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Proper use of autoclave for solid/liquid samples/glass sterilization and cleaning

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Science Education Title: Proper Use of Autoclave for Solid/Liquid Samples/Glass Sterilization and Cleaning

Overview:
Autoclaving is one of the most commonly used methods in the laboratory for the purpose of decontamination. The most common items decontaminated with an autoclave are those that have come in contact with a biological sample (typically containing microorganisms). An autoclave works by utilizing pressurized high temperature steam to kill microorganisms present in the loaded materials. Sufficient steam flow and heat transfer are essential for the highly efficient autoclaving, which are the key principles to consider when packaging materials. Safety needs to be concerned when working with autoclave due to the high pressure and temperature, which also sets limits to the compatible materials and requires attention when packaging, loading and unloading materials.

Principles:
Autoclaving utilizes high temperature (121 ^{o}C) and pressure steam (at least 15 psi) for at least 30 minutes to achieve the purpose of decontamination. It kills microorganisms by dehydration the cell under severe conditions. The rate of exhaust depends on the nature of the load. For dry solid materials, fast exhaust cycle can be reached. However, for liquid and biological waste, they require slow exhaust cycle to avoid boiling over super hooted liquid. Indicator tapes are often used to validate the effectiveness of autoclaving. The indicators work by using heat sensitive chemical markings to see if the autoclave meets the temperature requirement (121 ^{o}C). The tape color change implies an effective autoclaving whereas no color change indicates load is not decontaminated. However, don't use the indicator tape as the only source for decontamination since the tape won't reflect whether the microorganisms are killed or not but only if the autoclave meets the temperature requirements. The working conditions also set limits to the suitable material and yield safety concerns. In principle, avoid any materials that easily burn, melt, or explode under high temperature and pressure conditions. Take care when loading and unloading autoclaves to avoid burns by steam or hot water. To achieve sufficient decontamination, make sure there is enough heat transfer and flow within the autoclaves, bags, and materials when packaging.

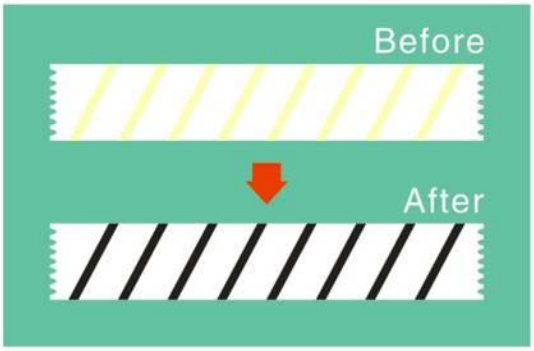

Figure1. Autoclave indicator tapes before and after effective autoclave.

Procedure:

1. Wear Proper Personal Protective Equipment (PPE)
   The high pressure and high temperature operation conditions encountered during autoclaving pose potential heat burn or explosion risks. Appropriate PPE includes a laboratory coat, safety glasses, heat resistant gloves, and close-toe shoes; these must be worn when loading and unloading materials from the autoclave. When dealing with liquid samples, wear an apron and face shield as additional PPE.

2. Preparation of Materials to be Loaded

   2.1. Identify Autoclave Compatible Material and Autoclave Incompatible Material
   Although autoclaving represents a commonly used and economic method for decontamination, not all materials are suitable for autoclaving due to the high pressure and temperature working conditions. In general, all materials that will evaporate or melt under the high-pressurized steam are not compatible with autoclaving. This includes materials like organic solvents, poor heat resistant plastic such as polystyrene and polyethylene, and non-stainless-steel metals. Never autoclave flammable, corrosive, toxic, reactive, or radioactive chemicals. Materials compatible for autoclaving include Pyrex or Type I borosilicate glass, polypropylene, polycarbonate, gloves, stainless steel, pipette tips, paper (put inside autoclave bags), and media solutions.

   2.2 Packaging Material
   After identifying autoclave-compatible materials, suitable packaging is also important since it ensures safety and efficient autoclaving. Here are the basic principles when packaging materials:

   2.2.1. Always use a secondary container made of polypropylene or stainless steel.

   2.2.2. For liquid samples, fill the vial around ½ full and loosen caps or use vented closures. Never fill the containers over 2/3 full and with the cap fully tightened.

   2.2.3 Inspect the glass vessel to make sure there are no cracks. If cracks are identified, dispose of glassware in a proper receptacle. NEVER autoclave a known broken glass vessel.

   2.2.4. Always use autoclave-compatible bags to package waste. The bags should have indication tapes on it and opened at the ends before loading to allow steam inside the bag.

   2.2.5. Never overload the bags or container to allow sufficient steam flow for complete sterilization. Leave space between items.

   2.1.6. Don’t mix incompatible materials.

2.1.7. Never place sharp items in the waste bag.

3. Load Material

3.1. Check the autoclave's interior to make sure there is no potential hazard left from previous user.

3.2. Clean the drain before loading materials to allow good circulation.

3.3. Make sure the bags do not touch the interior walls of autoclave to avoid melting.

3.4. Liquids and dry materials require different cycles and need to be autoclaved separately.

3.5. Make sure there is good flow of heat and steam and then close the door firmly.

4. Operation of Autoclave

4.1. Only trained people should be allowed to operate autoclaves and refer to the equipment manual if needed since the operation varies amongst different autoclaves.

4.2. After making sure the autoclave door is firmed closed, set the autoclaving temperature to 121 °C and maintain a pressure of at least 15 psi.

4.3. To set the autoclave time and cycle, the following factors need to be considered:

4.3.1 Equipment recommendation from manual.

4.3.2 The type of loaded materials inside: dry or liquid materials.

4.3.3 The amounts of material(s) loaded.

4.3.4 Shape and size of container used.

4.3.5 Heat conductivity of container and materials.

4.3.6 Purpose of decontamination.

5. Unloading of Autoclaved Material(s)

5.1. Wear proper PPE as described above.

5.2. Make sure the temperature and pressure of the autoclave have returned to safe range.

5.3. Stand behind the door and carefully open the door to release leftover steam inside the autoclave into the room.

5.4. Allow the materials inside the autoclave to stand for 10 min to release any steam or hot air trapped inside the material.

5.5. Do not shake any liquid material while removing it from the autoclave. If necessary, label the material as hot to avoid heat burns to an individual potentially unaware of the fact that the material has recently been autoclaved.

5.6. Wait for the material to cool down to room temperature before transporting within the laboratory or outside the laboratory. In both instances, be sure to utilize appropriate containment for transporting.

5.7. Record the details of the materials and autoclaving conditions in a user's log.

Summary:
Compared to chemical or radiation decontamination methods, autoclaving represents the most economic and applicable method in the laboratory. However, to ensure safety (avoid melting, heat burn, or explosion) identify compatible materials and practice proper packaging, loading, and unloading. To ensure sufficient decontamination efficiency, container or bag materials, enough space between materials, within the autoclave are required. Maintenance and inspection of the autoclave on a regular schedule are required to ensure autoclaves are operating properly.

References:

1. Autoclave Safety and Operation, 2015, Division of Research Safety, University of Illinois. at https://www.drs.illinois.edu/SafetyLibrary/AutoclaveSafetyAndOperation
2. Autoclave Use, Environmental Health & Safety, Princeton University. at https://ehs.princeton.edu/book/export/html/380
3. Proper Use of a Steam Autoclave, 2016, Environmental Health & Safety, Arizona State University.
4. Fact Sheet: Using Autoclaves Safely, Environmental Health & Safety, California State University, Los Angeles.
5. A Guideline for the Safe Use of Autoclaves, 2003, Environmental Health and Safety Service, University of Ottawa.