



**CSULB**

Department of Biological Sciences  
Common Use Equipment Training

***Autoclaves***

# Outline/Learning Objectives

- Understand how “gravity” autoclaves work
- Learn how autoclaves are ***SAFELY*** operated
  - What materials can and can not be autoclaved
  - The different autoclave cycle types
- How to keep autoclaves in operational condition
- Contact info for people that can help with issues
- **Lab is not a test. When in doubt:**
  - **Ask questions, look it up, refer back to this document**







# Secondary Containment

All items that are placed in the autoclave must be contained with in a

- Needs to be a \_\_\_\_\_ larger than the volume you wish to autoclave
- The primary function is to contain any spills or breakage
- Must be able to withstand elevated temperatures (heat stable)

## NOTE:

- When in doubt, ASK a knowledgeable person
- Never trust any sharpie marks such as “for autoclaving” unless is says “DO NOT Autoclave” - you can trust that



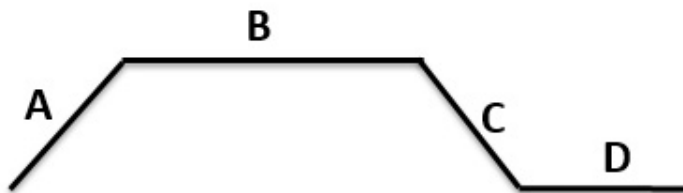
Melted Plastic: someone used non-heat stable plastic as secondary containment



# MLSC Common Use Autoclave Cycles

**Do not mix your cycles at CSULB.**

- **Unwrapped** - materials that are not wrapped or packaged
  - Metal equipment, tubing, mortar/pestle sets
  - 15 min at 121°C + quick pressure release + 5 minute drying step
- **Wrapped** - materials that are contained within packaging
  - Pipette tips, microfuge tubes, etc.
  - 15 min at 121°C + quick pressure release + 30 minute drying step
- **Glassware** - For items made of glass
  - 15 min at 125°C + quick pressure release + 30 minute drying step



- A) Increase of pressure and temp to 121°C and ~20psi
- B) Sterilization for 15 min at temp and pressure.
- C) Quick pressure release
- D) Drying time

# MLSC Common Use Autoclave Cycles, 2

- **Liquids** – (Liquid 15 or Liquid 20)

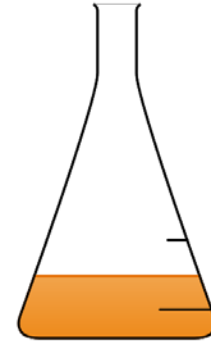
- 15 / 20 minutes at 121<sup>0</sup>C + **slow pressure exhaust**

- to prevent super-heated liquids from boiling over

- Never fill a vessel greater than **½ of its capacity**

- Keeps liquid from boiling out of your vessel

- Caps on vessels containing liquids **MUST** be loose

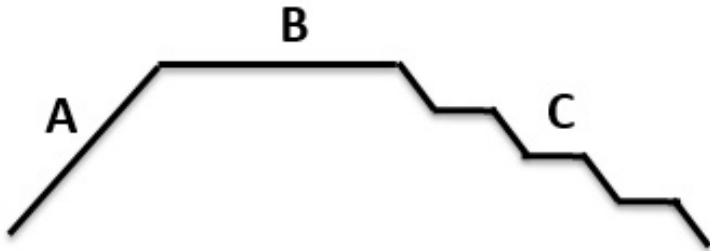


- **Cycle options:**

**Liq 15** < 500ml

**Liq 20** < 1L

- If you want to autoclave 2 liters you must separate your liquid into 2 1L vessels to properly autoclave



A) Increase of pressure and temp to 121<sup>0</sup>C and ~20psi

- Water boils at 100<sup>0</sup>C when it is not under pressure

B) Sterilization for 15/20 min at temp and pressure.

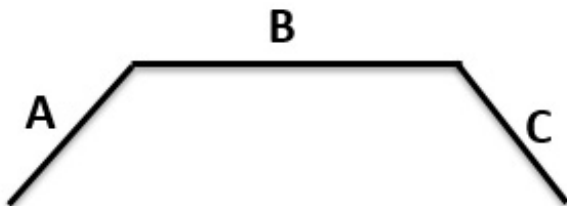
C) Slow step down pressure release

- This keeps your sample from violently boiling over



# MLSC Common Use Autoclave Cycles, 3

- **Waste** – For items prior to disposal
  - Standard Trash - no small particles or dirt
    - Leave unsealed so steam can circulate
  - Trash bags with small particles need to be sealed
    - Small particles blow around and end up in valve 3
    - Dry sealed trash – place some water into the bag
  - 45 minute at 121<sup>0</sup>C + quick pressure release
  - Waste bags must be made out of heat stable plastic
    - Do not use Target or Walmart bags
  - Waste bags must be completely contained in secondary containment. DO NOT over fill waste containers



- A) Increase of pressure and temp to 121<sup>0</sup>C and ~20psi
- B) Sterilization for 15 min at temp and pressure
- C) Quick pressure release

# Waste Containment



**Proper Waste  
Containment**



**Improper Waste  
Containment**

**Improper waste containment will lead to  
items falling into valve 3!**

# General Operating Guidelines

## Prior to Entering the Autoclave Room

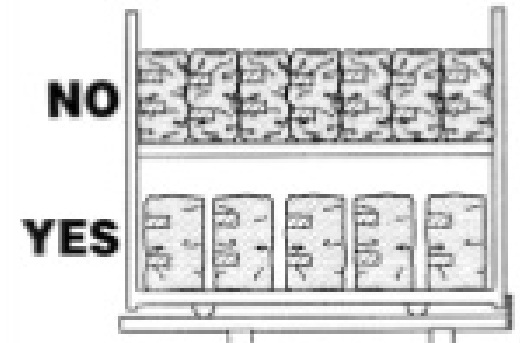
- **ALWAYS** use proper personal protective equipment (PPE)
  - At minimum, heat resistant gloves, lab coat, closed-toed shoes and eye protection

## Each Time You Enter the Autoclave Room

- 1) Make an entry on the user log (next to each autoclave) detailing your usage
- 2) Verify the chamber is empty clean prior to putting your items into the chamber
  - Never place items to be autoclaved directly on the chamber surface
  - **Your item must be in heat stable secondary containment**
- 3) Always verify that the door closes and seals properly at the start of your cycle
- 4) Pick up your items in a timely manner
  - The count down will help you estimate when you should return to pick up your items
- 5) **Never leave your items in the autoclave overnight!**
  - **This disrupts the shutdown procedure performed at midnight. Required for flushing!**

# Considerations When Autoclaving

- Do not overload the chamber
  - Make sure items are not touching sides or top of the chamber
  - Only surfaces that are contacted by steam will be sterilized
    - Steam kills
- Always loosen lids of plastic and glassware
- At CSULB do **NOT** mix your cycles.
- Never pour hot liquids down any sink
- When reporting autoclave issues/alarms
  - To do: Mute the alarm and read the autoclave screen
  - Tell Us:
    - Which autoclave
    - What does the screen display
  - Look for and read any new notes that have been posted
    - We communicate via notes. Note that have been posted it may tell you how to fix any ongoing issues until a repair is done



# Find the Bad Things

How many can you find?



- 1.
- 2.
- 3.
- 4.
- 5.

- 6.
- 7.
- 8.
- 9.
- 10.

# Find the Bad Things: Answers

Did you find them all?



1. Biohazard in the autoclave
2. Ethanol in the autoclave
3. Non heat stable My Little Pony
4. Non heat stable alligator
5. No secondary containment

6. Bleach
7. Coffee cup
8. Open toe shoes
9. No hot gloves
10. No lab coat

# Caution

- Autoclaves are HOT
  - Always wear personal protective equipment (PPE) and loading and unloading and autoclave
- Steam will burn
  - When opening the chamber keep head and arms away
- Autoclaved items are HOT
  - Allow hot items to cool for ~5-10 min prior to touching

**If you are burned seek medical treatment as soon as possible then inform your lab supervisor and the science safety office.**

# List of Important Contacts

**If you have any spills / melts report them immediately!**

## **Michelle Giffin – MLSC Biology Tech**

- MLSC Building Common Autoclaves (1<sup>st</sup> point of contact)
- 562-985-4038, [Michelle.Giffin@csulb.edu](mailto:Michelle.Giffin@csulb.edu)

## **Diane Graham – Microbiology Tech**

- Microbiology Building Common Autoclaves (1<sup>st</sup> point of contact)
- 562-985-4857, [Diane.Graham@csulb.edu](mailto:Diane.Graham@csulb.edu)

When no other is available contact CNSM Safety Office:

- John de la Cuesta: [John.delaCuesta@csulb.edu](mailto:John.delaCuesta@csulb.edu) 562-985-5623, or
- Chris Frost: [Chris.Frost@csulb.edu](mailto:Chris.Frost@csulb.edu) 562-985-5623



# MLSC Common Use Autoclave Cycles: Flush

- **Steam generator MUST be done before each day of use**
  - Record each flush on the user log
  - A flush is critical for continued proper autoclave operation
  - Flushes prevent buildup of mineral scale in the steam generator
  - **Follow the instructions below as well as on the autoclave screen**



- 1) Check the generator pressure gauge (must be at 0 psi)
- 2) Turn the generator drain valve (yellow) so that it is parallel with the pipe (open)
- 3) Start the flush (on screen prompt) and wait 5 min
- 4) Turn yellow handle back perpendicular (closed)



# Training - Next

## Complete the quiz on the [CSULB Autoclave web site](#)

- The goal is not to just pass this quiz. The goal is to learn how to properly use equipment and understand common issues with our equipment.

## MLSC Training Location → MLSC 100

- Training by Michelle Giffin - [Michelle.Giffin@csulb.edu](mailto:Michelle.Giffin@csulb.edu)
  - 1<sup>st</sup> Tuesday of each month @ 10:00am
  - 3<sup>rd</sup> Tuesday of each month @ 4:00pm
  - No appointment needed - you must be on time
    - Individual trainings may be requested in the eventuality of time conflict due to class schedules. Come to MLSC 100 with your class schedule.

## MICRO Training Location → Micro 104

- Training by Diane Graham - [Diane.Graham@csulb.edu](mailto:Diane.Graham@csulb.edu)
  - Dates: Tuesdays @ 8:30am
  - By appointment only