Microbiology 1

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Sterilization and disinfection

- **Terms:**
  - *Sterilization*
    - ✓ The inactivation of all self-propagating biological entities (e.g. bacteria, viruses, prions) associated with the materials or areas under consideration *100% killing*.
  - *Disinfection*
    - ✓ The *reduction* of pathogenic organisms to a level at which they no longer constitute a risk.
  - *Antisepsis*
    - ✓ Term used to describe *disinfection applied to living tissue* such as a wound.

- **Methods of Sterilization:**
  - *Heat*
    - ✓ Dry
    - ✓ Moist
  - *Radiation*
    - ✓ U.V. rays
    - ✓ Ionizing radiation
    - ✓ I.R. rays
  - *Filtration*
  - *Chemical agents* (Sterilization and Disinfection)

❖ Heat

- Less than 100^{o}C not kill T.B and spores
- 100^{o}C can kill all bacteria except spores
- More than 100^{o}C can kill all bacteria and spores but not destroy prions.

- **Types:**
  - **Dry heat**
    - ✓ Most common method
    - ✓ Dry heat kills microorganism by *destroying their oxidative processes*
      - Types:

1. *Red heat:*

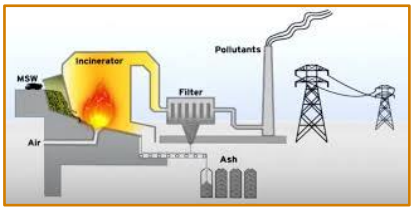
- The item to be sterilized is *directly* held in the flame and heated till it becomes red hot.
- *Application*: Bunsen burner used for sterilizing bacteriological loops, knives, blades

2. *Flaming:*

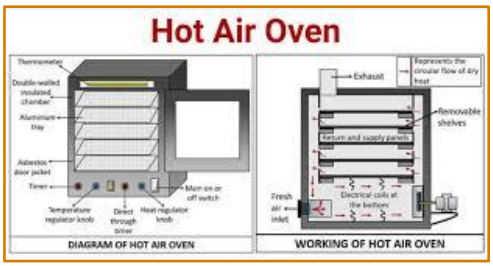
- *Killing* of organism present on the surface of slides , mouth of culture tubes

3. *Incineration*

- Is a huge metal compartment used for *burning* all the objects that can not be cleaned.
- *Used for* disposal of hospital waste



4. *Hot air oven*
   - Expose items to 160-170 C for 1-2 hour.
   - It has electric element in chamber as source of heat plus a fan to circulate air for even distribution of heat in chamber.
   - *Used to* sterilize items that are lacking water such as metals and glassware



➢ Moist heat

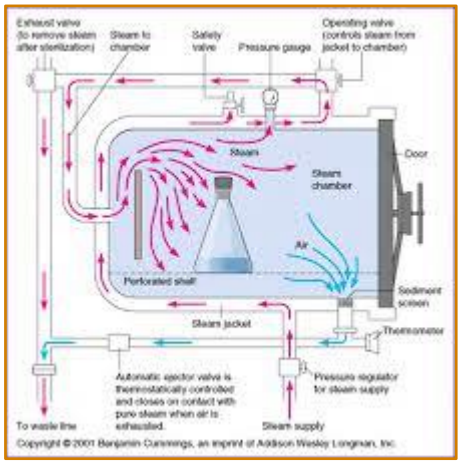
✓ *Steam* is non-toxic and non-corrosive, but for effective sterilization it must be:

1. *Saturated:* form of a transparent vapour.
2. *Dry*: it does not contain water droplets.

✓ ***Kills by*** denaturation of proteins:

- Types:

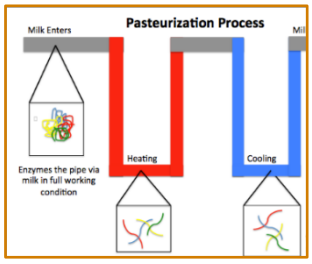
1. Moist heat at temperature below 100C:
   - Pasteurization
   - Inspissation
2. Moist heat at temperature at 100 C:
   - Boiling
   - Tyndallisation
3. Moist heat at temperature above 100 C:
   - Autoclaving



➢ Pasteurization

✓ Used heat at temperatures sufficient to *inactivate* harmful organism in milk

✓ Temperature may be 72 C for 20 sec. followed by rapid cooling (*flash method*) or 63C for 30 mins (*holder method*)



➢ Inspissation

✓ Exposure of the media to *humid heat* at 75C for 2 hours on 3 successive days (sterilization of media contain proteins e.g serum

➢ Boiling

✓ *Simple boiling* at 100C for 5-10 min. is used to sterilize some glassware, forceps, and scalpels.

➢ Tyndallisation:

✓ Exposure to *steam* (100C) for 20-30 min. for 3 *consecutive* days.

✓ It is *used for* materials which can not withstand prolonged boiling (media containing sugar and gelatine)

➢ **Autoclaving**

- ✓ *Most efficient* and reliable sterilization method in hospitals
- ✓ Is a tough double walled *chamber* in which air is replaced by pure saturated steam under pressure.
- ✓ ***Used to*** sterilize most of the instruments and culture media.

- ✓ *Monitoring of autoclaves:*

1. *Chemical*
   - It consists of heat sensitive chemical that changes colour at the right temperature and exposure time.
   - Types:
     - Autoclave tape
     - Browne's tube.
2. *Biological*
   - Where a spore-bearing organism is added during the sterilization process and then cultured later to ensure that it has been killed.
   - These biological indicators contain Bacillus stearothermophilus spores.

- **Radiation**

➢ *U.V. rays*

- ✓ *Bactericidal*
- ✓ Not efficient for complete sterilization
- ✓ Reduce number of bacteria in certain areas such as *operating theaters*

➢ *lonizing radiation:*

- ✓ Rays of *short wave* length and high penetration power e.g. Gamma rays.
- ✓ In moderate doses lethal to microorganisms.
- ✓ Sterilize pre-packed disposable plastic items that cannot stand heat such as plastic syringes, catheters, gloves.

➢ *Infra-red rays:*

- ✓ Act through *heating*, temp. reach 180°C.
- ✓ sterilize mainly glass ware and syringes

- **Filtration**

➢ *Mechanism*

- ✓ Mechanically removes microorganisms by *passage* c a liquid or gas through a screen like material with small pores.
- ✓ May be done under either negative or positive pressure:
  - *Positive Pressure Environment*
    - Air pressure in the room under positive pressure is *higher* than outside
    - Positive pressure rooms - also often known as a protective room.
  - *Negative Pressure Environment*
    - The air pressure in the room under negative pressure is *lower* than outside
    - Used for airborne infection control to protect people from patients with very contagious disease

- *Application*
  - ✓ Filtration is the preferred method of sterilizing certain solutions, that *likely to be damaged* by heat
    - Example: IV fluids. Antibiotic solutions, vaccines, enzymes and some culture media.
- *Most common types*:
  - ✓ Membrane Filters (Uniform pore size) have different sizes:
    1. 0.22 and 0.45um Pores:
       - Used to filter most bacteria.
       - Don't retain spirochetes, mycoplasmas and viruses.
    2. 0.01 um Pores:
       - Retain all viruses and some large proteins.

- **Chemicals**
  - *Ethylene oxide*
    - ✓ *Kill by:* denature proteins and DNA by cross-linking functional groups.
    - ✓ *Used in* sterilizing heat sensitive materials such as surgical instruments [endoscopes and anesthetic apparatus] and plastics.
    - ✓ *Warning*: Highly inflammable potentially explosive gas.
  - *Aldehydes:*
    - ✓ *Kill by:* ddenature proteins and inactivate nucleic acids.
    - ✓ Formaldehyde as gas *used to* sterilize operation theatres and other spaces.
    - ✓ Glutaraldehyde a chemical relative of formaldehyde less irritating and more effective than formaldehyde.
    - ✓ Glutaraldehyde *used to* disinfect hospital instruments, including endoscopes and respiratory therapy equipment
  - *Halogens*
    - ✓ The halogens, particularly; *iodine* and *chlorine*, are effective antimicrobial agents.
    - ✓ *Kill by:* damage enzymes via oxidation or by denaturing them
    - ✓ *Included*: iodophores (Betadine®), chlorine treatment of drinking water, bleach.
  - *Oxidizing agents*
    - ✓ *Peroxides* and *ozone*
    - ✓ *Kill by* oxidation of microbial enzymes
    - ✓ Hydrogen peroxide can disinfect and sterilize *surfaces of objects*
    - ✓ Ozone *treatment* of drinking water
  - *Alcohol*
    - ✓ Powerful disinfectant and antiseptic but does not inactivate spores
    - ✓ *Kill by:* denatures proteins, dissolves lipids and can lead to cell membrane disintegration
    - ✓ Swabbing of skin with 70% ethanol prior to injection
    - ✓ Most commonly used alcohols are *ethanol* and *Isopropanol*.

- **The choice of method of sterilization or disinfection depends on:**
  - The *nature* of the item to be treated
  - The likely microbial *contamination*
  - The risk of *transmitting* infection to patients or staff in contact with the item.

- **Resistance to Sterilization and disinfection**
  - Susceptible: Vegetative bacteria and viruses
  - Resistant: Bacterial spores
  - Prions: Highly resistance, use disposable instruments

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