STERILIZATION AND DISINFECTION

Ms. Ashwini Poghe
Clinical Instructor
KBNC, A'bad

Introduction

- Sterilization

  A physical or chemical process that completely destroys or removes all microbial life, including spores.
  It is process by which an article, surface or medium is made free of all microorganisms either in the vegetative or spore form.

- Disinfection

  It is killing or removing of harmful microorganisms. It means the destruction of all pathogens or organisms capable for producing infections but not necessarily spores. All organisms may not be killed but the number is reduced to a level that is no longer harmful to health

- **Disinfectant**

  Products used to kill microorganisms on inanimate objects or surfaces. Disinfectants are not necessarily sporicidal, but may be sporostatic, inhibiting germination or outgrowth

- **Antiseptic**

  A product that destroys or inhibits the growth of microorganisms in or on living tissue.

- **Aseptic**

  Characterized by the absence of pathogenic microbes.

Methods of Sterilization

1. Physical methods
   - Sunlight
   - Heat
     - Dry
     - Moist
   - Radiation
     - U.V. light
     - Ionizing radiation
     - Filtration

2. Chemical Methods
   - Alcohol
   - Phenols
   - Dyes
   - Aldehydes
   - Oxidizing agent
   - Halogens

Physical method

1. **Sunlight**

Sunlight has an active germicidal effect due to its content of ultraviolet rays. It is natural method of sterilization in cases of water in tanks, rivers and lakes.

Methods of Sterilization

2. Sterilization by Heat: Most common method

- Dry Heat
  - Simplest method is exposing the item to be sterilized to the naked flame e.g. Bunsen burner- for sterilizing bacteriological loops, knives, blades.

Principles of dry heat

1. Dry heat kills the organisms by denaturation of bacterial protein, oxidative damage and by the toxic effect of elevated levels of electrolytes

.

1. Moist heat kills the microorganisms by denaturation and coagulation of proteins

Dry heat sterilization

a) Red heat
b) Flaming
c) Hot air oven
d) incineration

Red heat:

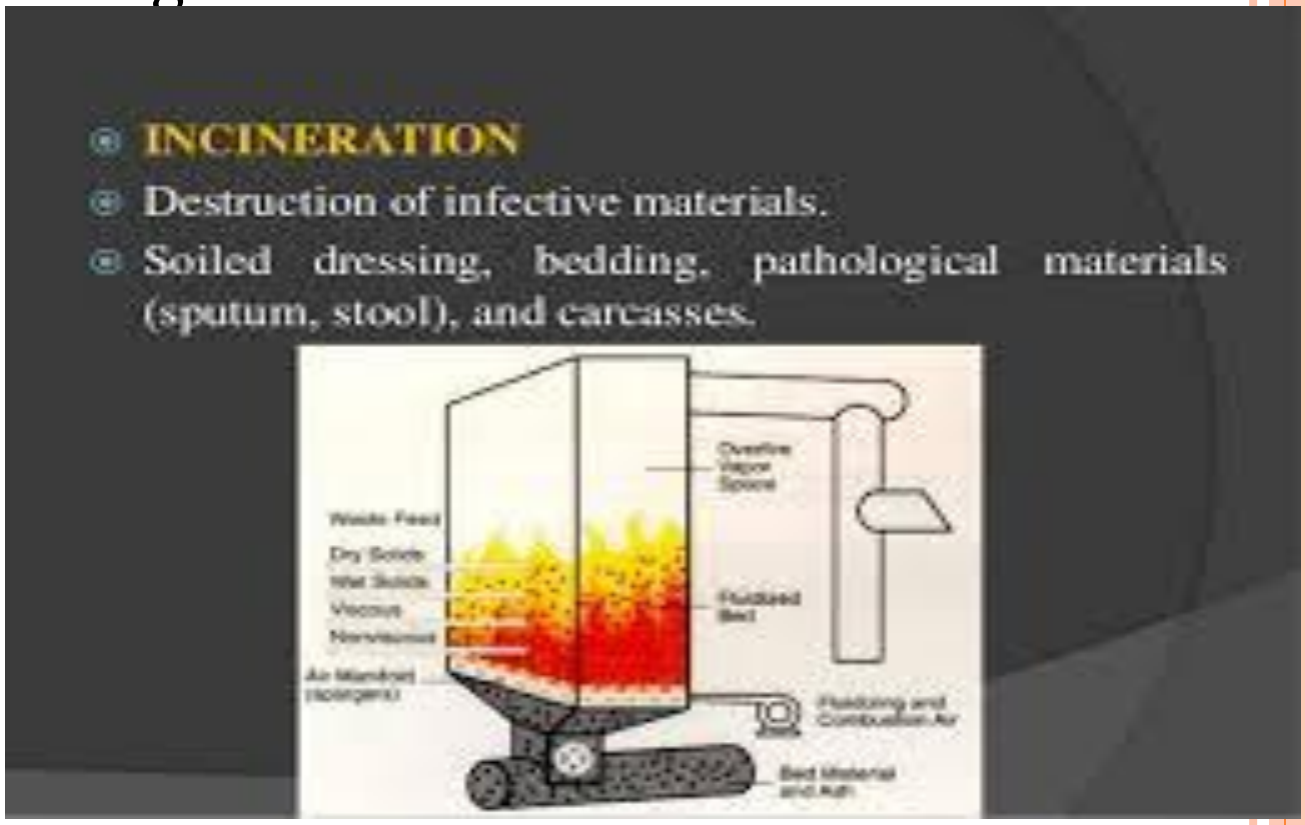
Inoculating wires or loops, tips of forceps and needles are held in the flame of a bunsen burner till they become red hot

Flaming

Glass slides , scalpels and mouths of culture tubes are passed through bunsen flame without allowing them to become red hot.

Incineration

By this method, infective material is reduced to ashes by burning. Instruments named by incinerator may be used for this purpose. Eg. Solid dressings, bleeding and pathological materials



Hot air oven

It is the most widely used method of sterilization by dry heat. The oven is electrically heated and is fitted with a fan to ensure adequate and even distribution of hot air in the chamber.

Temperature and time:

160 degree for two hours is required for sterilization

Uses:.

It is used for sterilization of

1. Glass wares like glass syringe, flasks, pipettes and test tube.
2. Surgical instruments like scalpels, scissors, forcep etc

Precaution

i) It should not be overloaded
ii) The material should be arranged in a manner which allows free circulation of air.
iii) Material to be sterilized should be perfectly dry.
iv) Test tube, flask etc. should be fitted with cotton plugs.
v) Petridishes and pipettes should be wrapped in papers.
vi) Rubber materials or any inflammable material should not be kept inside the oven.
vii) The oven must be allowed to cool for two hours before opening the doors, since the glassware may crack by sudden cooling

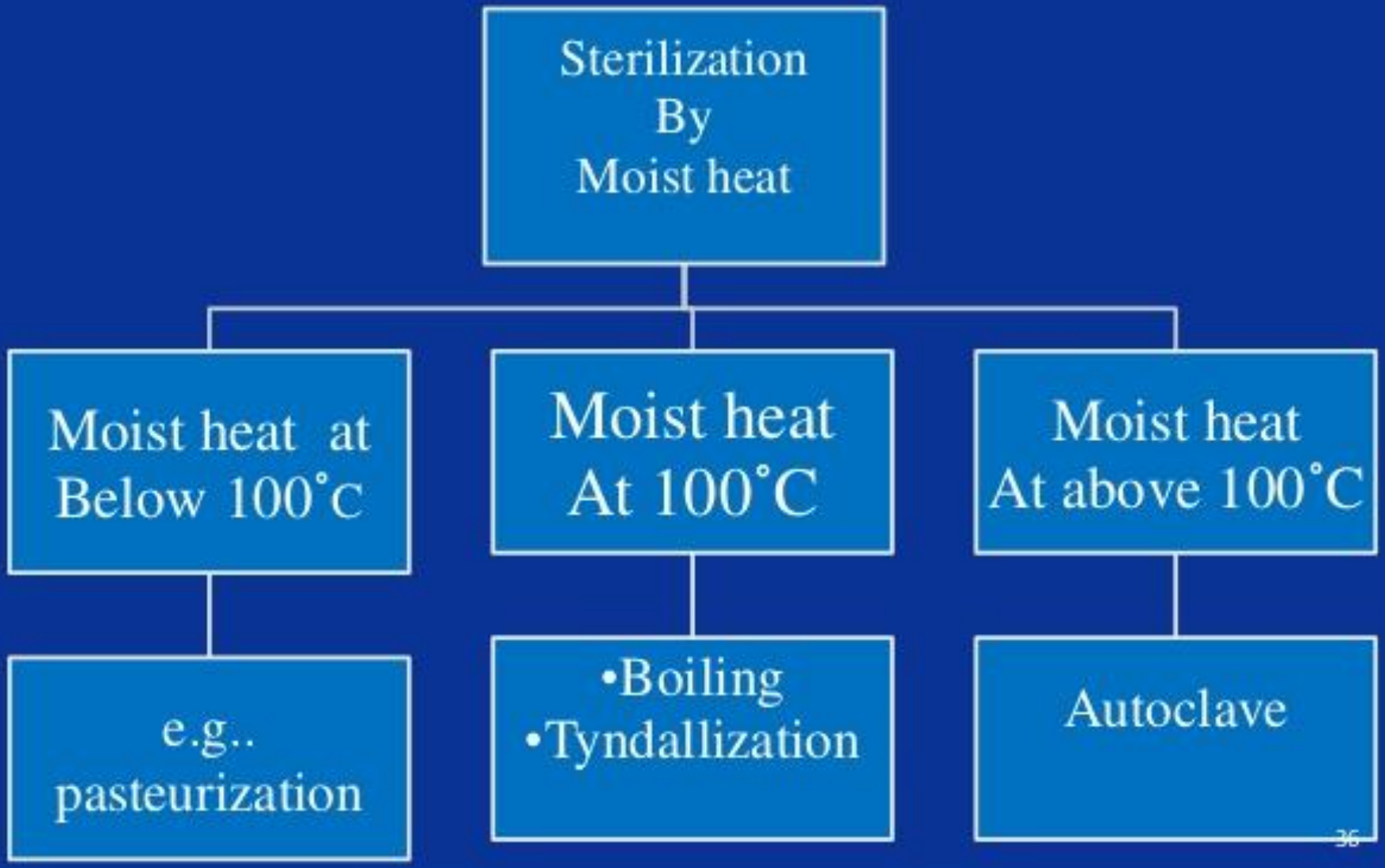

PRESTO
Mercury
Hot Air Oven

Sterilization control

- The spores of nontoxogenic strain of clostridium tetani kept inside the oven. This spores should be destroyed if the sterilization is proper
- **Thermocouples** may also be *used* (*A Thermocouple is a sensor used to measure temperature*.)

- Browns tube with green spot is available. After proper sterilization a green color is produced (after 2 hour at 160 degree.)

MOIST HEAT


Sterilization
By
Moist heat
Moist heat at
Below 100˚C
Moist heat
At 100˚C
Moist heat
At above 100˚C
e.g..
pasteurization
•Boiling
•Tyndallization
Autoclave

1.Pasteurization –

- The material is heated at
  - **Holder method** - 63^0C for 30 minutes
  - **Flash process** - 72^0C for 15-20 sec followed by sudden cooling 13^0C or lower.
- All non-sporing pathogens such as *mycobacteria, brucellae & salmonellae* are destroyed.

II.BOILING

- Boiling is highly unreliable as a sterilization technique; not recommended for sterilizing surgical instruments.

- Vegetative bacteria killed almost immediately at 90-100^{0}C, but sporing bacteria require prolonged periods of boiling.

- A minimum exposure period of 30min. is recommended to kill vegetative bacteria.

- Sodium bicarbonate 2% conc. is added to increase the efficiency of process.

TYNDALLIZATION

- Means intermittent exposure at 100^0C
- Principle : that one exposure kills vegetative organisms, between heatings the spores being in a favorable nutrient medium become vegetative forms which get killed during subsequent heating.
- Used for gelatin media, media containing sugars.

Methods of Sterilization

Moist heat:

Autoclaving –Standard sterilization method in hospitals.

- The Autoclave works under the same principle as the pressure cooker where water boils at increased atmospheric pressure *i.e.* because of increased pressure the boiling point of water is >100°C.

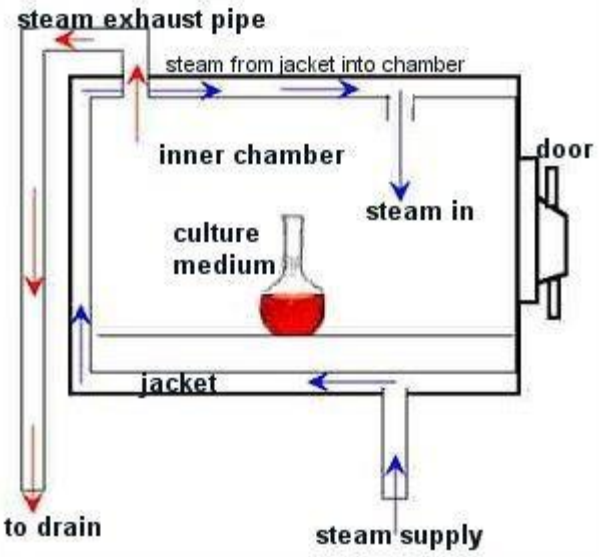
- The autoclave is a tough double walled chamber in which air is replaced by pure saturated steam under pressure.

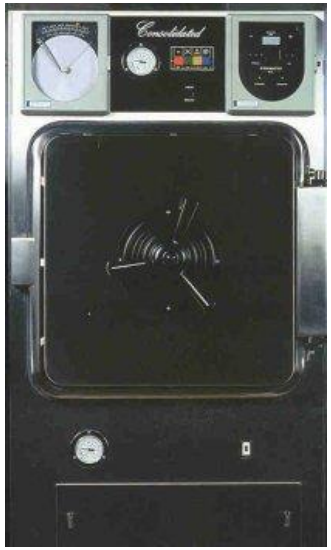
Methods of Sterilization

- The air in the chamber is evacuated and filled with saturated steam. The chamber is closed tightly the steam keeps on filling into it and the pressure gradually increases.

- The items to be sterilized get completely surrounded by saturated steam (moist heat) which on contact with the surface of material to be sterilized condenses to release its latent heat of condensation which adds to already raised temperature of steam so that eventually all the microorganisms in what ever form –are killed.

- The usual temperature achieved is 121 °C at a pressure of 15 pps.i. at exposure time of only 15-20 mins. By increasing the temperature, the time for sterilizing is further reduced.





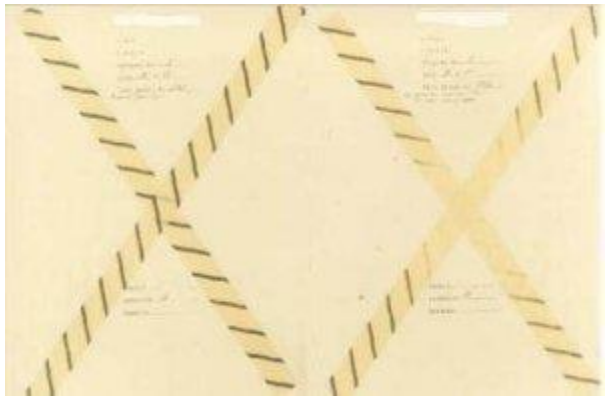
Methods of Sterilization

Advantages of Autoclave

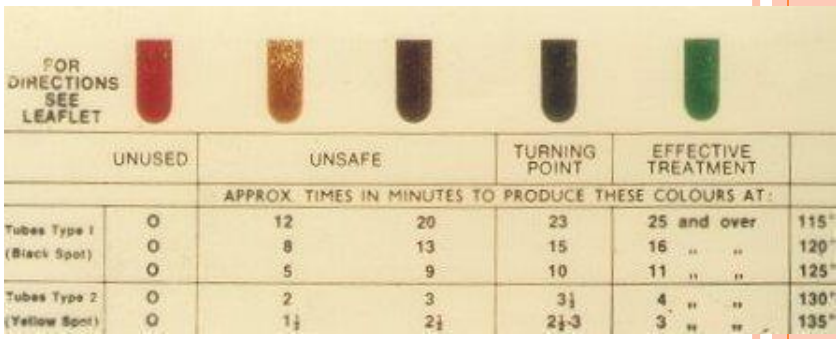
- Temperature is > 100°C therefore spores are killed.
- Condensation of steam generates extra heat (latent heat of condensation).
- The condensation also allows the steam to penetrate rapidly into porous materials.
- Note: that autoclavable items must be steam permeable. Can not be used for items that are lacking water.

METHODS OF STERILIZATION

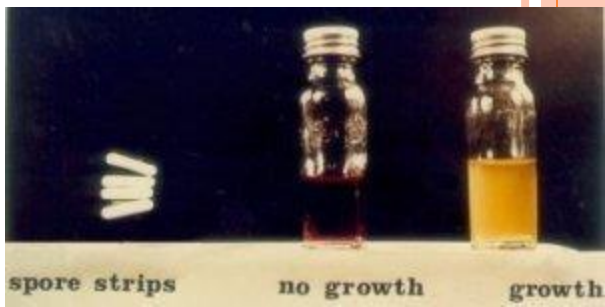
Monitoring of autoclaves



- Physical- use of thermocouple to measure accurately the temperature.
- Chemical- it consists of heat sensitive chemical that changes color at the right temperature and exposure time.
  - Autoclave tape
  - Browne's tube.



- Biological – where a spore-bearing organism is added during the sterilization process and then cultured later to ensure that it has been killed.



Methods of Sterilization

Sterilization by Chemical Methods

- Useful for heat sensitive materials e.g. plastics and lensed instruments endoscopes).
- **Ethylene Oxide Chamber:**
  - Ethylene oxide alkylates DNA molecules and thereby inactivates microorganisms.
  - Ethylene oxide may cause explosion if used pure so it is mixed with an inert gas e.g. Neon, Freon at a ratio of 10:90
  - It requires high humidity and is used at relative humidity 50-60% Temperature : 55-60°C and exposure period 4-6 hours.
- **Activated alkaline Glutaraldehyde 2%:**
  - Immerse item in solution for about 20 minutes if organism is TB. In case of spores, the immersion period is extended to 2-3 hours.

DISINFECTANTS

- **Factors influencing activity of Disinfectants**
  - Directly proportional to temperature.
  - Directly proportional to concentration up to a point – optimum concentration. After this level no advantage in further increases in concentration.
  - Time: Disinfectants need time to work.
  - Range of Action : Disinfectants are not equally effective against the whole spectrum of microbes. e.g. Chlorhexidine is less active against GNB than Gram Positive Cocci.
  - May be inactivated by
    - Dirt, organic matter
    - Proteins, Pus, Blood, Mucus, Faeces
    - Cork and some plastics.
- **Hypochlorites and Glutaraldehyde** are more active against hepatitis viruses than most other disinfectants.

DISINFECTANTS

Types of Disinfectants

Phenol and phenolics

- Phenol (carbolic acid) is seldom used today. Derivatives of the phenol molecule, however, are widely used.
- Phenolics injure plasma membrane, inactivate enzymes, or denature proteins. They are stable, persistent, and are not sensitive to organic matter.

O-Phenylphenol

- It is the main ingredient in most formulations of Lysol.

Hexachlorophene

- It is main ingredient of a prescription lotion, pHisoHex, used in nurseries and for surgical and hospital microbial control procedures to control gram positive skin bacteria such as staphylococci and streptococci.
- Excessive use can cause neurological damage.

Triclosan

- It is a widely used found in many household products. It has broad spectrum of activity, especially against gram positive bacteria. It is also effective against gram negative bacteria and fungi.

DISINFECTANTS

Biguanides

- Chlorhexidine, a member of the biguanide group, is not a phenol, but its structure and applications resemble hexachlorophene. It is frequently used for surgical skin preparation and surgical hand scrubs.

Halogens

- **Iodine** is effective against all kinds of bacteria, many endospores, fungi, and some viruses. Its mechanism of activity may be its combination with the amino acid tyrosine in enzyme and cellular proteins.
- An iodophore is a combination of iodine and an organic molecule. Iodophores do not stain and are less irritating than iodine. Examples are Isodine and Betadine.
- **Chlorine** is used as a gas or in combination with other chemicals. Chlorine gas is used for disinfecting municipal water supplies, swimming pools, and sewage. Sodium hypochlorite – ordinary household bleach- is good disinfectant.
- Chloramines consist of chlorine and ammonia. They are more stable than most chlorine. The U.S. military uses tablets for field disinfection of water.
- Chlorine dioxide in gaseous form is used for area disinfection, most notably to kill endospores of anthrax bacteria.

DISINFECTANTS

Alcohols

- Both ethanol and isopropanol (rubbing alcohol) are widely used, normally at a concentration of about 70%.
- Concentrations of 60% to 95% are effective.
- They are bactericidal and fungicidal but are not effective against endospores or non-enveloped viruses.
- Alcohols enhance the effectiveness of other chemical agents.

DISINFECTANTS

Heavy metals and their compounds

- Tiny amount of heavy metals (e.g. silver and copper) are effective antimicrobials. A silver coin on an inoculated nutrient medium will inhibit growth for some distance.

- 1% silver nitrate solution has been used to prevent gonorrheal ophthalmia neonatorum, which the infants might have contracted as they passed through the birth canal (recently been replaced by antibiotics).

- Silver-sulfadiazine is used in wound dressings. Available as topical cream for use on burns.

- Mercuric chloride is highly bactericidal, but is toxic and corrosive and is inactivated by organic matter. Organic mercury compounds such as Mercurochrome are less irritating and less toxic than inorganic mercury.

- Copper sulfate is often used to destroy green algae in reservoirs or other water.

- Zinc chloride is used in mouthwashes, and zinc oxide is used in paints as antifungal.