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Proper Use Of Autoclave For Solid/Liquid Samples/glass Sterilization And Cleaning

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Overview

Robert M. Rioux & Zhifeng Chen, Pennsylvania State University, University Park, PA

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 contact biological samples (typically those 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 highly efficient autoclaving, which are the key principles to consider when packaging materials. Safety needs to be considered when working with an autoclave due to the high pressure and temperature employed therein, which also sets limits on which materials are compatible and may necessitate special attention when packaging, loading, and unloading materials.

Principles

Autoclaving utilizes high temperature (121 °C), high pressure steam (at least 15 psi) for at least 30 minutes to achieve decontamination. It kills microorganisms by dehydrating the cell under severe conditions. The rate of exhaust depends on the nature of the load. For dry solid materials, a fast exhaust cycle can be reached. However, liquid and biological waste require slow exhaust cycles to avoid boiling over super-heated liquids. 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 requirements (121 °C). An indicator tape's color changing 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 evidence of 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 on which materials are suitable and which pose 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.

Procedure

1. Wear Proper Personal Protective Equipment (PPE)

The high pressure and temperature operating conditions encountered during autoclaving pose potential heat burn or explosion risks. Appropriate PPE includes a laboratory coat, safety glasses, heat resistant gloves, and closed-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

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, poorly heat resistant plastic such as polystyrene, polyethylene, and metals excluding stainless-steel. 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. 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:

1. Always use a secondary container made of polypropylene or stainless steel.
2. For liquid samples, fill the vial around ½ full and loosen the caps or use vented closures. Never fill the containers over 2/3 full with the cap fully tightened.
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.
4. Always use autoclave-compatible bags to package waste. The bags should have indication tapes on it and should be opened at the ends before loading to allow steam inside the bag.
5. Never overload the bags or container to allow sufficient steam flow for complete sterilization. Leave space between items.
6. Don't mix incompatible materials.
7. Never place sharp items in the waste bag.

3. Load Material

1. Check the autoclave's interior to make sure there is no potential hazard left from the previous user.
2. Clean the drain before loading materials to allow good circulation.
3. Make sure the bags do not touch the interior walls of the autoclave to avoid melting.
4. Liquids and dry materials require different cycles and need to be autoclaved separately.
5. Close the door firmly.

4. Operation of Autoclave

1. Only trained personnel should be allowed to operate autoclaves. Refer to the equipment manual if needed, since operation protocols vary among different autoclaves.
2. After making sure the autoclave door is firmly closed, set the autoclaving temperature to 121 °C and maintain a pressure of at least 15 psi.
3. To set the autoclave time and cycle, the following factors need to be considered:
 1. Equipment recommendations from the manufacturer's manual.
 2. The type of loaded materials inside: dry or liquid.
 3. The amounts of material(s) loaded.
 4. Shape and size of container used.
 5. Heat conductivity of container and materials.
 6. Purpose of decontamination.

5. Unloading of Autoclaved Material(s)

1. Wear proper PPE as described above.
2. Make sure the temperature and pressure of the autoclave have returned to a safe range.
3. Stand behind the door and carefully open the door to release any leftover steam inside the autoclave into the room.
4. Allow the materials inside the autoclave to stand for 10 min to release any steam or hot air trapped inside the material.
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 who may be potentially unaware of the fact that the material has recently been autoclaved.
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.
7. Record the details of the materials and autoclaving conditions in a user's log.

Applications and Summary

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

References

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3. Guideline for the Safe Use of Autoclaves, 2003, Environmental Health and Safety Service, University of Ottawa.