Autoclave Training

URSINUS COLLEGE

BIOLOGY AND CHEMISTRY DEPARTMENTS

Objectives

- What materials can be autoclaved?
- What Personal Protective Equipment (PPE) is necessary for autoclave use?
- How do you properly load and unload the autoclave?
- Who do you go to for assistance?

Importance of Proper Autoclave Use

- Tremendous pressure from steam in the chamber provides explosive potential.
- High temperatures (250^0F) and presence of extremely hot water creates potential for burns and scalding.
- Inadequate decontamination allows for the potential of biological hazards to contaminate personnel and the environment.

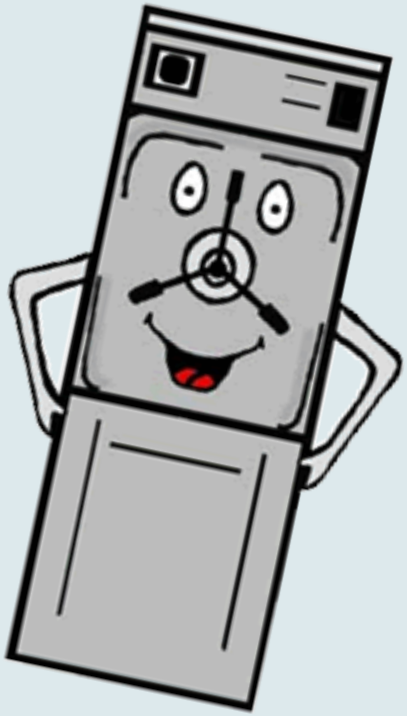
Autoclave Explosion

What can be autoclaved ?

- Glassware
- Polypropylene plastic tubes and pipette tips
- Aqueous Solutions and water
- Non-Biohazardous laboratory waste

Principles of Autoclave Operation

- Steam penetrates objects in the autoclave
- Condensation creates negative pressure and draws in additional steam
- Moist heat kills microorganisms via coagulation of proteins

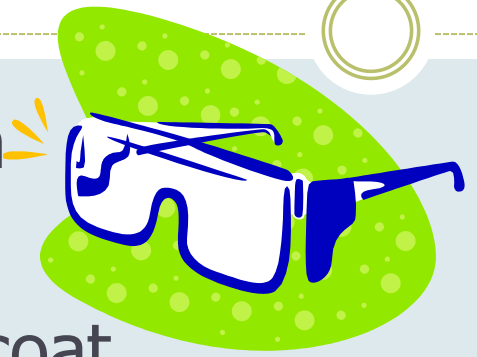


Autoclave Use Basics

- Personal Protective Equipment (PPE)
- Loading
- Operating
- Unloading
- Improper Autoclave Practices

PPE for Autoclave Users

- Eye Protection



- Buttoned lab coat is strongly recommended

- Closed-toed Shoes

- Heat-resistant Gloves

Prior to Loading the Autoclave

- Be sure the material can be autoclaved
  - No corrosives (acids, bases, phenol)
  - No solvents or volatile chemicals (ethanol, methanol, chloroform, bleach)
  - No sharps or red bag waste

- Choose appropriate containers and autoclave bags
  - Clear bags from inside the cardboard receptacles
  - Do not overfill bags or containers
  - Do not allow the plastic to touch the sides of the autoclave

Loading the Autoclave

- To allow for steam penetration
  - Loosely close autoclave bags
  - Loosen lids on bottles containing solutions to prevent the shattering of glass during pressurization

- Place items in an autoclavable polypropylene bin
  - Bottles should not be filled more than 2/3 & keep 1-2 inches between bottles
  - Cover open tops of glassware with foil
  - Label with heat sensitive indicator tape

Loading the Autoclave

- Always autoclave clean items and waste separately.
- Note the cycle end time and come back to collect your items promptly. Close the autoclave door and turn the autoclave OFF when you are finished.
- Items left unlabeled for an excessive period of time will probably disappear!

Operating the Autoclaves

- Be sure the autoclave is functioning properly before use
- Close door properly and securely
- Choose the correct cycle for your material

Thomas Hall autoclave cycles

- P07, gravity 15/15 for glassware
- P08, liquid 15/15
- P09, liquid 30/30
- P10, liquid 45/45
- P11, gravity 30/15 for lab waste

Operating the autoclaves

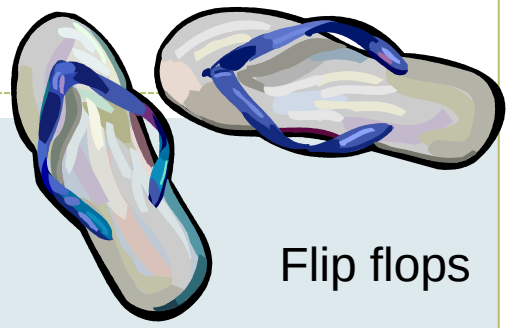
Pfahler Autoclave cycles

Program	Temp	Exhaust	Dry	
P01	250F	30 min	30 min	Standard dry materials (glassware/plastics)
P02	250F	10 min	5 min	
P03	275F	3 min	0 min	
P04	275F	10 min	0 min	
P05	250F	20 min	Liquid	Standard liquid run
P06	250F	15 min	Liquid	

Unloading the Autoclave

- Wear the recommended Personal Protective Equipment
- Allow the autoclave to completely finish cycle
  - Pressure gauge must read zero
- Open door slightly to allow steam to escape
- Carefully remove items
  - Be especially careful with liquids
  - Transport hot liquids in secondary containment
- Be considerate and return autoclave bins and gloves

Improper Autoclave Practices



Flip flops

Biohazards

Ethanol

Results of Improper Autoclave Use

Review

- What materials can be autoclaved ?

Glassware (beakers, graduated cylinders, flasks etc.)

Polypropylene plastic tubes, and pipette tips

Aqueous solutions and water

Non-Biohazardous Laboratory Waste (From inside the cardboard waste receptacles, may contain petri dishes, agar, bacteria, *C.eligans*)

Review

- What Personal Protective Equipment (PPE) is necessary for safe autoclave use?

Review

- How do you properly load the autoclave?

Load material to allow efficient steam penetration.

Autoclave clean items and waste separately.

Do not allow material to be autoclaved to touch the sides or top of the chamber.

Use secondary containment.

Review

How do you properly unload the autoclave?

Wear safety glasses, lab coat, closed-toed shoes, orange autoclave gloves.

Retrieve your items on time.

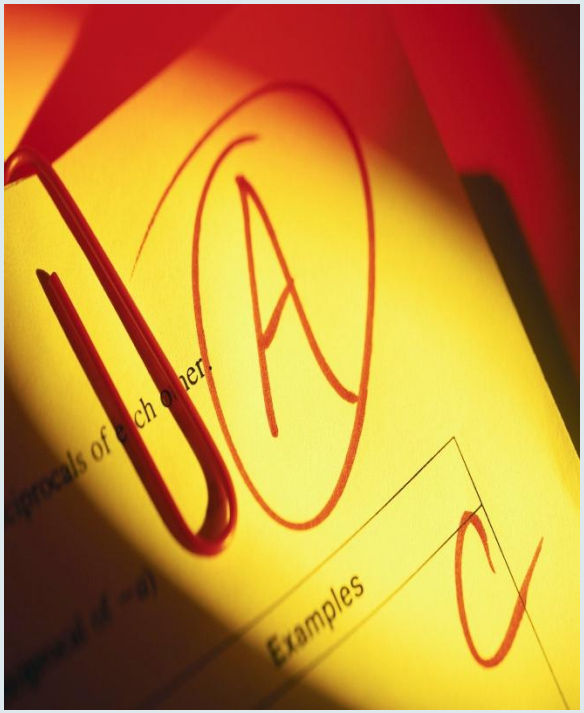
Transport the hot liquids in secondary containment.

Who to ask for assistance?

- Your faculty mentor
- Ann Breen, Biology Laboratory Manager x3072
- Brian Phillips, Chemistry Laboratory Manager x3346

Summary

- Contact your faculty mentor to schedule your hands-on autoclave training and quiz.
- You can operate the autoclave following hands-on training, a review of this PowerPoint presentation and passing the quiz.
- Your quiz will be kept on file in the Biology or Chemistry Dept.



References

University of Kentucky, Dept. of Biological Safety

University of South Carolina Autoclave Safety Policy

UNC Environmental Health and Safety Dept.