opening isn't blocked by the secondary container)
- when autoclaving empty glassware-turn on its side so air can escape as steam enters
- apply autoclave tape –which acts as a thermo-chemical indicator to show the autoclave achieved 121$^{o}$C. It does not indicate the set temp was maintained for the programmed time
- any spillage (other than water) must be reported immediately to Science Facilities

### WHAT CAN BE AUTOCLAVED:

- polypropylene (PP) plastic
- polycarbonate (PC) plastic --this type of plastic may crack after repeated autoclaving
- stainless steel
- solutions to be sterilized (water, media)
- glassware (heat resistant borosilicate glass)
- plastic tubes/tips
- biohazardous waste—if research approval has been granted & you have biosafety training
- sharps: if in a container specifically used for "sharps"
- always use an approved secondary container for all autoclave runs!

## DO NOT AUTOCLAVE:

Some chemicals and plastics may produce toxic gases or hazardous by products as a result of autoclaving—these are examples of some items that are NOT to be autoclaved.

- polystyrene (PS)
- high & low density polyethylene (HDPE/LDPE)
- polyethylene (PE)
- polyvinyl chloride (except PVC tubing)
- styrene acrylonitrile,
- acrylic
- polyurethane
- ethidium bromide
- chemotherapeutic agents
- solvents
- unstable organic materials generating fumes when heated (e.g. formaldehyde, formamide, beta-mercaptoethanol)
- flammable, combustible, volatile or corrosive chemicals (bleach, ethanol, methanol, acids, bases, phenol, trichloroacetic acid, ether, chloroform, etc.)
- any radioactive materials
- cafeteria trays

## Examples of "what not to do"

← A plastic secondary tray (likely HDPE)

➔ same tray

Most plastics are not intended for autoclave temperatures

What is wrong with this picture?

- autoclave rack was not used (do not place on floor of autoclave chamber)
- no secondary containment
- plastic bag used to contain load was not autoclaveable (melted)
- autoclave was overloaded & contents spilled & touched all sides (waste should have been split into multiple loads)

## WHAT ARE SECONDARY CONTAINERS?

- are containers that hold the goods to be autoclaved
- e.g. a biohazard bag should be placed into a tray in the event it ruptures during the run, or a flask of agar must be placed into a second container that would be able to hold the volume of the flask if it boils over
- always use a secondary container
- use glass and metal where possible as they conduct heat more efficiently than plastic
- ensure the container volume is suitable to hold a potential spills
- is the container safe for the autoclave (e.g. PP or PC type plastics or stainless steel)

## AUTOCLAVING LIQUIDS

- allow space between jars to prevent bumping and promote even heating
- if using a lid, simply set it on top or screw it on and loosen by ½ turn –if container is sealed it will over pressurize and explode
- or use a vented closure or loosely attached foil
- use Type 1 borosilicate glass (e.g. Pyrex™) & never used cracked or chipped glassware
- other types of glass may not be able to withstand the temp and break
- leave headspace in the container as liquid will expand and overflow the container
- do not fill container more than ½ full to allow for expansion
- volume is important –if you autoclave too large a volume, the autoclave will not be able to properly sterilize the liquid i.e. it won't get hot enough during the cycle. The liquid may show signs of contamination indicating improper sterilization.
- add 2.5 cm (1 inch) of water to secondary tray to facilitate even heating
- always allow liquids to sit for approximately 20 minutes prior to removal as contents are superheated and could boil over in container.
- if the contents are bubbling after autoclaving, do not remove them until cooled
- do NOT overload trays

## GENERAL GUIDELINES FOR AUTOCLAVING LIQUID VOLUMES PER CONTAINER

- liquid cycles are usually programmed at 121°C for 30 minutes
- if needed, divide your liquids into smaller volumes
- Do you need longer sterilization or drying times? Contact Angela for customized cycle
- Always ensure that the secondary container(s) can hold any boil-over or spillage
- Time parameters from Steris manual (*https://medschool.vanderbilt.edu/vbi-core-labs/files/vbi-core-labs/public_files/steris%20manual.pdf*)

| LIQUID VOLUME | RECOMMENDED AUTOCLAVE TIME (MINIMUM) |
|---|---|
| 75 mL | Use 30 minutes |
| 250 mL | 30 minutes |
| 500 mL | 40 minutes |
| 1000 mL | 45 minutes |
| 1500 mL | 50 minutes |
| 2000 mL | 55 minutes |
| >2000 mL | 55 minutes + 10 min/L |

## USE OF INDICATORS TO PROVE THE LOAD HAS BEEN STERILIZED

**Mechanical:** gauges, digital display, and paper printout from your cycle.

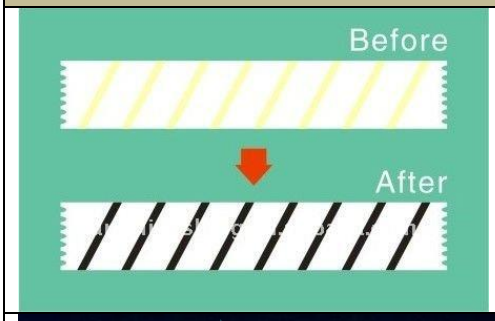
**Chemical:** use autoclave tape. The tape will display black lines after 121°C was attained, but does not indicate how long the temp was sustained

**Biological:** by using a bioindicator (BI) containing *Geobacillus stearothermophilus*.

- the spores used in BIs are more resistant and present in greater numbers than common microbial contaminants, an inactivated (negative) BI indicates that other potential pathogens in the load have also been killed
- a bioindicator verifies three parameters of an autoclave cycle: pressure, temperature & time. **It is important to run a BI to verify sterilization of a biohazardous load**

**Physical (Thermometer/probe):** recordable thermometer that can withstand a high temp for long period of time. The probe records the cycle temperatures and data can be uploaded/plotted to a computer.

## EXAMPLES:

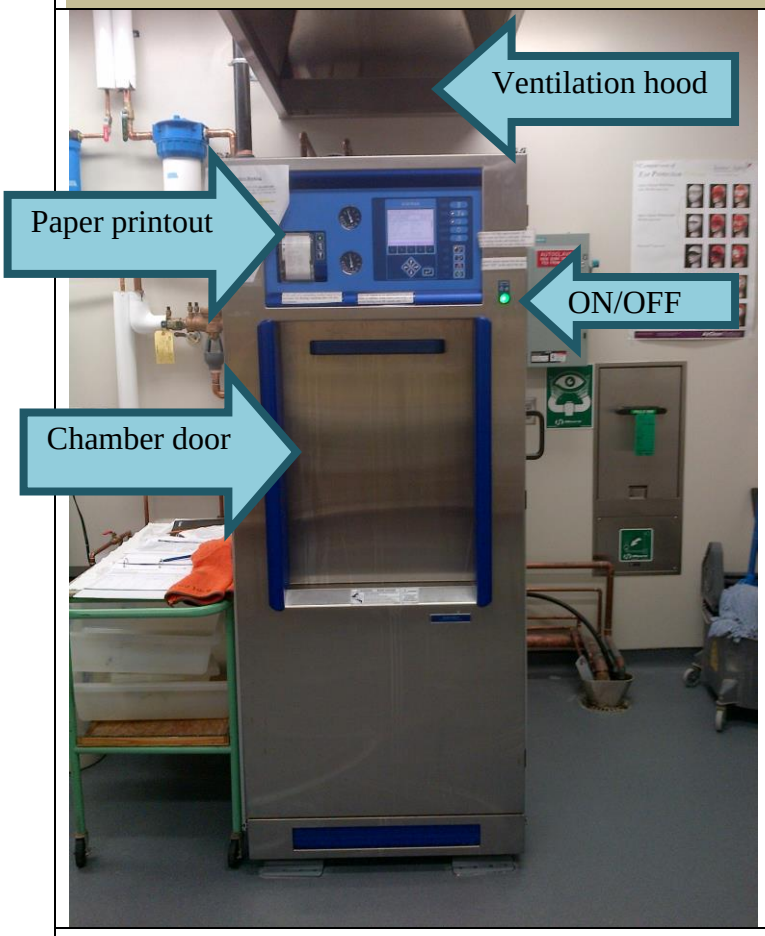



**Autoclave tape before & after autoclaving**


"Indicator ring" Pink colour means the indicator hasn't been autoclaved yet

**3M bioindicators**
**Employ both chemical & biological indicators**

## USING THE DNA A106 GETINGE AUTOCLAVE



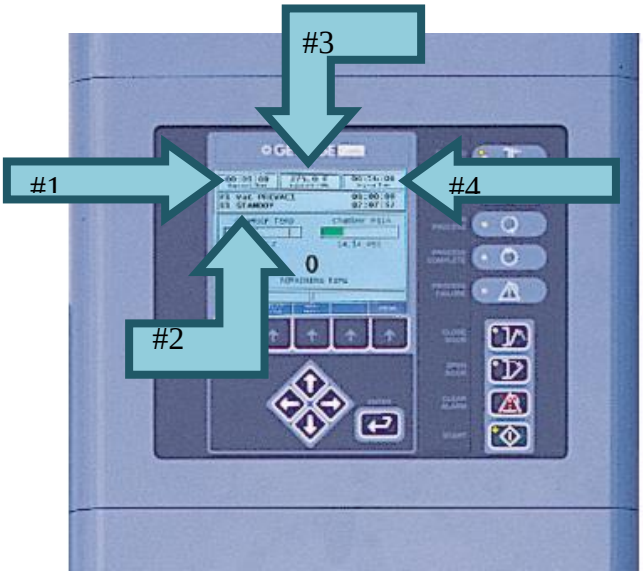

**DNA A106 Getinge Autoclave**

The autoclaves in DNA A106, LHS C261 and D205 are very similar
They look identical but have different features

**DNA A106**

- This unit has automatic on/off feature
- It is programmed to turn on/off at the same time 7 days per week
  e.g. 7AM and 8 PM

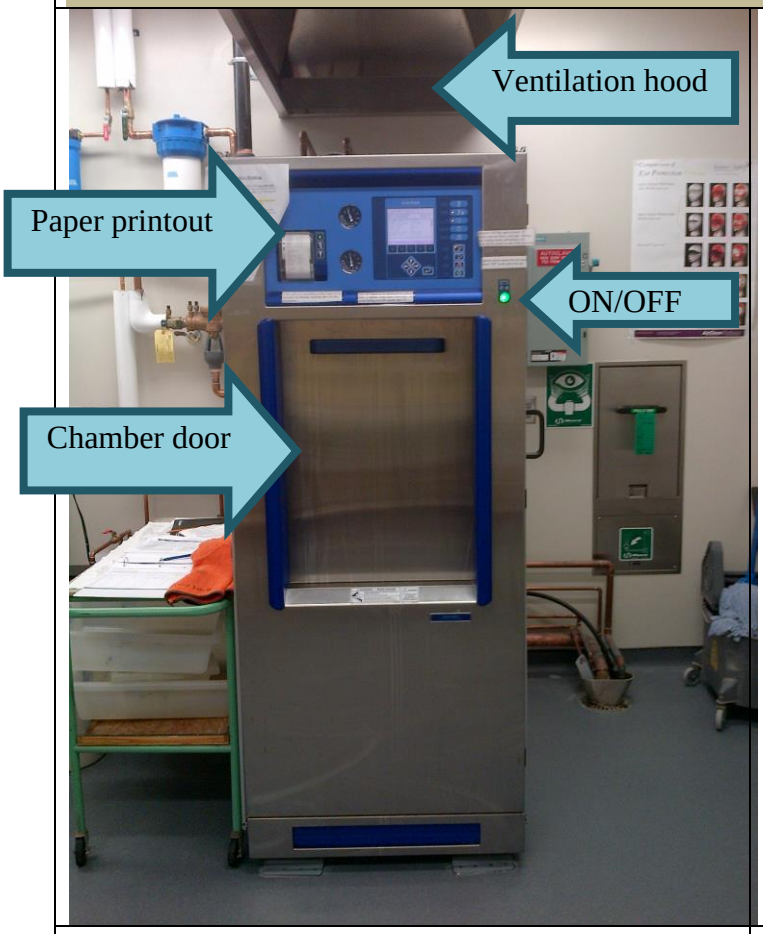
- the door has to be manually opened and closed
- ensure you have pushed it all the way to the top (closed) prior to starting cycle or chamber will not seal and cycle will not start




#1: display shows exposure (sterilization) time of cycle (e.g. 30 min)
#2 displays cycle name
#3 exposure (sterilization) temp
#4 drying time (not used for liquids)

Display may be slightly different depending on user's preference for graphics
e.g. bar, details or plot formats

## GENERAL INFO ON THE LHS C261 & D205 GETINGE AUTOCLAVE



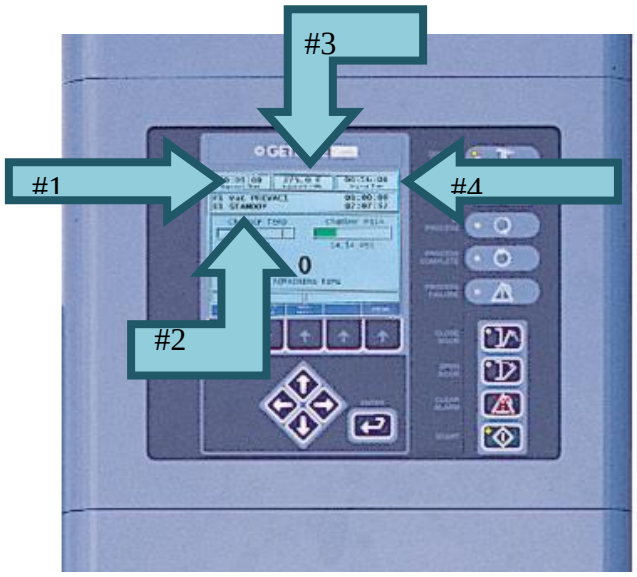

**LHS C261/D205 Getinge Autoclaves**

The autoclaves in DNA A106, LHS C261 and D205 are very similar

- they look identical but have some different features

**LHS C261 & D205**

- The autoclaves must be turned on manually
- Turn the units on 30-45 minutes prior to starting a cycle-the boiler must heat up
- Turn OFF at the end of the day (do not leave a cycle running if you are not available or overnight)
- The door can be opened by pressing the "open/close" door button
- If you need an access code contact Angela for C261 or Smolly Coulson/Debbie Lietz for D205



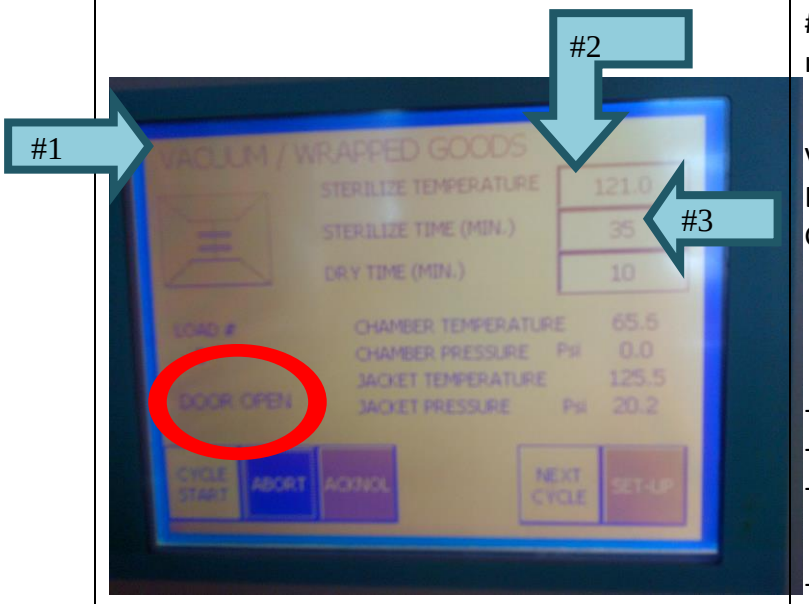

#1: display shows exposure (sterilization) time of cycle (e.g. 30 min)
#2 displays cycle name
#3 exposure (sterilization) temp
#4 drying time (not used for liquids)

Display may be slightly different depending on user's preference for graphics
e.g. bar, details or plot formats

## GENERAL INFO ON THE ESB A208 AMSCO AUTOCLAVE


**ESB A208 Amsco autoclave**

- the autoclave must be turned on manually
- turn the unit on 30-45 minutes prior to starting a cycle-the boiler must heat up
- turn OFF at the end of the day (do not leave a cycle running overnight or if you are not available to check on run)
- the chamber door is hinged and opens to the left
- The handle must be turned (clockwise) to seal the door. You can see the metal rods enter into the door frame when you tighten the handle
- If you need an access code for the lab door, contact Dorothy Howard Gill, ESB C201 x7426




#1: display shows cycle name,

#2 Sterilization temperature: should always be 121°C

#3 Sterilize time (minutes): minimum 30 minutes

- Press “next cycle” to select

Vacuum/wrapped goods
Liquid cycle
Gravity/hard goods

- Screen indicates “door open”

- Confirm that you have;

-selected the correct cycle
-sterilization parameters e.g. time are correct
-sealed door

- The display is a touch screen

-if a parameter e.g. sterilization time needs changed, you can adjust the time
-ensure that the settings are correct
Press “cycle start” once you have loaded contents into autoclave

## GENERAL GUIDELINES FOR USE OF AUTOCLAVES

Fill out the sign-out sheet & log book next to each autoclave

- this is a shared facility, be respectful of other users
- If something goes wrong with your autoclave load, we need to know who to contact
- LHS D205 is for Biology use only (teaching has priority use)

Prior to starting your run, check the inside of the chamber to ensure there were not any spills or debris from previous run e.g. pipette tips, foil, etc. Report any issues to Science Facilities.

- make sure nothing is blocking the drains in the bottom of the autoclave

If the building ventilation system (air supply/exhaust, fume hoods are not working, then do NOT use the autoclave). The vent over the autoclave is necessary to remove exhaust from the autoclave

- **DNA A106 autoclave has automatic ON/OFF features. It will turn on/off at pre-set times each day**
- **LHS C261/D205 Getinge** and **ESB A208 Amsco** units must be **manually turned on/off** each day.
- place goods to be autoclaved in secondary container
- close door and select cycle--ensure that your selected cycle has been confirmed.
- press start
- after the cycle has is complete, ensure your run was successful. Check the print out to ensure the run achieved the correct temperature and time—typically 121$^{o}$C for 30 minutes
- Need longer than 30 minutes? We can customize a programmed cycle
- If not successful, do not remove contents. Repeat the cycle. If the 2$^{nd}$ cycle does not finish, place a "do not use/out of order" sign on autoclave and contact angelasikma@trentu.ca or cwilliams@trentu.ca
- Allow liquids to sit for approximately 20 minutes prior to removal. Contents will be superheated and could boil over. If liquid contents are bubbling, do not remove until cooled.
- Lift each liquid container out individually. Do not lift out the entire plastic secondary tray with the hot liquids in it. Heat can compromise the strength of the secondary tray and it might break.
- When unloading-stand to the side and open the door slowly to allow any residual steam to escape from chamber. Keep hands & face back from door to prevent steam burns.
- Always use personal protective equipment (PPE) when removing load e.g. thermal gloves etc.
- Rinse out secondary tray with water
- Complete all columns in the autoclave log book
- **Remember DNA A106 turns itself on/off each day! All other units must be manually turned on/off each day of use**

**Print out paper**—if the roll needs changed, you can follow the diagrams inside the paper holder or contact Angela for instructions

## Contact Science Facilities personnel

- If you have any question or concerns
- Report any spills inside the autoclave immediately (even water if it goes into the drain inside the chamber). Report any water leaks from the outside of the unit.
- If you suspect the autoclave isn't operating normally, contact:
  Angela ext. 6253**,** **angelasikma@trentu.ca** (see autoclave for cell #) or
  Chris ext. 7061**,** **cwilliams@trentu.ca**

## Autoclaving biohazardous material?

- prior to working with biohazards, you must take the Biosafety course offered by Science Facilities
- contact Chris Williams (cwilliams@trentu.ca) x7061 for details
- ensure you have received lab specific training from your supervisor


Examples of biohazard bags
-they will withstand the autoclave temperature & exposure times


Examples of waste containers specifically for autoclaved waste

## HOW TO PACK A BIOHAZARD BAG?

- use a bag specifically designed for autoclaving biohazardous waste
- regular bags will melt at 121°C
- do not pack too tightly—or steam will not penetrate contents
- pack no more than ½ if densely packed
- if contents are loosely packed, fill no more than ¾ full
- do not seal the bag closed—ensure there is an opening (minimum "3 finger" width) to allow steam to enter
- prior to autoclaving a biohazard bag add approximately 1/2 -1 cup water to the bag—this will help to displace air and create additional steam which will displace the dry air from the bag, increasing the rate of heat penetration.

## SELECTING A CYCLE FOR BIOHAZARDOUS WASTE

- Solid wastes only: use **pre-vacuum** when sterilizing only solids e.g. paper towel, disposable pipette and tips, gloves etc. (minimum 30 minutes sterilization time)
- Use **liquid cycle** if the bag contains any **media/agar** that will liquidize upon heating e.g. petri dishes etc. (minimum 30 minutes sterilization)

## BIOLOGICAL INDICATORS

We use 3M Attest bioindicators (BI)

- they contain *Geobacillus stearothermophilus*
- the spores used in BIs are more resistant and are in higher concentrations than common microbial contaminants
- an inactivated (negative) BI indicates that other potential pathogens in the load have also been killed.
- a bioindicator verifies three parameters of an autoclave cycle: pressure, temperature & time

**WHEN TO USE A BI?**

Autoclave a bioindicator ***once per month as a minimum*** and after:

- any repair to the autoclave
- any changes in your loading/packing process including training new personnel
- regular basis with biohazardous loads

**HOW TO PLACE A BI IN LOAD**

- prior to autoclaving, include a BI in your load by placing on the tray or inside empty glassware, taped to the side etc.
- biohazard bag- when the waste is ready for autoclaving, use the copper pipe next to the autoclave and push the pipe into the centre of the biowaste bag. Tie a cotton string around the indicator and suspend the bioindicator inside the copper pipe, leaving the string extending out through the top of the bag. Tape the string to the outside of the bag. Use the string to remove the bioindicator after autoclaving. After autoclaving, remove the pipe and place it next to the autoclave.

**HOW TO CHECK A BI**

- After autoclaving, wait 10 minutes before removing bioindicator from load to allow all contents to cool.
- check the chemical indicator on the ***label*** of the biological indicator. A color change from *rose* to *brown* confirms that the biological indicator has been exposed to the steam sterilization process
- if the load was biohazardous waste, place the autoclaved bag in grey garbage bin provided & flip the sign on the lid to “red” or “do not dispose”. Once the indicator is verified as a PASS, the sign can be flipped to permit disposal.
- incubate the indicator within 2 hours of removal from autoclave load (or refrigerate until it can be incubated)
- the incubator is a small 56$^{o}$C incubator manufactured specifically for 3M Attest indicators. They are stored in DNA 106, LHS C261 cupboards –DO NOT REMOVE from lab
- the 3M Attest incubator is a dry block unit. DO NOT add water to it and always unplug the unit after use
- to insert indicator, wear safety glasses & gloves, place bottom of vial into slot
- push top of vial into place. This will break the media vial inside the tub
- label a 2nd vial “C” or “control. Insert into the incubator
- ensure the control and the test indicators are from the same lot number and manufacturing date.
- the control vial is not autoclaved and is used to ensure: correct incubation conditions, that the indicators are still viable and capable of supporting growth

- place a note next to incubator with your name & contact information
- **<u>Incubate both 3M Attest indicators for 48 hours- checking for colour changes at 8, 12 and 48 hours</u>**
- if the autoclaved indicator turns yellow, this is a "positive" test and indicates the contents did not achieve 121$^{o}$C for at least 15 minutes
- If both indicators pass, flip the sign on the garbage to "green" indicating the waste can be disposed
- check to ensure the log out sheet is completed and includes indicator results
- the indicators can be placed in your next waste bag and autoclaved prior to disposal

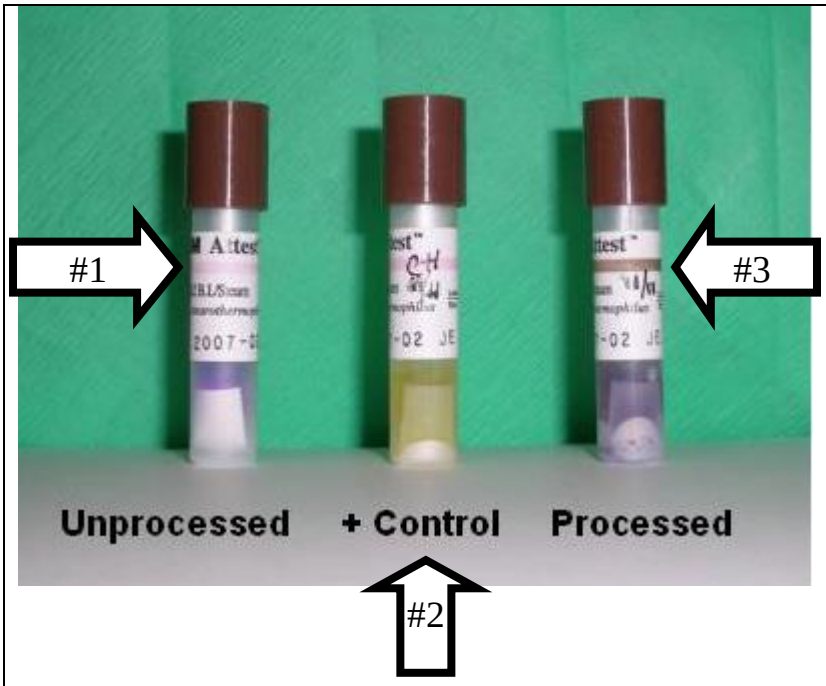
If the bioindicator(s) turn yellow

- you must determine which variable may have resulted in a non-sterile product.
- do not discard your waste until a pass is achieved

Examples:
-waste packed too tight to allow steam penetration
-bag sealed shut
-choice of packaging materials
-packaging technique
-sterilizer loading techniques
-inappropriate cycle parameters for the items being processed
-autoclave not working properly

**How to analyze 3M Attest 1262 bioindicator results**

| Bioindicator | Colour after incubation (48 hrs) | Result | |
|---|---|---|---|
| Autoclaved bioindicator | Purple | **PASS** | Bacterial spores are not viable |
| Control | Yellow | **PASS** | Bacteria spore are viable |
| | | | |
| Autoclaved bioindicator | Yellow | **FAIL** | Indicates bacterial spores are still viable—sterility not achieved. Repeat process. |
| Control | Purple | **FAIL** | Means media not viable or error in labelling. Repeat process. |




**Examples of *3M 1262* bioindicators:**

#1: unprocessed: pink coloured ring shows the tube was not autoclaved

#2: control: pink ring shows that the bioindicator was not autoclaved. It was incubated to be used as a "control" against #3. Shows the bacteria are still viable.

#3: the ring at the top is brown indicating that the tube was autoclaved. The bioindicator has been incubated and is purple to prove that the contents were successfully sterilized i.e. purple/no bacterial growth