



Sterilization of Biological Waste

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Definition of Sterilization

- Sterilization – eliminates all microbes by a chemical or physical means

Importance of Sterilizing Biohazardous Materials

- Good stewardship
 - Protects landfill and environment
- Required by law

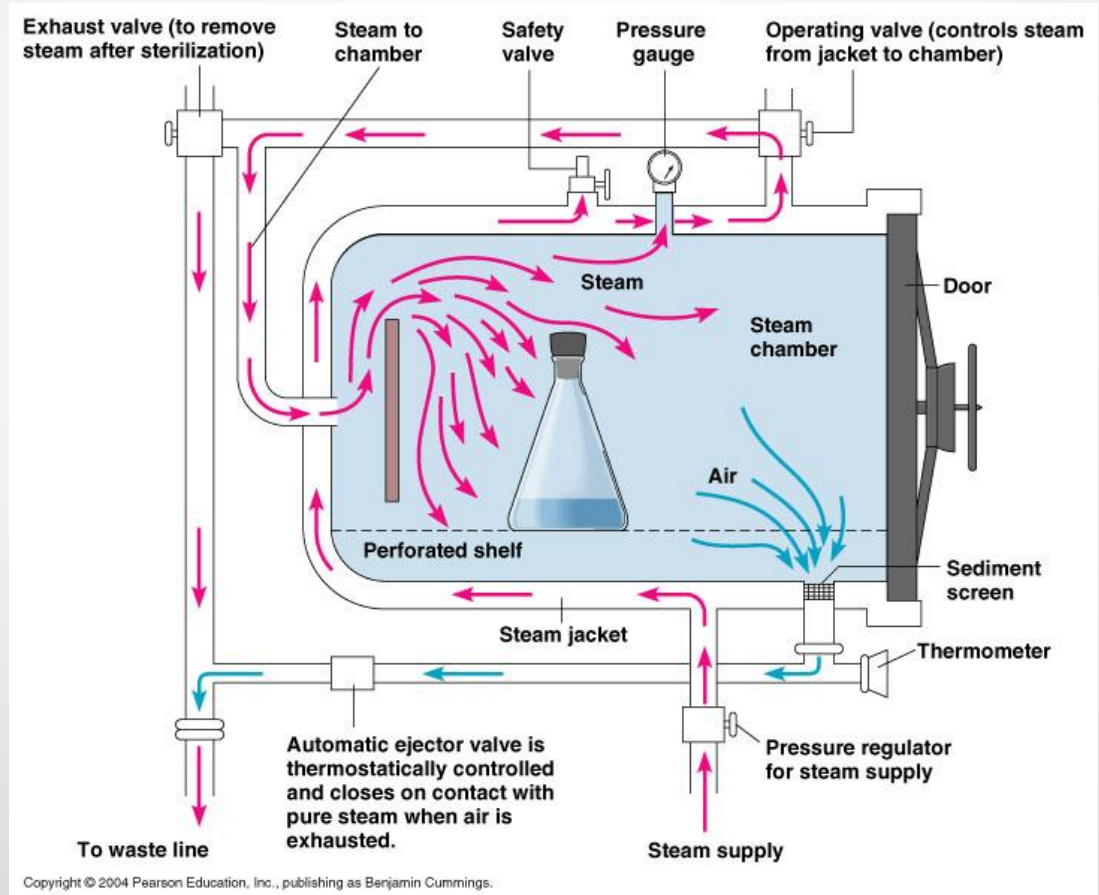


Methods of Sterilization

- Autoclave
 - Most commonly used in our labs
- Chemical digestion
- Irradiation
- Gaseous vapor treatment
- Dry heat
- Incineration
- Chlorine dioxide

Gravity Displacement Autoclave

- Steam enters jacket
- Steam enters chamber
- Lighter air is forced out
- Steam is forced into autoclave
- Pressure builds up
- Sterilization occurs



Mechanism of Autoclave Sterilization

- Contributors:
 - Steam
 - Pressure
 - Time
- Microbes are killed or inactivated by denaturation of their proteins and/or nucleic acids

Autoclave Hazards

- Burns
 - Explosions
 - Inhalation of harmful chemicals*
 - If hazardous chemicals are autoclaved
 - Lift hazards
 - Minimal if materials are loaded properly
- *Occurrence at CSU

Autoclave Incidents

Uncommon, but when they occur, the results are devastating!



Autoclave Incident 1

- 2001, several bottles inside autoclave exploded because they were tightly capped
- In response, 3 workers investigated the explosion, and opened the autoclave door.
- *While standing in front of the open door, additional bottles exploded and glass flew 25 feet out of the autoclave*
- *The employee standing in front of the door received second degree burns and injuries to arms and face from the glass shards*
 - **Employee was wearing safety glasses and was in the process of donning a thermal suit*

Lessons Learned

- Leave caps on bottles loose!
- Stand away from the door when you open it
- Wear a face shield, steam apron, and steam gloves

Autoclave Incident 2

- A postdoctoral fellow was removing an autoclave pan containing bottles of media with water surrounding them.
- The Nalgene tub buckled, pouring near boiling water over the person's trunk and legs
- The person sustained serious burns, especially in areas where clothes were not removed
 - The person was wearing autoclave gloves

Lesson Learned

- Do not place water in Nalgene tubs
- Nalgene tubs weaken over time and can collapse if full of water or heavy items
- Use metal tubs to collect water or boil over liquids
- Adding water to tubs is not needed if you have type I borosilicate glassware (Pyrex)

Lessons Learned

- Lab coats will not protect you from steam burns
- Use a steam protecting apron
- In case of an emergency with boiling water or steam – remove ALL clothes covering affected skin and put on a clean lab coat

Autoclave Incident 3

- Person seriously injured after starting the autoclave when the autoclave door was not securely closed
- The locking mechanism was not aligned and interior pressure blew the door off and it struck the person
- The person operating the autoclave was 50 feet away when he was hit by the door
 - Note that there have been several fatalities from persons being struck by autoclave doors

Lessons Learned

- Be sure that the door is aligned and sealed properly

Remember.... Some chemicals can not be autoclaved

- Oxidizers – EXPLOSION HAZARD!
- Flammables – BURN/EXPLOSION HAZARD
 - Alcohols, etc.
- Phenols
- Bleach
- Carcinogens
 - Ethidium bromide, etc.
- Caustic materials
- Contact EHS at (970)491-6745 if you are unsure whether a chemical can be autoclaved

Autoclave Explosion

- Door not properly closed



Which Autoclave Type Do You Have?

- There are many types
- Operation may vary
- Loading and cycles may vary



Older Autoclave Example (Microbiology Building)



Control Panel



4 cycles are available to choose from:
2 Liquid cycles
2 Gravity cycles

Older Autoclave Cycles

- There are 4 autoclave cycles but older autoclaves have 2- noted by orange circle

- Gravity
- Vacuum/
Pre-vacuum
- Liquid
- Flash



Newer Autoclave Example (Scott Engineering Building)



Touch screen control panel-
choose cycle here

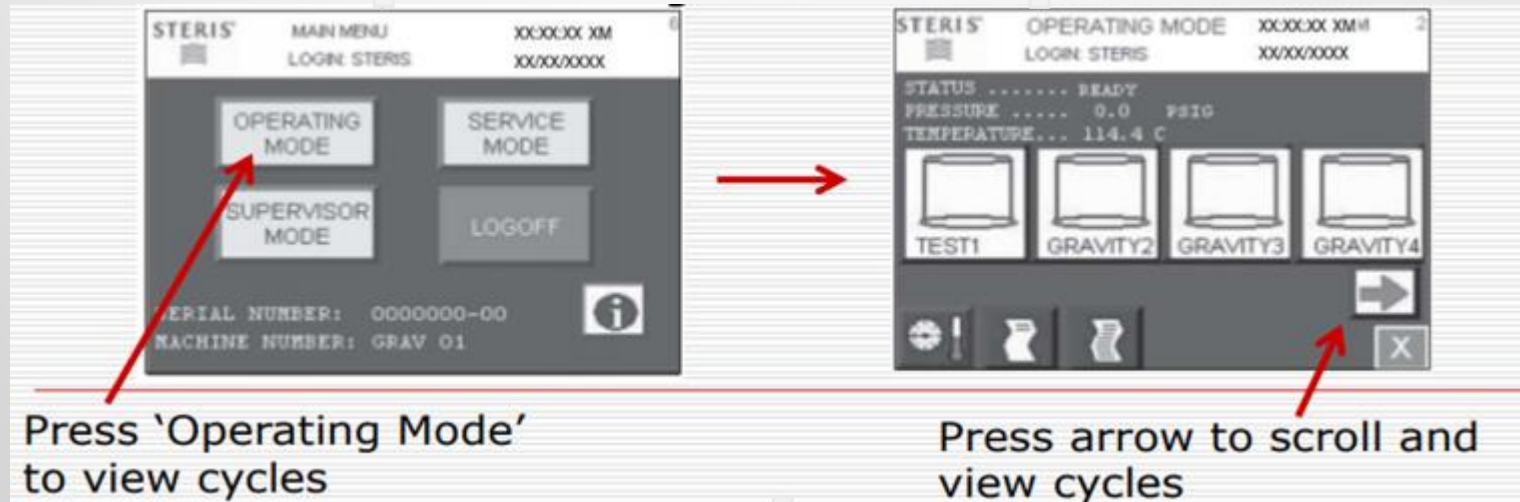
Emergency stop

Chamber pressure
gauge

Door

Newer Autoclave Cycles

- Have at least 3 cycles- prevacuum, Liquids, Gravity
- Can customize cycle and name it
 - Example: Scott Bldg. has 'waste' cycle



Newer Autoclave Cycles

- Screen will move to another window
- It will ask you if you want the chosen cycle
- Input 'yes'
- Cycle will begin

Disclaimer

- These are **guidelines**- your autoclave may be different, so check with the person in charge of the autoclave to make sure that you know how to operate it properly.

Table top Autoclave



Self-contained unit present in some laboratories- ask PI for instructions before using/ read the manual

Other Autoclave Cycles

- Rarely used at CSU:
 - Pre-vacuum



Porous loads, partly vented containers, animal cages with bedding, wrapped goods, surgical packs – pulls 'air blanket' out from around materials, allowing steam to penetrate

- Flash



Shorter (3-10 min), higher temperature (270°C-275°F) treatment – used for dental tools, unwrapped instruments – NOT ROUTINE

Phases during Autoclave Run

- Pre-conditioning
 - Air removed
 - Chamber charged
- Exposure
 - Time
 - Contact with steam
 - Post conditioning
- Drying – pull vacuum
 - Removes moisture

Best Practices - Liquids

- Use type I borosilicate glass
 - Avoid cracked, nicked glass
- Do not fill more than 2/3
- Use autoclave tape
 - visual indicator that the liquid was heated
 - *Does not confirm sterility*
- Loosen caps!



Recommended Method: Loading Liquids for Autoclaving

- Place flask/bottle on rack
- Put metal pan on lower rack to catch boil-overs
 - Media that boils over will clog the drain resulting in pressure buildup and/or flooding



Metal pans are recommended for catching spillover- they are less likely to degrade over time

Alternate Method:

Loading Liquids for Autoclaving

- Place flask in a metal 'jelly roll pan' with sides of ~1 inch
 - Jelly roll pans are recommended because they will not degrade with continued autoclaving
 - Short sides allow steam to envelope the material



Alternate Method:

Loading Liquids for Autoclaving

- Add water to ~1/4 inch height in pan
 - Water is needed to generate steam and push air out of the pan.
- This method is less preferred because air can be trapped in the pan unless water is added
- If materials are placed in a pan, add water

Autoclaving Liquids – ‘Slow Exhaust’*

- 121°C, 15-18 psi- for Colorado it is 18 psi
- Time can vary with volume-see [chart-note-existing autoclaves have been set for generic times](#)

Volume of Liquid in One Container	Minimum Recommended Sterilize Time* at 121°C (250°F) minutes
75 mL	25
250 mL	30
500 mL	40
1000 mL	45
1500 mL	50
2000 mL	55
> 2000 mL	55 + 10 min/L



*Autoclave conditions are the same for liquids and glassware, however, with liquids the pressure is released slowly at the end of the run to avoid boil-over

Packaging Biohazard Bags for Autoclaving

- *DO NOT place tips/ pipettes directly into an autoclave bag for autoclaving unless they are protected by a puncture proof container (see slides 39 and 40 and <http://www.ehs.colostate.edu/dv2.aspx?id=426>)*
 - Tips/ Pipettes have punctured the bags and injured personnel
 - Don't overfill bag -2/3 is enough
 - Do not double bag your waste if using standard autoclave times, because..
 - Autoclave time must be increased substantially to achieve sterility
 - *Exceptions: Animal carcasses*
 - *Note: Other conditions may apply in the BSL₃ facility*

Packaging Biohazard Waste Bags for Autoclaving

- Add water to bag so steam will be generated
- Tape bag closed- use autoclave tape
- Label bag- PI name and date



Loading Biohazard Waste Bags

- Biohazard labeled autoclave bags must be placed in a pan because they may break and spill over materials
- Do not overfill bag- spill over will occur
- Place the pan/ bin with the bag on the wire rack



Loading Empty Glassware

- Load dry glassware on side or invert when possible
 - Air is displaced more easily by steam than if the glassware is upright
- Place glassware on metal rack

Autoclaving using Gravity Cycle

Table 7. Minimum cycle times for steam sterilization cycles

Type of sterilizer	Item	Exposure time at 250oF (121oC)	Exposure time at 270oF (132oC)	Drying time
Gravity displacement	Wrapped instruments	30 min	15 min	15-30 min
	Textile packs	30 min	25 min	15 min
	Wrapped utensils	30 min	15 min	15-30 min
Dynamic-air-removal (e.g., prevacuum)	Wrapped instruments		4 min	20-30 min
	Textile packs		4 min	5-20 min
	Wrapped utensils		4 min	20 min

Modified from Association for the Advancement of Medical Instrumentation. 813, 819.

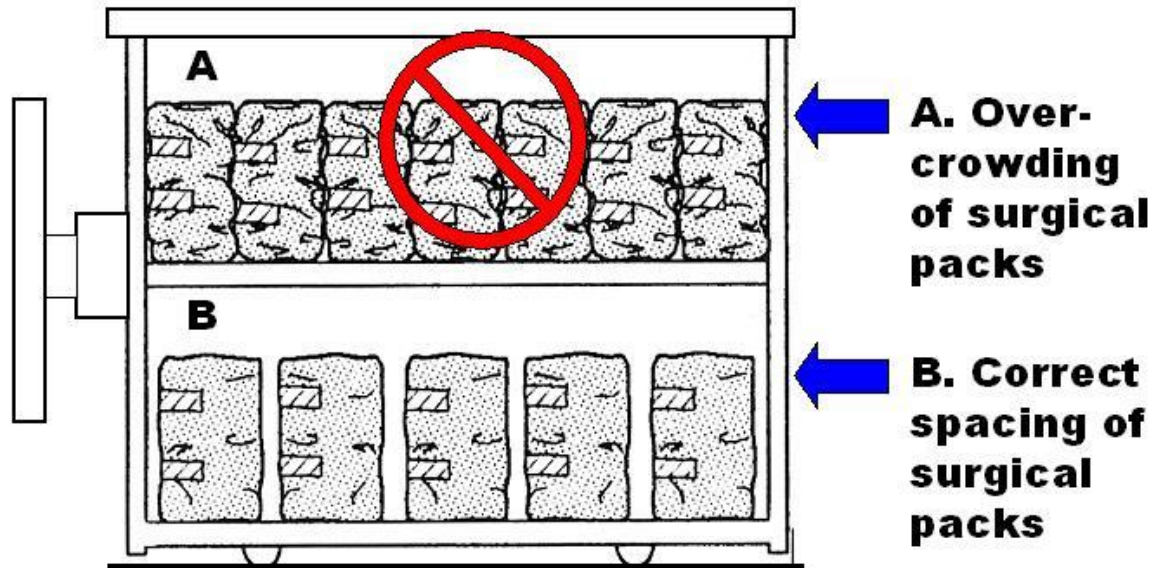
Table 8. Examples of flash steam sterilization parameters.

Type of sterilizer	Load configuration	Temperature	Time
Gravity displacement	Nonporous items only (i.e., routine metal instruments, no lumens)	132°C (270°F)	3 minutes
	Nonporous and porous items (e.g., rubber or plastic items, items with lumens) sterilized together	132°C (270°F)	10 minutes
Prevacuum	Nonporous items only (i.e., routine metal instruments, no lumens)	132°C (270°F)	3 minutes
	Nonporous and porous items (e.g., rubber or plastic items, items with lumens) sterilized together	132°C (270°F)	4 minutes
Steam-flush pressure-pulse	Nonporous or mixed nonporous/porous items	132°C (270°F) Manufacturers' instruction	4 minutes

Modified from Association for the Advancement of Medical Instrumentation. 812, 819.

These are general guidelines and autoclave times may vary by site

Avoid Overcrowding



Avoid touching autoclave bags to sides of autoclave

Autoclaving Pipettes and Pipette Tips



Place pipettes and tips in cardboard box lined with bag



Put cardboard box inside biohazard bag



Autoclave a minimum of 30 minutes



When cool, place in secondary cardboard box and dispose in autoclave waste bin

For information, see <http://www.ehs.colostate.edu/dv2.aspx?id=426>

Alternative Method for Autoclaving Pipettes and Tips



Step 1: Dispose of pipettes in pipette boat Tape boat closed.



Step 2: Place boat in biohazard bag



Step 3: Put labeled materials in bin and autoclave

Step 4: Remove autoclaved pipettes and place in plastic lined box for disposal



Alternative Method for Autoclaving Pipettes and Tips

- Place used pipettes in pipet keeper box



- Seal
- Autoclave and discard

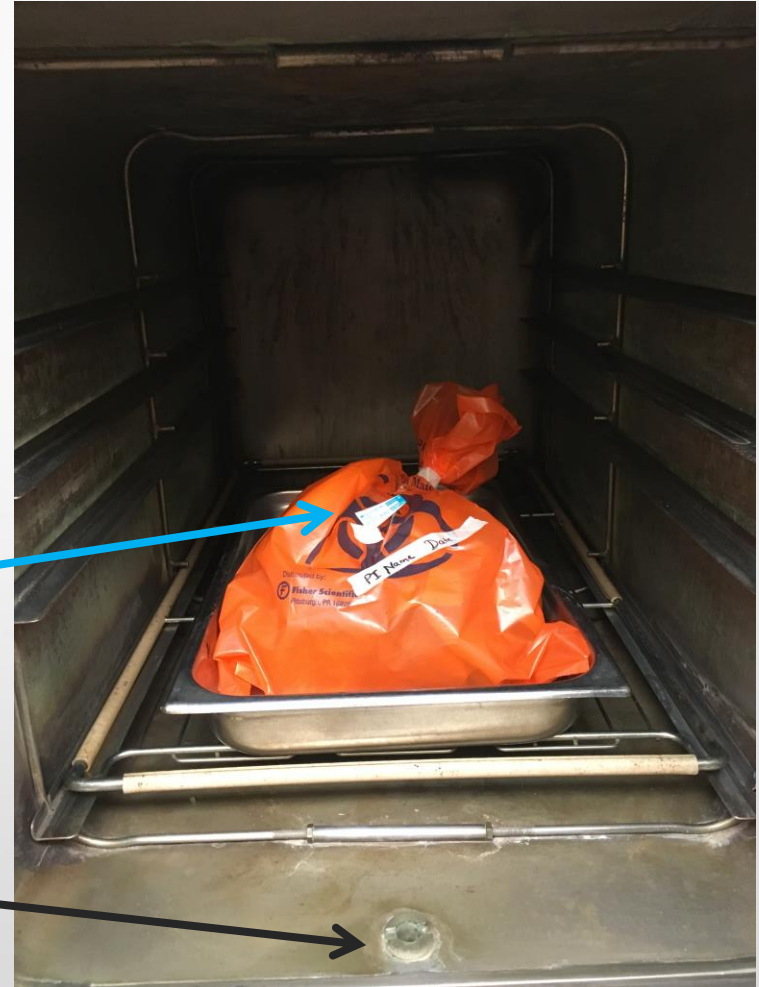
For more information, see <http://www.ehs.colostate.edu/dv2.aspx?id=426>

Operating Procedure-Microbiology

- Check the drain and make sure it is clear – no debris or dried media allowed
- Place materials in autoclave with sterility validation strip

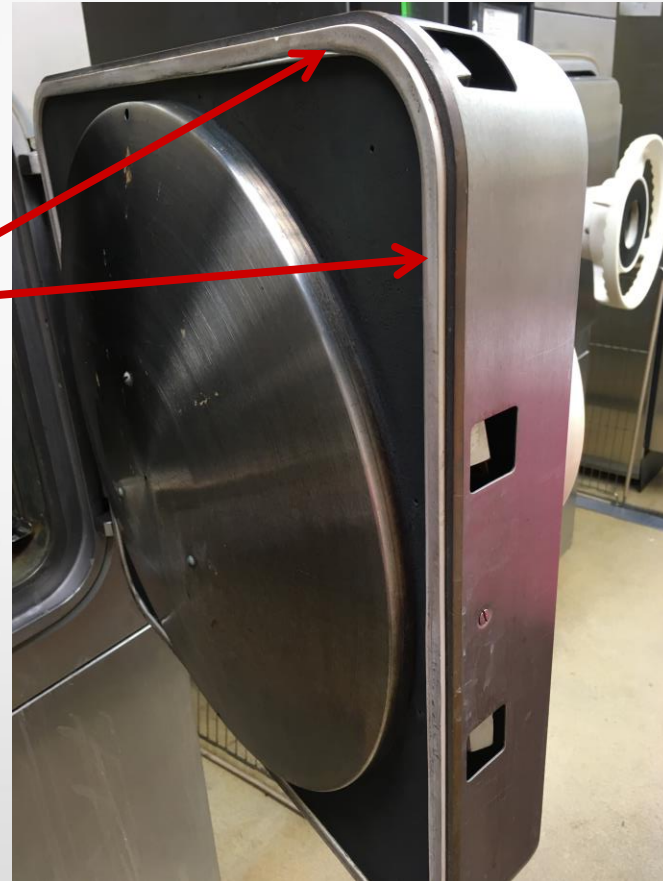


DRAIN



Operating Procedure- Older Autoclaves

- Check that the door seal is intact and not cracked/broken



Operating Procedure-Older Autoclaves

- Place materials in autoclave
- Close door firmly, making sure the door hinges are pushed in for a good fit
- Close door



Push
firmly
here

Operating Procedure- Older Autoclaves

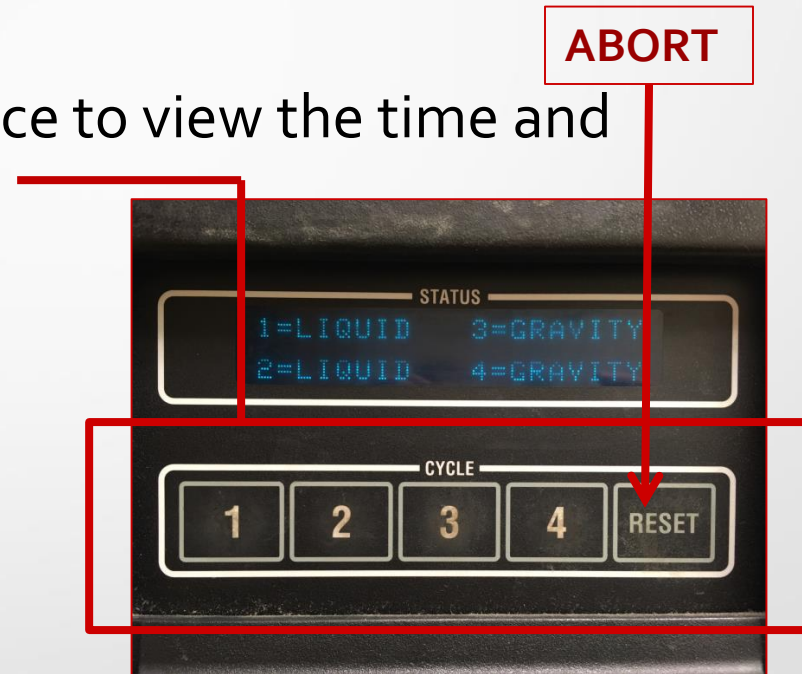
- Close using door wheel
- Check to make sure all door rods are inside the rim of the autoclave



Door wheel

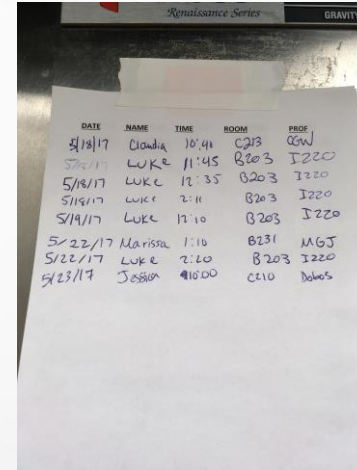
Operating Procedure-Older Autoclaves

- Push the cycle selector button once to view the time and pressure setting
- Push the cycle selector button twice to start the autoclave
- **Abort the run IMMEDIATELY** if steam is escaping or if you hear whistling or screeching



Operating Procedure-

- Sign up in logbook/
sign up sheet
- Place Sterigage Strip/
chemical indicator in
log book/ on sign up sheet



Renaissance Series GRAVITY

DATE	NAME	TIME	ROOM	PROF
5/18/17	Claudia	10:41	C213	CGW
5/18/17	LUKE	11:45	B203	I220
5/18/17	LUKE	12:35	B203	I220
5/18/17	LUKE	2:11	B203	I220
5/19/17	LUKE	12:10	B203	I220
5/22/17	Marissa	1:16	B231	M6J
5/22/17	LUKE	2:20	B203	I220
5/23/17	Jessica	4:00	C110	Dobbs



Removing Materials from the Autoclave

- Look at the Autoclave Control Panel
- The pressure must read 0 psi before opening the autoclave door



Removing Materials from the Autoclave

- Put on PPE
 - Steam apron
 - Protective gloves
 - Face shield
- Open door, **let materials cool for 10 min** before removing them
- Do not jolt bottles



Verification of Sterility

- Chemical indicator
 - Example: 3M Comply™ Steam Chemical Integrator 'Sterigage' strips
 - Use 1 chemical indicator per run and place in log book after run

Each of these indicate sterilizing conditions were met

Unprocessed



Accept

When the color bar moves anywhere into the "ACCEPT" area, all the critical parameters of steam sterilization have been met.



Reject

If the bar does not reach the "ACCEPT" area, the necessary conditions for sterilization have not been met. The pack should be reprocessed and the cause of the sterilization process failure should be investigated.



Verification of Sterility

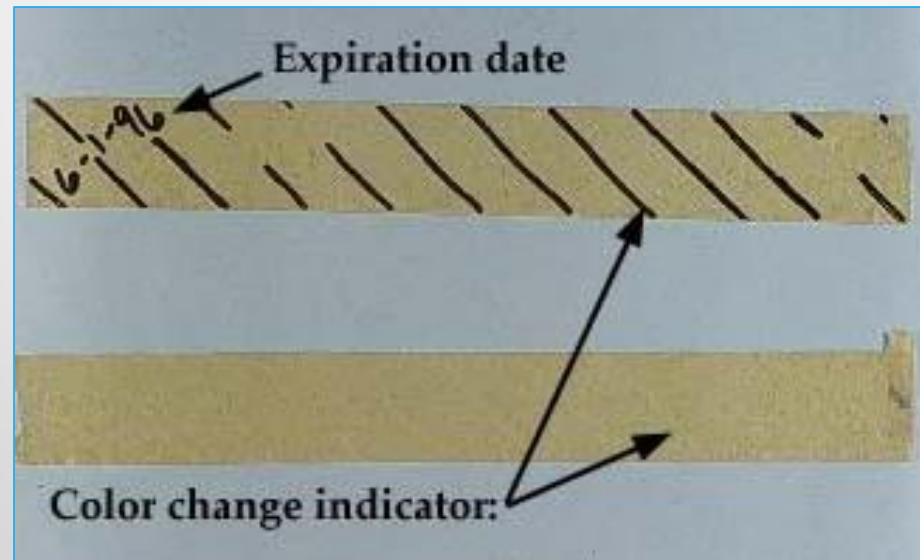
- Biological Indicator- ProTest Self-Contained Biological Indicator
 - Weekly use recommended by CDC*
 - Example: ProTest Self-contained Biological Indicator
 - Vial with bacterial spores (*Geobacillus stearothermophilus*), glass ampoule with growth media, and pH indicator
 - If autoclave did not sterilize properly, spores will germinate and media will turn yellow
 - If autoclave sterilizes properly, spores are killed and media will remain purple



*https://www.cdc.gov/oralhealth/infectioncontrol/faq/sterilization_monitoring.htm

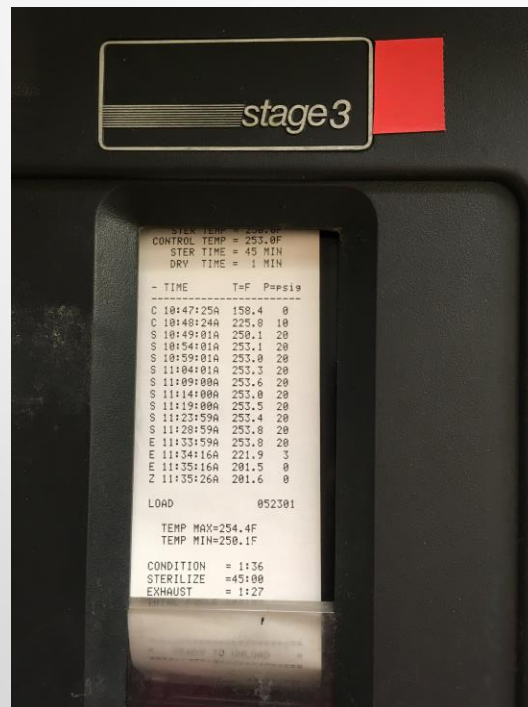
NOT a Verification of Sterility

- Does not indicate sterility
- Black bars are a visual indicator that the tape as exposed to elevated temperature



Not a Verification of Sterility

- Displays on autoclave or on recording of run DOES NOT indicate sterility



Discard Autoclaved Biohazard Bags



Place
autoclaved
bags in bin



Autoclave Safety

- Remember....Steam causes more severe burns than hot air or surfaces due to transfer of heat on condensation
- Wear appropriate PPE
- Open the autoclave door only when the pressure has dropped to 0 psi
- Do not remove materials from the autoclave until cooled 10 min

Autoclave Safety

- Keep your face and body away from the autoclave when opening the door
- Loosen caps on bottles
- Make sure that the door is properly closed

Quiz

Be Safe and Don't Get Steamed!

