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
- Air is forced out
- Steam is forced into autoclave
- Pressure builds up
- Sterilization occurs
Mechanism of Autoclave Sterilization

- 2 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 faceshield, steam apron, and steam gloves
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 boilover liquids
- Adding water to tubs is not needed if you use type I borosilicate glass (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)

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 a cycle and name it
  - Example: Scott Bldg. has ‘Waste’ cycle

Press ‘Operating Mode’ to view cycles
Press arrow to scroll and view cycles
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
  - Flash

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

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 (by gravity or vacuum)
  - Chamber charged

- **Exposure**
  - Time
  - Contact with steam
  - Post conditioning

- **Drying – pulls vacuum**
  - Removes moisture
Best Practices - Liquids

- Use type I borosilicate glass
  - Avoid cracked, nicked glass
- Do not fill more than 2/3 full
- 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 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 materials
Alternate Method: Loading Liquids for Autoclaving

- Add water to ~ ¼ 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

<table>
<thead>
<tr>
<th>Volume of Liquid in One Container</th>
<th>Minimum Recommended Sterilize Time* at 121°C (250°F) minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 mL</td>
<td>25</td>
</tr>
<tr>
<td>250 mL</td>
<td>30</td>
</tr>
<tr>
<td>500 mL</td>
<td>40</td>
</tr>
<tr>
<td>1000 mL</td>
<td>45</td>
</tr>
<tr>
<td>1500 mL</td>
<td>50</td>
</tr>
<tr>
<td>2000 mL</td>
<td>55</td>
</tr>
<tr>
<td>&gt; 2000 mL</td>
<td>55 + 10 min/L</td>
</tr>
</tbody>
</table>

*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 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 spillover materials
- Do not overfill bag – spillover will occur
- Place pan with bag on 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

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

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

Table 8. Examples of flash steam sterilization parameters.

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

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

These are general guidelines and autoclave times may vary by site.
Avoid Overcrowding

A. Overcrowding of surgical packs

B. Correct spacing of surgical packs

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 more 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

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

- Check that the door seal is intact and not cracked/broken.
Operating Procedure - Older Autoclaves

- Place materials in autoclave
- Push door firmly at hinges first, make sure the door hinges are pushed in for a good fit
- Close door
Operating Procedure-Older Autoclaves

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

- Sign up in log book/sign up sheet

- Place Sterigage strip in log book
Removing Materials from the Autoclave-Microbiology

- 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

Note that you may want to use the orange autoclave gloves for removing materials.
Verify Sterility

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

Each of these indicate sterilizing conditions were met
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 was 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 red 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
Thank you for viewing these slides

Be Safe and Don’t Get Steamed!