Quadrant II – Transcript and Related Materials

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Module Name: Physical Methods of Sterilization

Module No: 02

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Notes:

METHODS OF STERILIZATION

- There are two methods of sterilization
  1. Physical methods and 2. Chemical methods
- Physical methods of sterilization includes:
  A. Sunlight, B. Heat, C. Radiation, D. Filtration and E. Vibration
- Chemical methods of sterilization includes
  1. inorganic chemicals, 2. alcohol and 3. aldehyde

PHYSICAL METHODS OF STERILIZATION:

A. STERILIZATION BY SUNLIGHT

- Responsible for spontaneous sterilization in natural conditions.
- Microbicidal activity of sunlight is due to the presence of ultra violet rays in it.
- It is more effective in killing germs due to combination of UV rays and heat.
- Since it does not kill spores, it is not a perfect sterilization method.

B. STERILIZATION BY HEAT

- Use of heat is the most reliable method of sterilization.
- The killing effect is due to the denaturation and coagulation of protein.

- The method used depends on number of microorganisms present, characteristics of microorganisms and the type of material.

METHODS OF HEAT STERILIZATION:

Heat sterilization can be done by two methods i.e. **Moist heat** and **Dry heat**.

B.1. Moist heat sterilization - can be done at different temperatures i.e. below 100^{o}C, at 100^{o}C and above 100^{o}C.

I. MOIST HEAT STERILIZATION - Temperature below 100°C

1. Pasteurization:

- Method used for sterilization of milk, serum and heat sensitive materials.
- Sterilization is achieved by 2 methods:
  i. **Holder method** (63°C for 30 minutes)
  ii. **Flash method** (72°C for 15-20 seconds) followed by quick cooling to 15°C or lower temperature.

✓This method destroys all non-sporulating pathogens, but is not suitable for spore producing bacteria.

2. Vaccine bath:

- Contaminating bacteria in a vaccine preparation can be inactivated by heating in a water bath at **60°C** for **1 hour**.
- Vegetative cells of bacteria are killed, but spores survive.

3. Serum bath:

- Contaminating bacteria in a serum preparation can be inactivated by heating in a water bath at **56°C** for **1 hour** on several successive days.
- Proteins in the serum will coagulate at higher temperature.
- Vegetative cells of bacteria are killed, but spores survive.

4. Inspissation:

- Technique to solidify as well as disinfect egg and serum containing media.
- The medium is placed in the slopes of an inspissator and heated at **80-85°C** for **30 minutes** on three successive days.
- On the first day, vegetative bacteria would be killed; those spores that germinate by next day are then killed the following day.

II. MOIST HEAT STERILIZATION - Temperature at 100°C

1. Boiling: Boiling water (100°C) kills most vegetative bacteria and viruses.

- Some bacterial spores are resistant to boiling and survive; hence this is not a substitute for sterilization.
- Metal articles and glassware can be disinfected by placing them in boiling water for **10-20 minutes**.
- Lid of the boiler is closed during this period.

III. MOIST HEAT STERILIZATION - Temperature at 100°C

1. Steam at atmospheric pressure - free steam at 100°C using steamer.

- Steamer is a metal cabinet with perforated trays to hold the articles and a conical lid.
- Bottom of steamer is filled with water and heated.
- Steam sterilizes the articles when exposed for a period of **90 minutes**.
- Vegetative bacteria are killed in the first exposure and the spores that germinate by next day are killed in subsequent days.

IV. MOIST HEAT STERILIZATION - Temperature above 100°C

1. Steam under pressure - Autoclaving.

- Principle – as pressure inside a closed vessel increases, the temperature at which water boils also increases thereby killing the microbes present.
- A temperature of 121°C at 15psi with 20-30 minutes holding time is commonly used.
- Used to sterilize culture media, distilled water and glassware.
- Items like Petri plates, plastic tubing, cotton, filters etc. are wrapped in brown paper before putting in autoclave.
- Mouths of test tubes, flasks, pipettes are plugged with non-absorbent cotton plugs. Capped bottles are loosely screwed.
- Then all these are loaded in autoclave with adequate space in between items for free circulation of steam.
- Autoclave at **15 psi, 121°C for 20-30** minutes.
- Allow the pressure to drop gradually to 0 psi and then only open autoclave and unload.
- Tighten the caps of bottles immediately.
- Transfer glassware to oven at **50°C** for removing moisture and complete drying. Then transfer to dust free, sterilized cabinet.

B.2. Dry heat sterilization - can be done by red heat, flaming, incineration and hot air oven

I. Red Heat:

- Articles such as bacteriological loops, straight wires, tips of forceps and searing spatulas are sterilized by holding them in a Bunsen flame till they become red hot.

II. Flaming:

- ✓ The article to be sterilized is passed a few times over a Bunsen flame, but not heated to redness.
- ✓ Articles sterilized include mouth of test tubes, flasks, glass slides etc.

III. Incineration:

- This is a method of destroying contaminated material by burning them in an incinerator.
- This method results in the loss of the article.

IV. Hot Air Oven:

- Articles are exposed to high temperature **(160°C)** for **one hour** in an electrically heated oven.
- Heat distribution is by a fan.
- Articles sterilized include metallic instruments, glassware, swabs, oils, grease, and some pharmaceutical products.
- *Advantages:*
  - ✓It is an effective method of sterilization of heat stable articles.
  - ✓The articles remain dry after sterilization.
  - ✓This is the only method of sterilizing oils and powders.
- *Disadvantages:*
  - ✓Since air is poor conductor of heat, hot air has poor penetration.
  - ✓Cotton wool and paper may get slightly charred.
  - ✓Glasses may become smoky.
  - ✓Takes longer time compared to autoclave.

C. STERILIZATION BY RADIATION:

- Two types of radiation are used: ionizing and non-ionizing.
- Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power.
- Since radiation does not generate heat, it is termed "cold sterilization".

I. Non-ionizing rays – (Infra red rays and UV rays)

- These are electromagnetic radiations with wavelengths longer than those of visible light.

1. IR rays – bring about sterilization by generation of heat.

- Articles to be sterilized are placed in a moving conveyer belt and passed through a tunnel that is heated by infrared radiators to a temperature of 180°C for 7.5 minutes.
- Used for rapid mass sterilization of syringes, instruments & glassware.

2. UV rays: Used for disinfecting enclosed areas such as laboratories, inoculation rooms and operation rooms.

- UV radiation in the form of UV tube or UV lamp is used in Laminar Air Flow Cabinet or inoculation chamber
- Working area is exposed to UV radiation for 25 minutes before starting work

Disadvantages:

- Low penetrative power.
- Limited life of UV bulb.
- Some bacteria have DNA repair enzymes.
- Does not penetrate glass, paper or plastic.
- Eye protection is needed since it can cause severe damage.

II. Ionizing rays - (Gamma rays)

- This method is also called cold sterilization as there is no appreciable increase in temperature; sterilization is accomplished in few seconds.
- Radiations with very high penetrating power
- Highly lethal to DNA and other vital cell constituents
- Cause biological damage by producing hyper reactive ions.
- Gamma rays are used for sterilizing plastics, syringes, dressing packs, swabs, culture plates etc.

D. STERILIZATION BY FILTRATION

- Heat sensitive substances like enzymes, antibiotics, amino acids, vitamins etc. cannot be sterilized by autoclaving.
- Contaminants like bacteria are filtered out by using millipore filters.

- Filters are made up of cellulose acetate or polycarbonates that contain small sized pores (0.22 microns).
- The filters are placed in the filtration assembly and sterilized by autoclaving prior to use.
- The solution to be sterilized is passed through the filtration assembly and collected in a sterilized container.

TYPES OF FILTERS:

a. *Earthenware filters* - made up of diatomaceous earth or porcelain.
- Usually baked into the shape of a candle.

b. *Asbestos filters* - made from chrysotile type of asbestos, chemically composed of magnesium silicate.
- Pressed to form a disc; used only once.
- Disc is held inside a metal mount, which is sterilized by autoclaving.

c. *Sintered glass filters* - made from finely ground glass that are fused sufficiently to make small particles adhere to each other.
- Available in the form of disc fused into a glass funnel.
- Filters of Grade 5 have average pore diameter of 1-1.5 µm.
- Washed in running water in reverse direction and cleaned with warm concentrated H_2SO_4.
- Sterilized by autoclaving.

d. *Membrane filters* - made from cellulose nitrate, cellulose diacetate, polyester and polycarbonate.
- Pore diameter ranges from 0.015 µm to 12 µm.
- Filters are sterilized by autoclaving.
- Advantages of membrane filters are known porosity, reusable after autoclaving and compatible with many chemicals.

E. STERILIZATION BY VIBRATIONS

Sonic and ultrasonic vibrations:
- High frequency sound waves of frequency >20,000 cycle/second are generated using a sonicator.
- These vibrations are known to kill bacteria by causing cell disruption.
- Used to clean and disinfect instruments.
- Not a reliable method, since many viruses are not affected by these waves.