

Sterilization and Disinfection

Definition

- Sterilization means destruction of all microorganisms including spores
- Disinfection means the destruction of vegetative organisms which might cause disease or putrefaction
- **Sterilization Methods**
- Red heat (Flaming)
- Dry heat (Hot air)
- Moist heat
- Steam under pressure (autoclaving)
- Steam not under pressure (Tyndallization)
- Filtration

Dry Heat

- Instruments inoculating wires and loops are sterilized by in a flame
- Incineration
- Glass spreaders, forceps, scissors to flaming after dipping in alcohol allowing the alcohol to flame
- The sterilizing effect of dry heat is due to oxidation of intracellular components and extreme dehydration
- very high temperature
- glass petridishes, pipettes, flasks and metal objects in hot air oven

- operation or use of hot air oven includes, the heating up period
- the holding period 1 h at 160° C or 2 hours at 140° C
- the cooling down period - to be cooled to less than 80° C



Moist Heat

- The sterilizing effect of moist heat is due to coagulation of proteins
- **Steam under pressure**
- Done by autoclaving at 121°C for 15 min
- suitable for culture media, aqueous solution, treatment of discarded cultures and specimens
- Boiling point of water increases with increasing pressure of steam

| Steam pressure (psi) | Temperature |
|----------------------|-------------|
| 0 | 100° C |
| 5 | 109° C |
| 10 | 115° C |
| 15 | 121° C |

Laboratory autoclaves

- Pressure cooker type
- Gravity displacement



Steam not under pressure (at 100°C)

- **Moist heat at 100°C**
- Arnold's steam sterilizer
- **Tyndallization** is performed
- at 100°C for 20 – 30 min for three successive days
- **Moist heat below 100°C (Pasteurization)**
- Not a sterilization method
- Pasteurization is adopted in preserving milk intended to destroy the vegetative pathogenic organisms
 - Holder method
 - Flash method

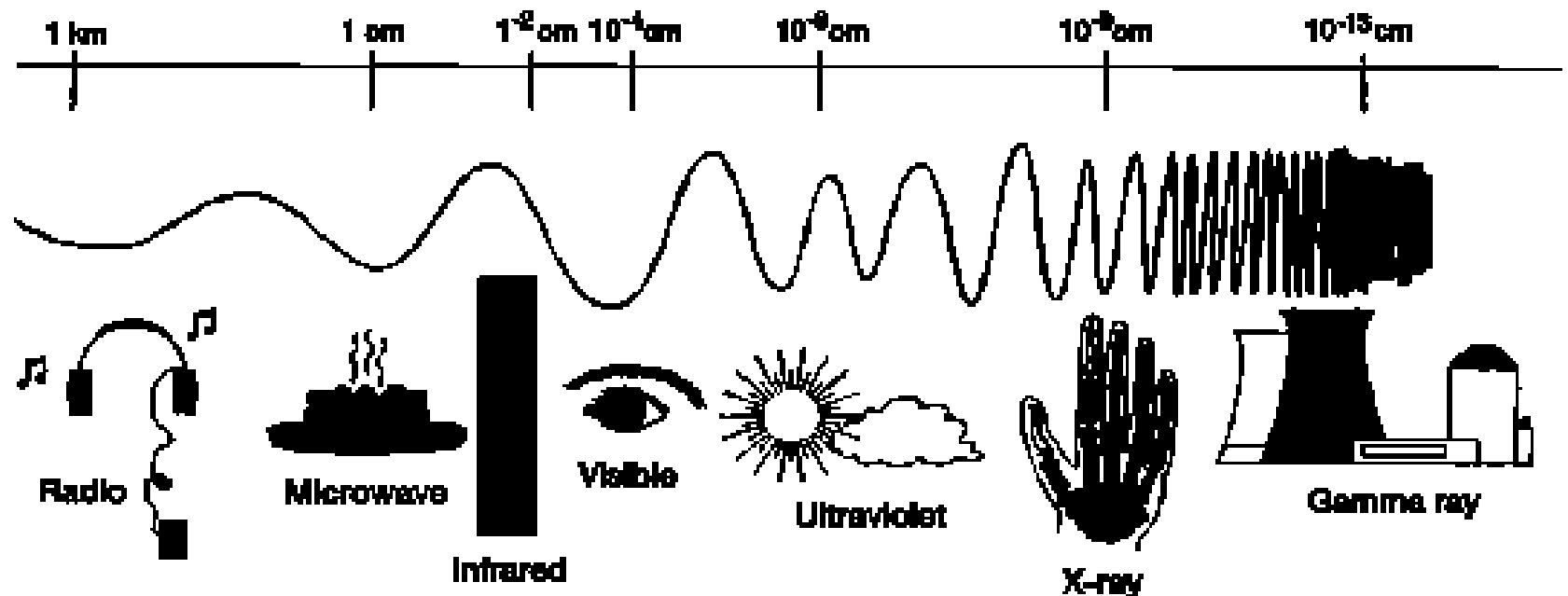
Filtration

- Bacteria can be removed from liquids by passing them through filters
- Useful for sterilizing heat labile fluids like vitamins, antibiotics, sera, urea, protein solution etc
- Types of filters
 - Earthenware candle filters - eg. Berkefeld filter, Chamberland filter
 - Asbestos pad filters - eg. Seitz filters
 - Sintered glass filter
 - Membrane filters
 - made from cellulose acetate or cellulose nitrate
 - membranes of $0.45\ \mu\text{m}$ or $0.22\ \mu\text{m}$ are used

Radiation

- Many forms of electromagnetic radiation are harmful to microorganisms
- Gamma rays and x-rays are more energetic than visible light or infra-red waves
- Radiation is the emission and propagation of energy through a space or material medium
- Two types
 - Ionising radiation
 - Non ionising radiation

- X-rays
- γ (gamma) rays
- Destruction of DNA is the most important cause of death of organisms



Ultra – violet (UV) radiation

- UV has maximum bactericidal effect or is lethal at a wave length of 260 nm
- Primary mechanism of UV damage is the formation of thymine dimers in DNA
- Killing is appreciable at 330 nm and below
- **Sterilization using radiation is called cold sterilization**

Other methods

- **Photodynamic sensitization**
 - presence of certain fluorescent dyes, strong visible light denatures proteins and sterilizes bacteria and viruses
- **Ultrasonic and Sonic waves**
 - ultrasonic range (frequency of 15,000 hz & more)
sound waves denature proteins
- **Freezing**
 - crystallization of the water results in the formation of tiny pockets of concentrated solution of salts
 - damage the bacteria

Chemical sterilization

- Ethylene oxide (EtO)
- Used for sterilization of plasticwares, tubings, etc
- EtO at the concentration of 700 mg / lit for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C
- Excessive aerations is required after the treatment
- Betapropiolactone (BPL), vapour phase of H₂O₂

Disinfection

- Most disinfectants are effective against vegetative bacteria but not spores
- While selecting disinfectants, factors such as their toxicity and harmful effects on the skin, eyes and respiratory tract should be considered
- **Desirable properties of a disinfectant**
- Effective and capable of rapidly killing microorganisms
- Reasonably stable
- Non-corrosive and non-staining
- Odourless or have an inoffensive odour
- Non toxic and non-irritating to skin and eyes
- Readily soluble in water and readily rinsable
- Cost – effective

Commonly used Disinfectants

- **Clear phenolics**
- Effective against bacteria and fungi, inactive against spores
- 2 – 5% solutions are recommended
- **Hypochlorites**
- activity is due to chlorine and is effective against bacteria, spores
- Reasonably clean surfaces – 1000 ppm
- Pipette and discard jars - 2500 ppm

- **Aldehydes**

- Formaldehyde, glutaraldehyde

- Formalin is diluted to 1:10

- Aldehydes are however toxic and cause eye and skin irritation

- **Alcohol and Alcoholic mixtures**

- Ethanol and propanol at 70 – 80% concentrations are effective

- 10% formalin in 70% alcohol or 2000 ppm of available chlorine in alcohol is effective

- **QAC (Quarternary Ammonium Compounds)**
- cationic detergents effective against vegetative bacteria, fungi, 1 – 2% dilutions
- **Iodophores**
- Iodines are effective vegetative bacteria, spores, fungi, virus
- at 75 – 150 ppm iodine

Chemical Disinfectants

| Agent | Mode of Action |
|---|---|
| Halogens Chlorine and its compounds Iodine and iodophores | Oxidation of proteins and enzymes Protein inactivation by iodination |
| Heavy metals Mercuric chloride and organomercurials Silver nitrate | Enzyme inactivation by coupling to sulphhydryl groups of proteins Denaturation of proteins and enzymes |
| Phenolic compounds | Disruption of cell membrane, inactivation of proteins and enzymes |

| | |
|--------------------------------|--|
| Alcohols | Solubilization of lipids, denaturation of proteins and inactivation of enzymes |
| Quarternary Ammonium compounds | Disruption of cell membranes; denaturation of proteins and inactivation of enzymes |
| Formaldehyde | Strong reducing agent, inactivates enzymes |
| Ethylene oxide | Inactivates enzymes |