Technical Note:

Protocols for the Safe Autoclaving of Laboratory Glassware.

Introduction

Sterilization of Glassware, Plasticware, Solutions, media, etc. by autoclaving is a well-established process that is commonly used in laboratories across many industries and academia. The protocols used for autoclave sterilization will differ depending upon the type of vessel, material and whether the glassware is to be sterilized empty or if it contains liquid. This Application note focuses on laboratory glassware.

The critical parameters that govern the effectiveness of autoclaving are:

Temperature. The most common sterilization temperatures are 121°C (250°F) and 134°C (273°F). At these temperatures steam effectively penetrates materials, denatures proteins and kills microorganisms.

Pressure. To achieve these high temperatures autoclaves must operate under pressure. At 121°C, the pressure is usually set to 15psi (pounds per square inch), while at 134°C pressure will reach 30psi. This elevated pressure allows steam to exceed the normal boiling point of water and ensures sterilization.

Time. The duration of exposure to these temperatures is crucial to achieving sterilization. Sterilization cycles last between 15 to 30 minutes depending on the load size and type. Larger or denser loads will take longer.

"Always follow the instructions and safety precautions detailed in the operators manual provided by the autoclave manufacturer"

"Always ensure that the autoclave is regularly calibrated to ensure that the correct operating parameters are being achieved". Failure to do so will result in unsterile products.

Do's and Don'ts of Autoclaving

1. Do not autoclave vessels containing corrosive materials acids-bases, volatile solutions, solvents or radioactive materials
2. Aways place vessels to be autoclave in a heat-resistant plastic or metal autoclave tray, never place them directly on the shelf or rack in the autoclave.
3. Always maintain a minimum of 5cm of pace between objects that are being autoclaved to allow for heat expansion.
4. Add ¼" to ½" of water in the tray to ensure consistent heating of vessel.
5. Check the material of construction of any caps, tubing, drip rings and gaskets to make sure they are autoclavable at the required temperature.
6. Add DDI water to vessels to be autoclaved to generate steam during sterilization
7. When autoclaving vessels that do not contain media/solution, use the dry cycle
8. Always use liquid cycles for media/solutions to be sterilized
9. Do not mix solutions or volumes when autoclaving media/solutions.
10. When autoclaving media/solutions consider the volume of liquid being autoclaved, a 500mL media bottle will take less time to sterilize than a 1L media bottle

11. To ensure the solution reaches 121°C during the autoclave cycle suspend an autoclave thermometer by a thin wire in the middle of the solution to be sterilized. When the cycle is complete, check the highest temperature reached. If the temperature is less than 120°C increase the autoclave cycle time.
12. When autoclaving vessels with caps, always completely loosen the caps to prevent the vessel from shattering.

Removal from the Autoclave

1. When opening the autoclave stand to the side to allow any remaining steam/heat to escape.
2. Wait 5 minutes prior to removing trays containing dry glassware from autoclave.
3. Wait 10 to 20 minutes prior to removing trays containing glassware containing solutions/media from autoclave. Larger volumes will need more time prior to removal from the autoclave.
4. Use heavy duty autoclave gloves to remove trays from the autoclave
5. Do Not tighten caps until the media/solution is cooled. Tightening too soon might result in the cap liner being pulled into the vessel as a condensation vacuum could be created.
   NOTE. An extreme vacuum may cause the glassware to crack.
6. Do Not place hot glassware on a cold benchtop, the temperature difference might stress the glass and crack the bottom of the vessel.
7. Allow at least 15 minutes prior to touching the autoclaved vessels with ungloved hand.
8. For vessel containing media/solutions allow 1 hour prior to touching with ungloved hand.

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