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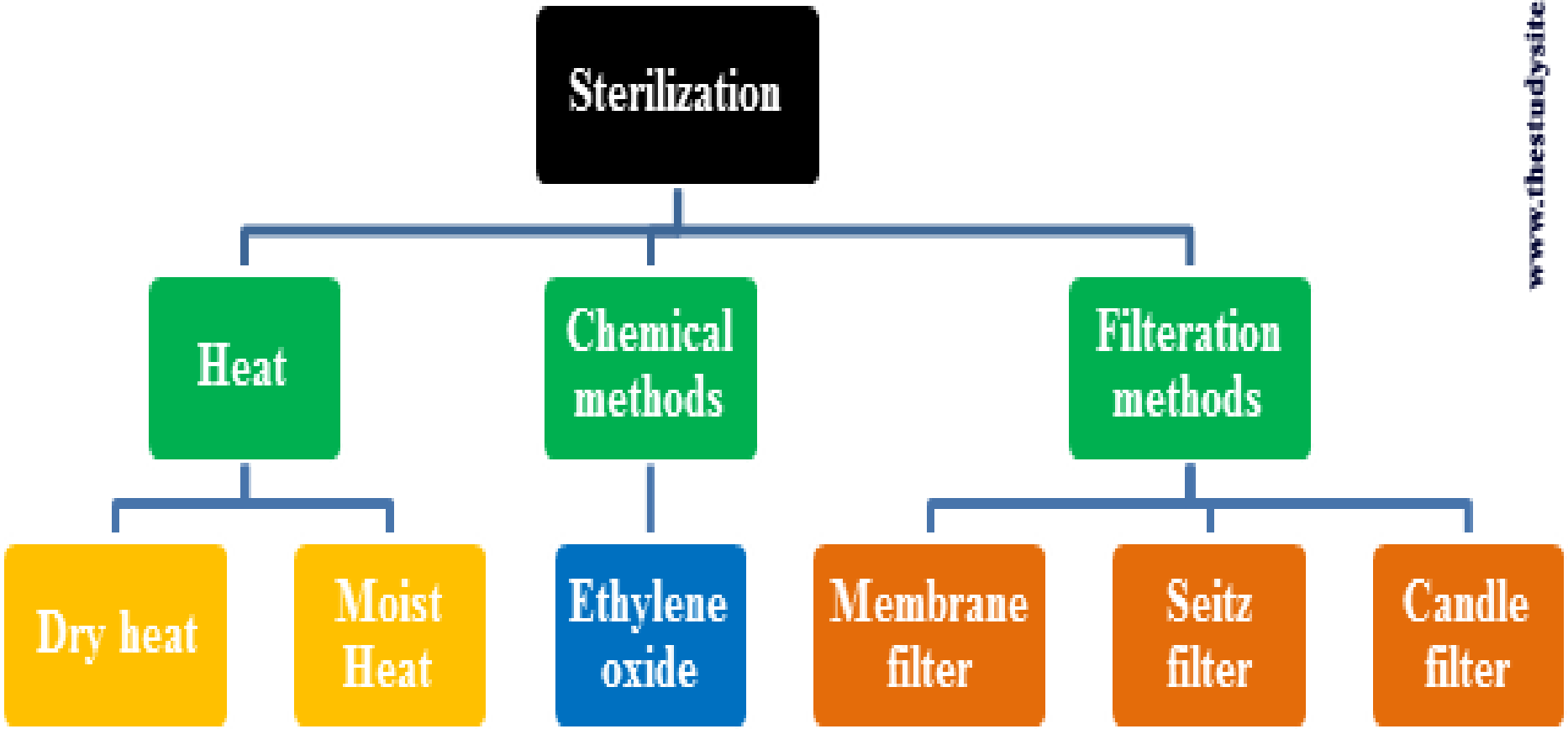
STERILIZATION

INTRODUCTION

- A physical or chemical process that completely destroys or removes all microbial life, including spores present in a specified region, such as a surface, a volume of fluid or in a compound such as biological culture media
- Sterilization can be achieved with one or more of the following:
  - heat
  - chemicals
  - radiation
  - high pressure and
  - filtration



METHODS OF STERILIZATION: PHYSICAL

- **Sterilization by Heat:** Mostcommon 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.
  - Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents.
  - It employs higher temperatures in the range of 160-180°C and requires exposures time up to 2 hours, depending upon the temperature employed.
  - The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glass-wares and metal surgical instruments.

METHODS OF STERILISATION

- It is also used for sterilizing non-aqueous thermo-stable liquids and thermostable powders.
- Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically.
- Examples of Dry heat sterilization are:
    1. Incineration
    2. Red heat
    3. Flaming
    4. Hot air oven

METHODS OF STERILIZATION

- **Hot-air oven :**
  - Dry heat sterilization is usually carried out in a hot air oven, which consists of the following:
    - (i)An insulated chamber surrounded by an outer case containing electric heaters.
    - (ii) A fan
    - (iii) Shelves
    - (iv) Thermocouples
    - (v) Temperature sensor
    - (vi) Door locking controls.

HOT AIR OVEN

- **Operation**

  (i)Articles to be sterilized are first wrapped or enclosed in containers of cardboard, paper or aluminium.

  (ii)Then, the materials are arranged to ensure uninterrupted air flow.

  (iii)Oven may be pre-heated for materials with poor heat conductivity.

  (iv)The temperature is allowed to fall to 40°C, prior to removal of sterilized material.

HOT AIR OVEN

- **Principle**
  - Sterilizing by dry heat is accomplished by **conduction**.
  - The heat is absorbed by the outside surface of the item, then passes towards the centre of the item, layer by layer.
  - The entire item will eventually reach the temperature required for sterilization to take place.
  - Dry heat does most of the **damage by oxidizing molecules.**
  - The essential cell constituents are destroyed and the organism dies.
  - The temperature is maintained for almost an hour to kill the most difficult of the

FLAMING

- Thisis an emergency method, the forceps-tips, the surfaces of the scalpels and the needles
- may be sterilized by holding the items directly in the flame of a Bunsen burner.

Advantages of dry heat sterilization

- A dry heat cabinet is easy to install and has relatively low operating costs;
- It penetrates materials
- It is nontoxic and does not harm the environment;
- And it is **noncorrosive** for metal and sharp instruments.

Disadvantages for dry heat sterilization

- Time consuming method because of **slow rate of heat penetration** and microbial killing.
- High temperatures are not suitable for most materials.

METHODS OF STERILIZATION

Moist Heat (or Steam):

- Uses hot water.
- Moist heat kills microorganisms by denaturing proteins.
- Moist heat may be used in three forms to achieve microbial inactivation
  1. Dry saturated steam – Autoclaving
  2. Boiling water/ steam at atmospheric pressure
  3. Hot water below boiling point
  4. Pasteurisation

- Moist heat sterilization involves the use of steam in the range of 121-134°C.
- Steam under pressure is used to generate high temperature needed for sterilization.
- Saturated steam acts as an effective sterilizing agent.
- Steam for sterilization can be either wet saturated steam (containing entrained water droplets) or dry saturated steam (no entrained water droplets).

- **Boiling** –
  - quite common especially in domestic circumstances.
  - Boiling is done for metallic devices like surgical scissors, scalpels, needles etc like instruments. Here substances are boiled to sterilize them.
- **Pasteurisation**
  - Pasteurization is the process of heating the milk at a temperature of 60 degrees or 72 degrees 3 to four times.
  - Here alternative heating and cooling kills all the microbes and molds without boiling the milk.

METHODS OF STERILIZATION

Moist heat: Tyndallization

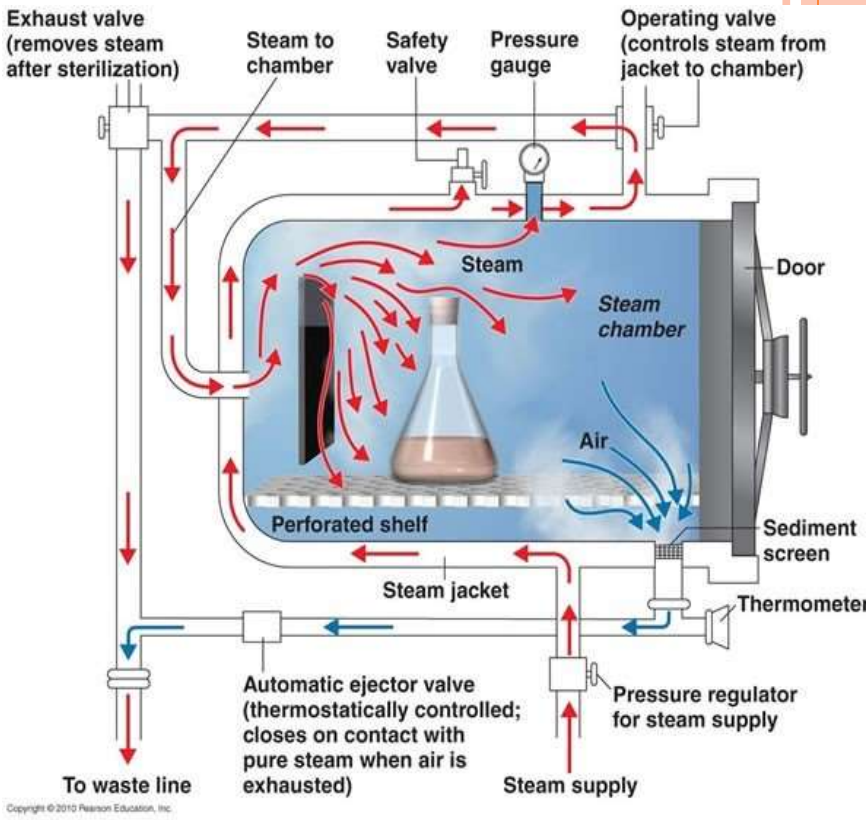
- The process involves boiling for a period (typically 20 minutes) at atmospheric pressure, cooling, incubating for a day, boiling, cooling, incubating for a day, boiling, cooling, incubating for a day, and finally boiling again.

- The three incubation periods are to allow heat-resistant spores surviving the previous boiling period to germinate to form the heat-sensitive vegetative (growing) stage, which can be killed by the next boiling step.

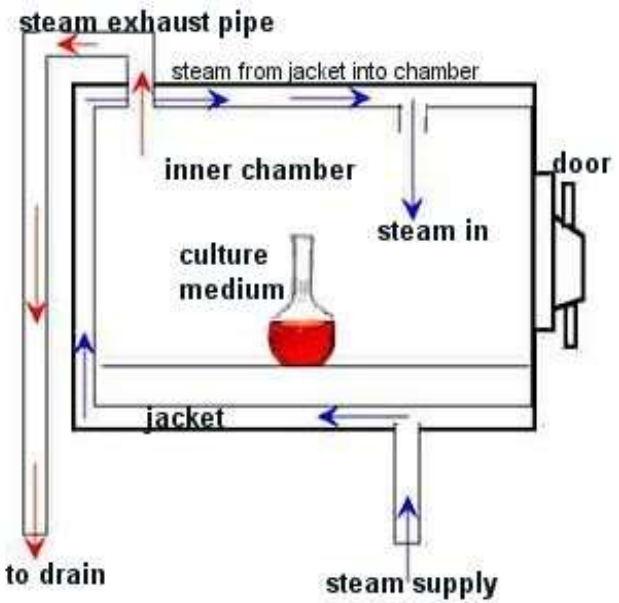
METHODS OF STERILIZATION

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.
- Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient



AUTOCLAVE



- The air in the chamber is flushed out and filled with saturated steam.
- Water is boiled to produce steam, which is released through the jacket and into the autoclave's chamber.
- Hot, saturated steam enters the chamber and the desired temperature and pressure, usually 121°C.
- At this temperature saturated steam destroys all vegetative cells and endospores.
- Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins.
- It also may disrupt cell membranes. 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.ie. 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.

RADIATION

- Electromagnetic radiation
  - Gamma rays
  - UV rays
- Particulate radiation
  - Accelerated electrons

- The major target for these radiation is microbial DNA.
- Gamma rays and electrons cause ionization and free radical production while UV light causes excitation.
- U.V. light has limited sterilizing power because of poor penetration into most materials. Generally used in irradiation of air in certain areas eg. Operating Rooms and T.B. laboratories.
- Ionizing radiation- *e.g.* Gamma radiation: Source Cobalt^{60} has greater energy than U.V. light, therefore more effective. Used mainly in industrial facilities e.g. sterilization of disposable plastic syringes, gloves, specimens containers and Petri Dishes.

RADIATION

- **Gamma ray Sterilizer:** Gamma rays for sterilization are usually derived from cobalt-60 source, the isotope is held as pellets packed in metal rods, each rod carefully arranged within the source.
- This source is housed within a reinforced concrete building with 2 m thick walls.
- Articles being sterilized are passed through the irradiation chamber on a conveyor belt and move around the raised source.
- Penetrates deep into objects and is an excellent sterilizing agent.
- It destroys bacterial endospores and vegetative cells of both prokaryotic and eukaryotic origin but not against viruses.
- Gamma radiation from a cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures and plastic disposable supplies such as syringes, and Petri dishes.

RADIATION

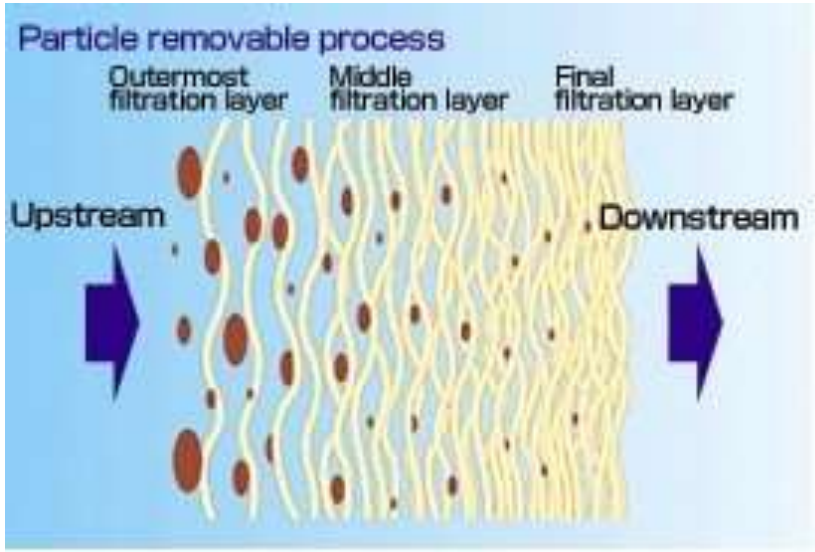
- **Ultraviolet radiation:** The optimum wavelength for UV sterilization is 260 nm. A mercury lamp giving peak emission at 254 nm is the suitable source of UV light in this region.
- But this does not penetrate glass, dirt films, water and other substances very effectively.
- UV radiation is used as a sterilizing agent only in a few specific situations, like UV lamps are placed on the ceilings of rooms or in biological safety cabinets to sterilize air and other exposed surfaces.
- Commercial UV units are available for water treatment. Pathogens and microorganisms are destroyed when a thin layer of water is passed under the lamps (water purifiers).

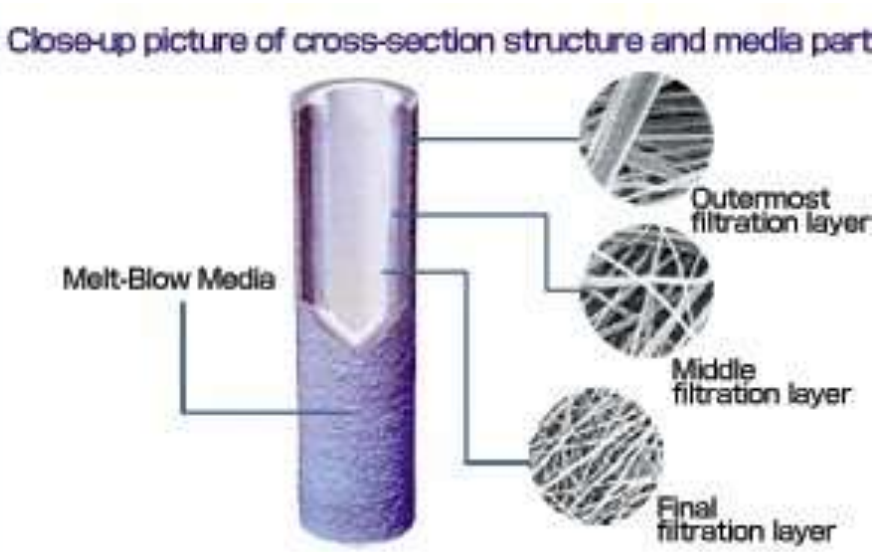
INFRARED RADIATION

- Infrared radiation (IR) is a thermal radiation, i.e. when absorbed by some article its energy is converted to heat and therefore it is often known as radiant energy.
- A tunnel having an IR source is used for this purpose.
- The instruments and glass wares are kept in trays are passed through this tunnel keeping on the conveyor belt, at a controlled speed exposing them to a temperature of 180^0C for 17 minutes, thereby achieving the sterility.

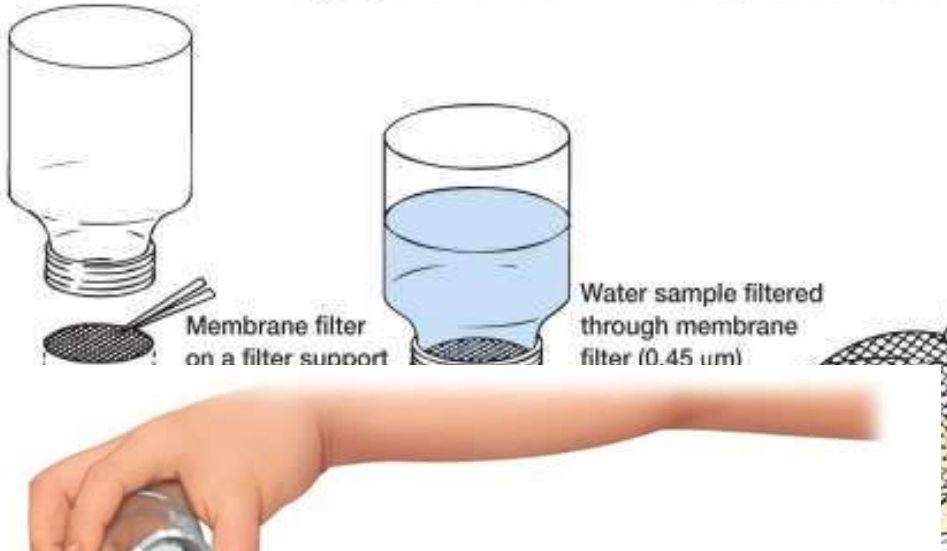
- This is a continuous process and is used in hospitals for regular supply of sterile syringes and other apparatus.
- Heating at or above 200^0C by IR in vacuum is employed as a means of sterilizing surgical instruments.
- Cooling is hastened, (after the heating cycle) during the cooling period, by admitting filtered $N_2$to the chamber.

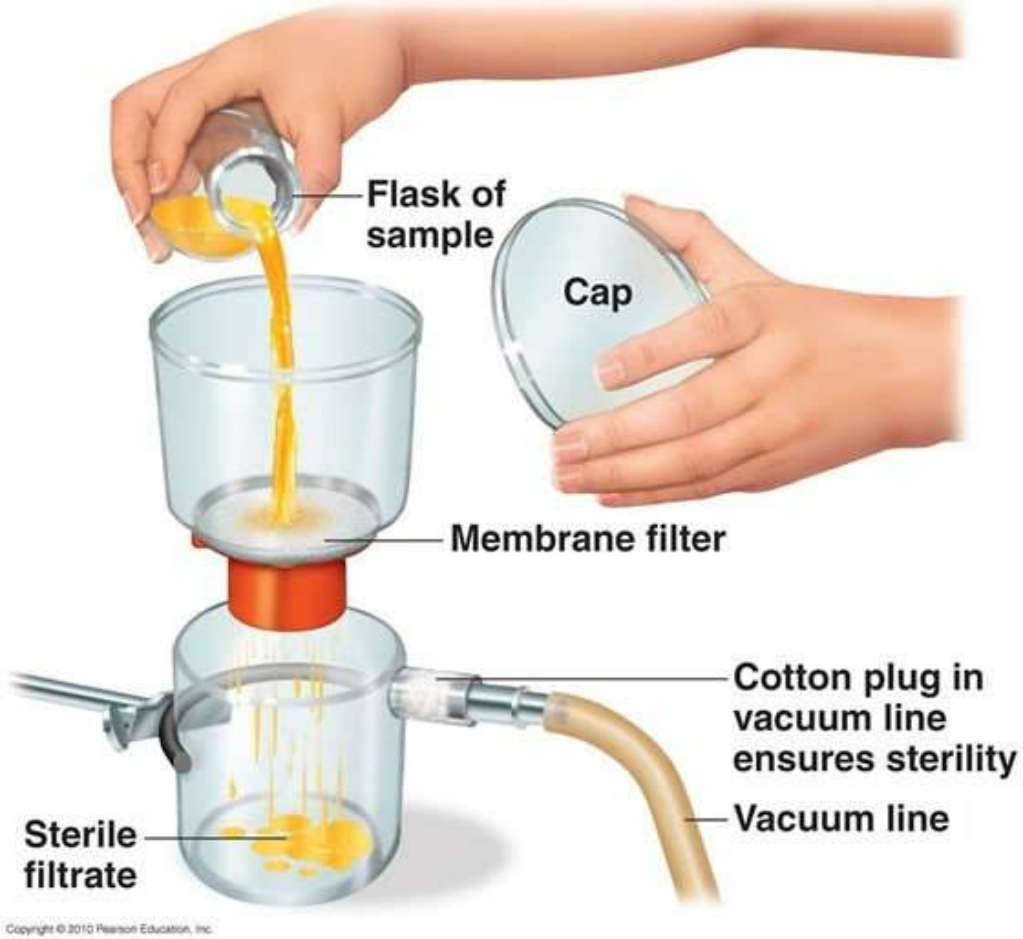
FILTRATION





- Consists of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter.
- The solution is passed through the filter which is sucked through this layer under vacuum and microbial cells are removed. The material used mostly is unglazed porcelain, asbestos or other similar materials





ı pore sizes mostly of 0.2 to
to remove most vegetative
olutions ranging in volume

tly used to sterilize
solutions, culture media,
t sensitive solutions.

FILTRATION

- The other way this met
laminar flow biological s
air is sterilized by filtra
- These cabinets contain
air (HEPA) filters, whic
0.3µm particles.
- The safety cabinets are
culturing of any organis
contamination free air t
other undesired organis
of media, examining tiss

- Phenol and Phenolics
- Halogens
- Alcohols
- Heavy Metals
- Quaternary Ammonium Compounds
- Aldehydes
- Gaseous
- Peroxygens

STERILIZATION : CHEMICAL METHODS

- Useful for heat sensitive materials e.g. plastics and lensed instruments endoscopes).
- The sterilising agent must be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms, and have a low surface tension so that it can enter cracks in surfaces.

- **Peracetic Acid liquid sterilization:**
- Peracetic acid was found to be sporicidal at low concentrations.
- It was also found to be water soluble, and left no residue after rinsing. It was also shown to have no harmful health or environmental effects.
- It disrupts bonds in proteins and enzymes and may also interfere with cell membrane transportation through the rupture of cell walls and may oxidize essential enzymes and impair vital biochemical pathways.

CHEMICAL STERILISATION

- **Hydrogen Peroxide Sterilization**:

- This method disperses a
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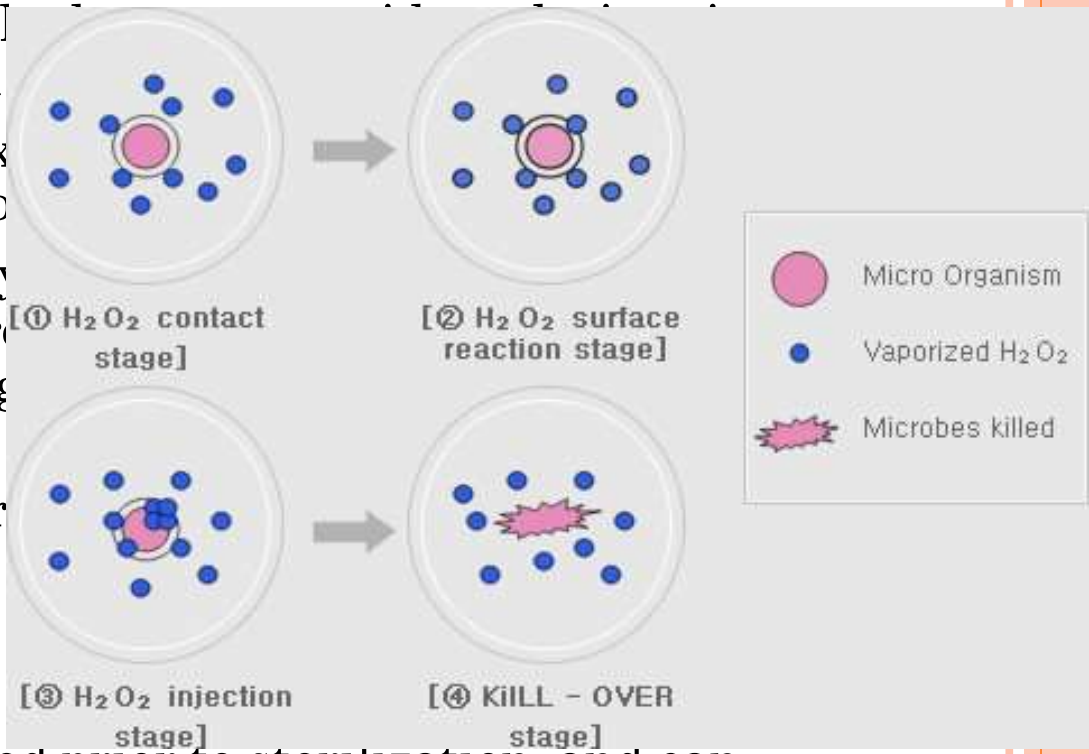
ge,
se with hea
s are wrapped prior to sterilization, and can
or used immediately.



GASEOUS STERILIZATION

Ethylene Oxide Sterilizer:

- Ethylene oxide gas readily pe
  plastic wraps and is both micr
  by combining with cell protein
- Ethylene oxide alkylates DNA
  inactivates microorganisms.
- Ethylene oxide may cause expl
  with an inert gas e.g. Neon, Fr
- It requires high humidity and
  60% ; Temperature : 55-60°C a
- An ethylene oxide sterilizer co
  Litre capacity and surrounded
- Air is removed from sterilizer
  and preheated vaporized ethyl
- After treatment, the gases are
  outside atmosphere or through a special exhaust system.

Multiple-load capable sterilizing cabinet

GASEOUS STERILIZATION

- **Activated alkaline Glutaraldehyde 2%:**
  - Immerse item in solution for about 20 minutes f organism is TB. In case of spores, the immersion period is extended to 2-3 hours.
- **Batapropiolacetone (BPL)**
  - is occasionally used as a sterilizing gas in the liquid form to sterilize vaccines and sera.
- Recently **vapour-phase hydrogen peroxide** has been used to decontaminate biological safety cabinets.