3. STERILIZATION TECHNIQUE USED IN MICROBIOLOGY

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Introduction

Sterilization is the process of killing all microorganisms (bacterial, viral, and fungal) with the use of either physical or chemical agents. A disinfectant is a chemical substance that kills microorganisms on inanimate objects, such as exam tables and surgical instruments. Skin can never be completely sterile. Sterilization in the microbiological laboratory denotes sterilization process implemented in preparation of culture media, reagents and equipment where the work warrants maintaining sterile condition. Sterilization in microbiology laboratory is done by following methods Physical method i.e., use of heat, filters, radiation Chemical method i.e., by use of chemicals Heat sterilization a. Dry heat sterilization.

a. Dry heat sterilization

Inoculation loops or needle are sterilized by heating to 'red' in Bunsen burner or spirit lamp flame. Sterilization in hot air oven is performed at a temperature of 160C and maintained or holding for one hour. Spores are killed at this temperature and this is the most common method of sterilization of glassware, swab sticks, pestle and mortar, mineral oil etc. Dry heat sterilization causes protein denaturation, Oxidative damage, toxic effect of elevated electrolyte in absence of water.

b. Wet heat or moist heat sterilization

Moist heat sterilization is accomplished by

1). **Boiling** at 100°C for 30 minutes is done in a water bath. Syringes, rubber goods and surgical instruments may be sterilized by this method. Almost all bacteria and certain spores are killed in this method

2). **Steaming** at 100°C for 20 to 30 minutes under normal atmospheric pressure are more effective than dry heat at the same temperature because bacteria are more susceptible to moist heat, Steam has more penetrating power and sterilizing power as more heat is given up during condensation. Suitable for sterilizing media which may be damaged at a temperature higher than 100°C

3).**Tyndallization** (Fractional Sterilization) is the steaming process performed at 100°C is done in steam sterilizer for 20 minutes followed by incubation at 37°C overnight and this cycle is

repeated for successive 2 days. Spores, if any, germinate to vegetative bacteria during incubation and are destroyed during steaming on second and third day. Heat labile media containing sugar, milk, gelatin can be sterilized using this method.

4). **Autoclaving** is done by steam under pressure. Steaming at temperature higher than 100°C is used in autoclaving. This is achieved by employing a higher pressure. The autoclave is closed and made air-tight for pressure development and at 15 lbs per sq. inch pressure, 121°C temperatures will be reached and this temperature is given as sterilizing holding time for further 15 minutes. This process kill spores and this works like a pressure cooker and one of the most common methods of sterilization.

5). **Pasteurization** is another one method of moist heat sterilization which works below 100°C heat. This process is used in heating of milk and other liquid food. The product is held at temperature and for a period of time to kill pathogenic bacteria that may be present in the product. This process does not destroy complete organism including spores.

All these moist heat sterilization causes denaturation and coagulation of protein, breakage of DNA strands, and loss of functional integrity of cell membrane.

c). **Filtration**: This method of sterilization is used for media particularly heat labile in nature (e.g. sera an media containing proteins or labile metabolites. If the study warrants bacteria-free filtrates it can be obtained through 0.45micron sized filter membranes and if the study requires viral particle free solution, then 0.22micron sized filter membranes are use. In earlier days absorptive filters of asbestos or diatomaceous earth were replaced by unglazed porcelain or sintered glass are used. Nowadays these are replaced by nitrocellulose membrane filters of graded porosity, PVDF etc.

d). **Ultraviolet Radiation:** at wavelength between 330nm and 400nm causes sterilizing effect. This method is used in surface sterilization of laminar airflow, biosafety cabinet and in certain cases in laboratory.

In microbiology laboratory autoclaving, hot air oven sterilization, filtration and UV radiation are commonly used.

Standard operating procedure for the setting up of autoclave

- Pack your media, reagents, plastic wares, in their appropriate autoclavable resistant polypropylene or borosilicated glassware
- Screw the lid of the tube and leave one thread loose in case of closed containers or plastics

- Stick at random autoclavable indicators for each run in any of the items to be autoclaved
- Check for the water level in the autoclave machine
- Donot jam pack the items in the autoclave machine
- Switch on the machine
- Keep the lid of the machine tightly closed with one valve open until it reaches boiling
- Leave heated air to escape for few minute through valve
- Completely close the valve and wait to reach the temperature for 121^0C at 15lbs pressure.
- Hold the sterilization cycle for 15 minutes
- Once the sterilization cycle end, switch off the heating and leave the machine to reach to 65^0C
- Then open the lid and take out the items back after sterilization

Standard operating procedure for the setting up of hot air oven

- Pack all the glassware such as pipette with pipette can, glass petridishes, sample dish, test tubes, pestle and mortar, mineral oil to be sterilized by hot air oven sterilization with suitable wrapping
- Switch on the hot air oven until to reach 160^0C
- Hold on in that temperature for 1 hour
- Switch off the heating of hot air oven and open the door once come below 65^0C

Standard operating procedure for the setting up of filtration

- Once the bio safety cabinet is ready for filtration
- Switch on the blower
- Filtration unit should be inside the cabinet
- Vacuum or positive pump should be kept outside of the cabinet
- Filtration assembly should be with the suitable filters
- Pour the media or reagents to be sterilized in the top of the filtration assembly
- Connect the bottom assembly to vacuum pump or top of the assembly to the positive pump