CHAPTER-V
STERILIZATION

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Sterilization: It is defined as the process where all the living microorganisms, including bacterial spores are killed.

Sterilization can be achieved by physical, chemical and physiochemical means. Chemicals used as sterilizing agents are called chemisterilants.

Sanitization is the process of chemical or mechanical cleansing, applicable in public health systems. Usually used by the food industry. It reduces microbes on eating utensils to safe, acceptable levels for public health.
- **Asepsis** is the employment of techniques (such as usage of gloves, air filters, U.V. rays etc) to achieve microbe-free environment.

- **Antisepsis** is the use of chemicals (antiseptics) to make skin or mucus membranes devoid of pathogenic microorganisms.

- **Bacteriostasis** is a condition where the multiplication of the bacteria is inhibited without killing them.

- **Bactericidal** is that chemical that can kill or inactivate bacteria. Such chemicals may be called variously depending on the spectrum of activity, such as bactericidal, virucidal, fungicidal, microbicidal, sporicidal, tuberculocidal or germicidal.

- **Antibiotics** are substances produced by one microbe that inhibits or kills another microbe. Often the term is used more generally to include synthetic and semi-synthetic antimicrobial agents.
Methods of sterilization/disinfection

Physical
- Sunlight
- Heat
- Vibration
- Radiation
  - Non-ionizing
    - Electromagnetic
  - Ionizing
    - Particulate
  - Filtration
    - Earthenware
    - Asbestos
    - Sintered glass
    - Membrane

Chemical
- Liquid
  - Alcohols
  - Aldehydes
  - Phenolics
  - Halogens
  - Heavy metals
  - Surface active agents
  - Dyes

Physicochemical
- Gaseous
  - Formaldehyde
  - Ethylene oxide
  - Plasma
PHYSICAL METHODS OF STERILIZATION:

**Sunlight:** The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it. It is responsible for spontaneous sterilization in natural conditions. In tropical countries, the sunlight is more effective in killing germs due to combination of ultraviolet rays and heat. By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes. Sunlight is not sporicidal, hence it does not sterilize.

**Heat:** Heat is considered to be most reliable method of sterilization of articles that can withstand heat. Heat acts by oxidative effects as well as denaturation and coagulation of proteins. Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure.
Factors affecting sterilization by heat are:-

**Nature of heat:** Moist heat is more effective than dry heat.

**Temperature and time:** temperature and time are inversely proportional. As temperature increases the time taken decreases.

**Number of microorganisms:** More the number of microorganisms, higher the temperature or longer the duration required.

**Nature of microorganism:** Depends on species and strain of microorganism, sensitivity to heat may vary. Spores are highly resistant to heat.

**Type of material:** Articles that are heavily contaminated require higher temperature or prolonged exposure. Certain heat sensitive articles must be sterilized at lower temperature.

**Presence of organic material:** Organic materials such as protein, sugars, oils and fats increase the time required.
**Action of heat:**
Dry heat acts by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes. The moist heat acts by coagulation and denaturation of proteins. Moist heat is superior to dry heat in action. Temperature required to kill microbe by dry heat is more than the moist heat.

**Thermal death time** is the minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.
**DRY HEAT:**

**Red heat:** Articles such as bacteriological loops, straight wires, tips of forceps and searing spatulas are sterilized by holding them in Bunsen flame till they become red hot. This is a simple method for effective sterilization of such articles, but is limited to those articles that can be heated to redness in flame.

**Flaming:** This is a method of passing the article over a Bunsen flame, but not heating it to redness. Articles such as scalpels, mouth of test tubes, flasks, glass slides and cover slips are passed through the flame a few times. Even though most vegetative cells are killed, there is no guarantee that spores too would die on such short exposure.

This method too is limited to those articles that can be exposed to flame. Cracking of the glassware may occur.
**Hot air oven:** This method was introduced by Louis Pasteur. Articles to be sterilized are exposed to high temperature (160° C) for duration of one hour in an electrically heated oven. Since air is poor conductor of heat, even distribution of heat throughout the chamber is achieved by a fan. The heat is transferred to the article by radiation, conduction and convection. The oven should be fitted with a thermostat control, temperature indicator, meshed shelves and must have adequate insulation.

**Articles sterilized:** Metallic instruments (like forceps, scalpels, scissors), glassware (such as Petri-dishes, pipettes, flasks, all-glass syringes), swabs, oils, grease, petroleum jelly and some pharmaceutical products.
**Infra red rays:** Infrared 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. The articles are exposed to that temperature for a period of 7.5 minutes. Articles sterilized included metallic instruments and glassware. It is mainly used in central sterile supply department. It requires special equipments, hence is not applicable in diagnostic laboratory.
MOIST HEAT

Moist heat acts by coagulation and denaturation of proteins.

At temperature below 100oC:

**Pasteurization:** This process was originally employed by Louis Pasteur. Currently this procedure is employed in food and dairy industry. There are two methods of pasteurization, the holder method (heated at 63oC for 30 minutes) and flash method (heated at 72oC for 15 seconds) followed by quickly cooling to 13oC. Other pasteurization methods include Ultra-High Temperature (UHT), 140oC for 15 sec and 149oC for 0.5 sec. This method is suitable to destroy most milk borne pathogens like Salmonella, Mycobacterium, Streptococci, Staphylococci and Brucella, however Coxiella may survive pasteurization. Efficacy is tested by phosphatase test and methylene blue test.
**Vaccine bath:** The contaminating bacteria in a vaccine preparation can be inactivated by heating in a water bath at 60°C for one hour. Only vegetative bacteria are killed and spores survive.

**Serum bath:** The contaminating bacteria in a serum preparation can be inactivated by heating in a water bath at 56°C for one hour on several successive days. Proteins in the serum will coagulate at higher temperature. Only vegetative bacteria are killed and spores survive.
At temperature above 100ºC:

- **Autoclave**: Sterilization can be effectively achieved at a temperature above 100ºC using an autoclave. Water boils at 100ºC at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases. In an autoclave the water is boiled in a closed chamber. As the pressure rises, the boiling point of water also raises. At a pressure of 15 lbs inside the autoclave, the temperature is said to be 121ºC. Exposure of articles to this temperature for 15 minutes sterilizes them. To destroy the infective agents associated with spongiform encephalopathy (prions), higher temperatures or longer times are used; 135ºC or 121ºC for at least one hour are recommended.
Construction And Operation Of Autoclave
FILTRATION:

- Filtration does not kill microbes, it separates them out. Membrane filters with pore sizes between 0.2-0.45 μm are commonly used to remove particles from solutions that can't be autoclaved. It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution. Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and
- purifying hydrated fluid. Filtration is aided by using either positive or negative pressure using vacuum pumps. The older filters made of earthenware or asbestos are called depth filters.
DIFFERENT TYPES OF FILTERS

1. **Earthenware filters**: These filters are made up of diatomaceous earth or porcelain. They are usually baked into the shape of candle. Different types of earthenware filters are:

   A. **Pasteur-Chamber land filter**: These candle filters are from France and are made up of porcelain (sand and kaolin). Similar filter from Britain is Dolton. Chamber land filters are made with various porosities, which are graded as L1, L1a, L2, L3, L5, L7, L9 and L11. Doulton filters are P2, P5 and P11.

   B. **Berkefeld filter**: These are made of Kieselguhr, a fossilized diatomaceous earth found in Germany. They are available in three grades depending on their porosity (pore size); they are V (veil), N (normal) and W (wenig). Quality of V grade filter is checked using culture suspension of *Serratia marcescens* (0.75 μm).

   C. **Mandler filter**: This filter from America is made of Kieselguhr, asbestos and plaster of Paris.
2. **Asbestos filters:** These filters are made from chrysotile type of asbestos, chemically composed of magnesium silicate. They are pressed to form disc, which are to be used only once. The disc is held inside a metal mount, which is sterilized by autoclaving. They are available in following grades; HP/PYR (for removal of pyrogens), HP/EKS (for absolute sterility) and HP/EK (for clarifying).

3. **Sintered glass filters:** These are made from finely ground glass that are fused sufficiently to make small particles adhere to each other. They are usually available in the form of disc fused into a glass funnel. Filters of Grade 5 have average pore diameter of 1-1.5 μm. They are washed in running water in reverse direction and cleaned with warm concentrated H2SO4 and sterilized by autoclaving.

4. **Membrane filters:** These filters are made from a variety of polymeric materials such as cellulose nitrate, cellulose diacetate, polycarbonate and polyester. The older type of membrane, called gradocol (graded colloidion) membrane was composed of cellulose nitrate. Gradocol membranes have average pore diameter of 3-10 μm. The newer ones are composed of cellulose diacetate. These membranes have a pore diameter ranging from 0.015 μm to 12 μm. These filters are sterilized by autoclaving. Membrane filters are made in two ways, the capillary pore membranes have pores produced by radiation while the labyrinthine pore membranes are produced by forced evaporation of solvents from cellulose esters.