STERILIZATION

- Egyptians used fire for sterilisation
- Greeks burned sulphur for fumigation
- Advanced techniques

To Prevent

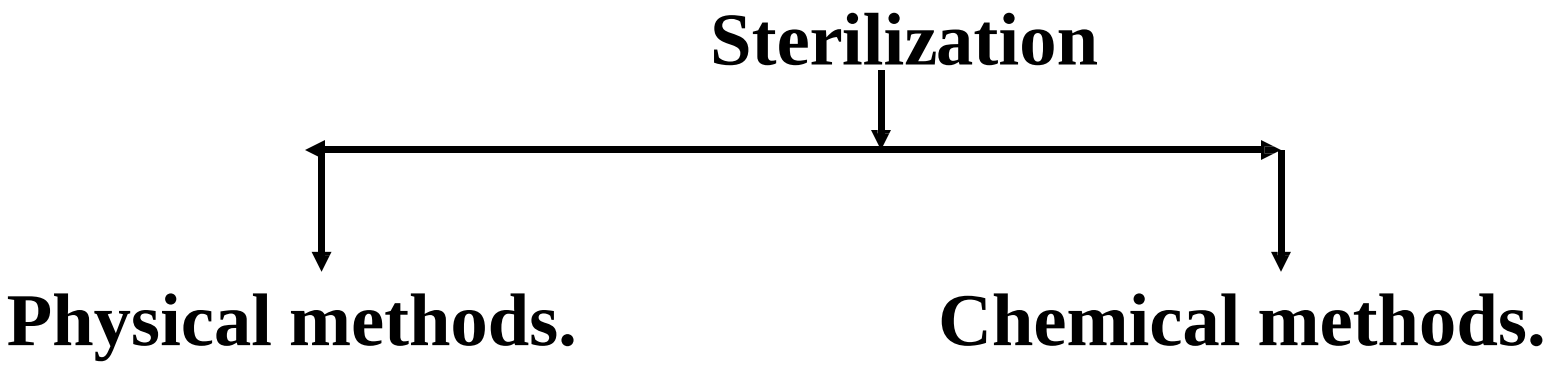
- Transmission of diseases.
- Spoilage of food.
- Contamination of pure cultures in laboratories.
- Interference of unwanted microbes in industrial process.
- Research studies.

Choice of Anti microbial agents depends on

- Type of microbe
- Stages of growth and number
- Surroundings

METHODS/AGENTS OF STERILIZATION

Classification of Sterilization:



Physical agents:

- **Sunlight**
- **Drying**
- **Heat**

  Dry heat: flaming, incineration, hot air

  Moist heat: pasteurization, boiling, steam under pressure.
- **Filtration: candles, asbestos pads, membranes**
- **Radiation**
- **Ultrasonic vibrations.**

Sun light:

- Sun light:
  - Active germicidal effect due to its content of ultraviolet rays .
  - Natural method of sterilisation of water in tanks, rivers and lakes.

Drying:

Moisture is essential for growth of bacteria.

Drying in air has deleterious effect on many bacteria.

However, spores are unaffected.

Heat:

- **Most reliable method of sterilization and should be the method of choice.**

Dry Heat & Moist Heat.

The factors influencing sterilization by heat:

- **Nature of heat-dry or moist**
- **Temperature and time**
- **Number of microorganisms present**
- **Characteristics of organisms –species, strain, sporing capacity**
- **Type of material from which organism have to be eliminated.**
- **Killing effect is due to protein denaturation, oxidative damage and toxic effect of elevated level of electrolytes.**
- **Killing effect of moist heat due to denaturation and coagulation**

Thermal Death Time:TDT

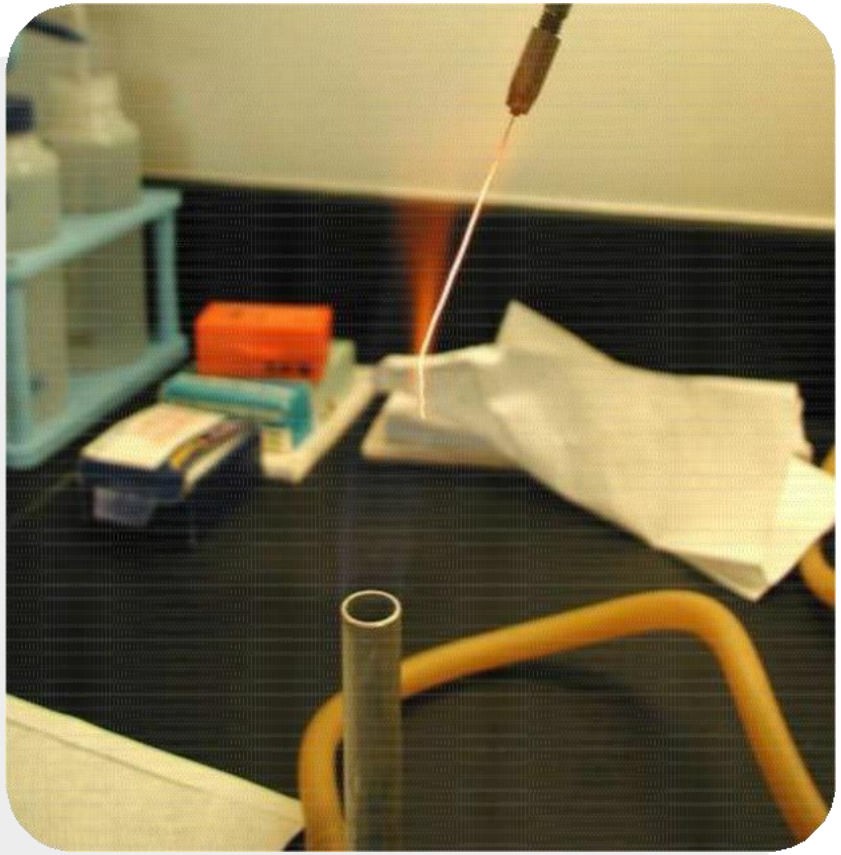
“Minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.

Thermal death time is inversely proportional to temperature.

TDT is increased in presence of organic substance, proteins, nucleic acid, starch, gelatin , sugar , fats, oils.”

Heat :

- Dry heat:
- 1.Red heat
  2.Flaming
  3.Incineration
  4.Hot air oven



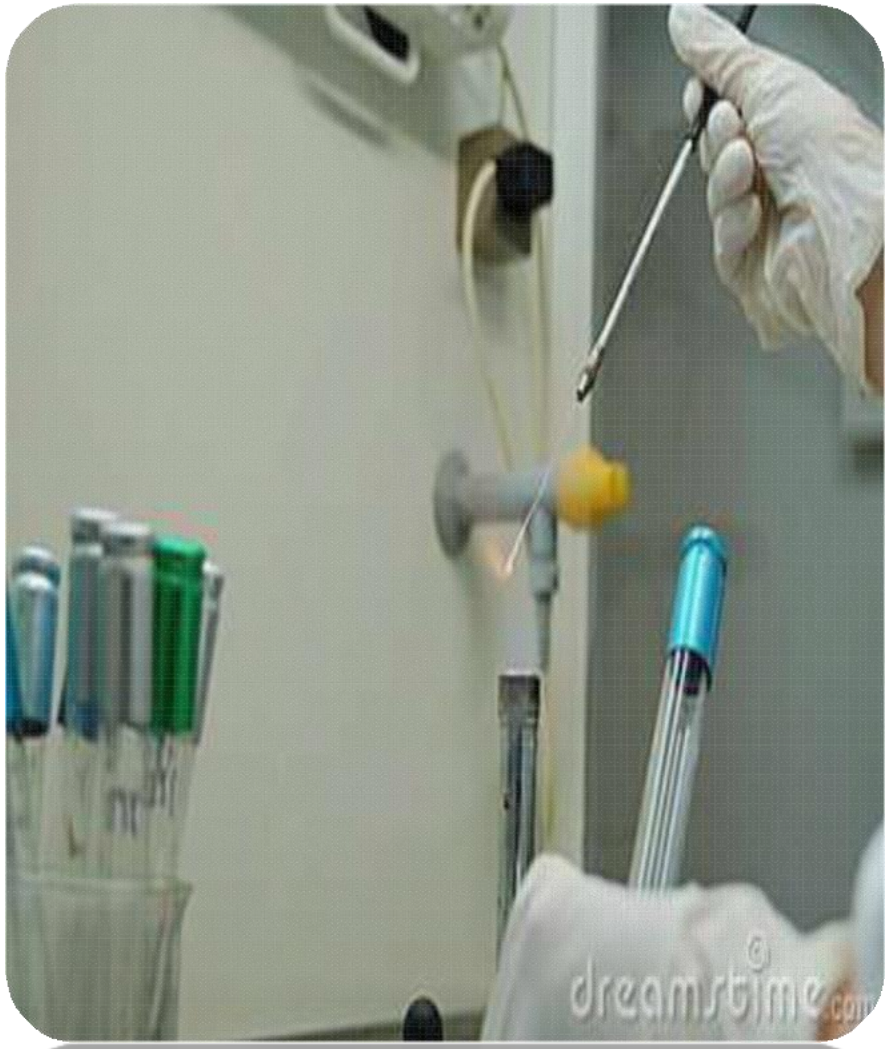
Dry heat:

1. Red heat: Materials are held in the flame of a bunsen burner till they become red hot.

» Inoculating wires or loops

» Tips of forceps

» Needles

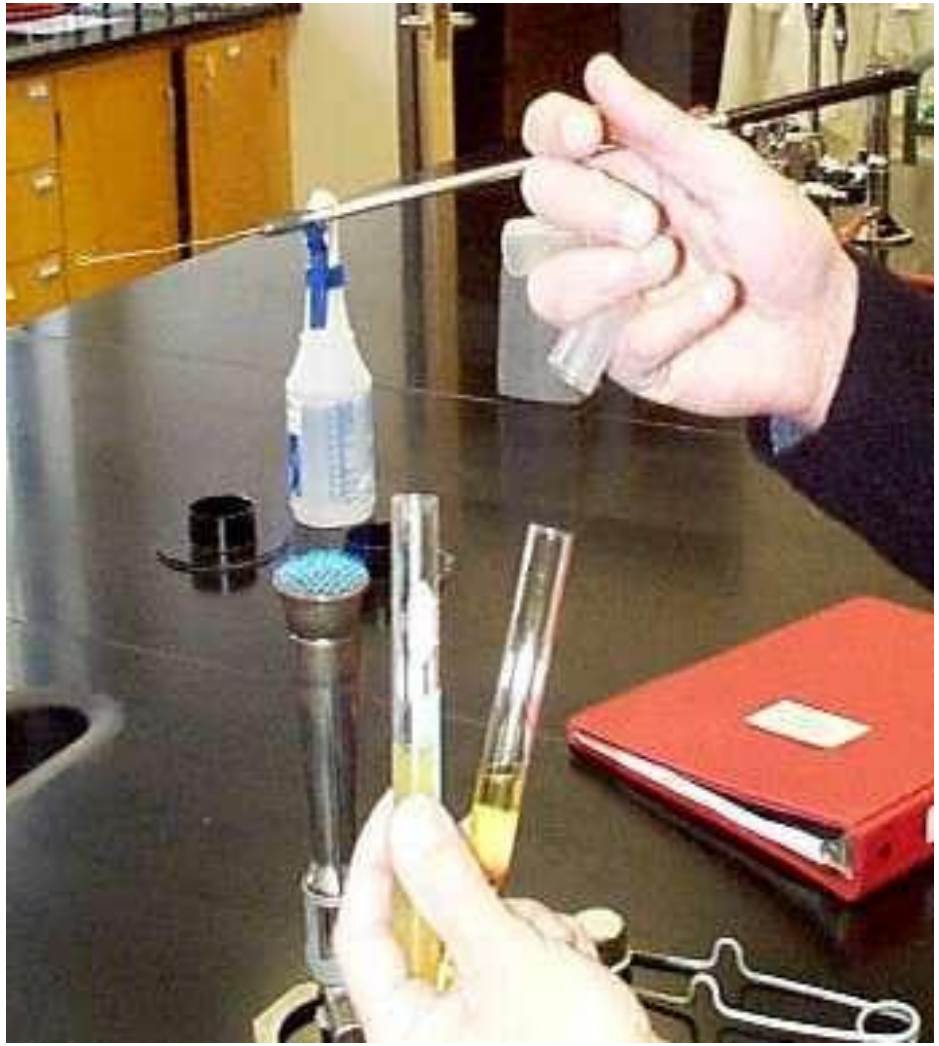


Dry heat:

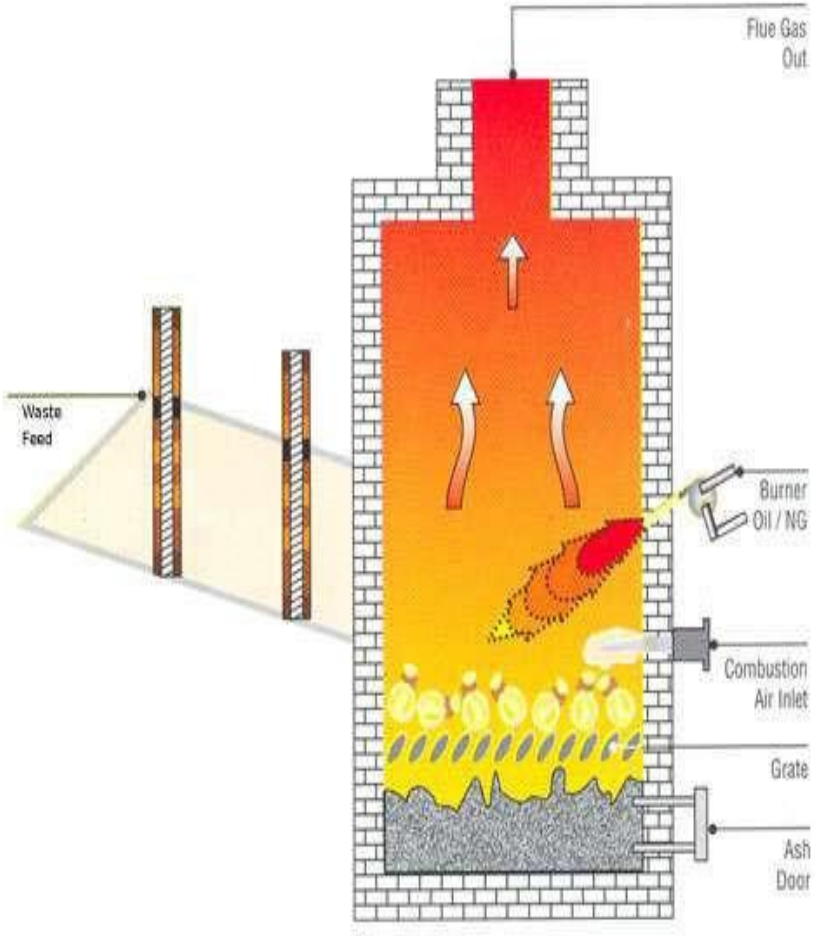
2. Flaming:

Materials are passed through the flame of a bunsen burner without allowing them to become red hot.

- **Glass slides**
- **scalpels**
- **Mouths of culture tubes**



Incineration:



- Materials are reduced to ashes by burning.
- Instrument used was incinerator.
- Soiled dressings
- Animal carcasses
- Bedding
- Pathological material

Dry-Heat Sterilization

Disadvantages

–Less reliable than autoclaving

–Large temp difference may arise within device.

–sharp instruments get dulled

–Many materials do not tolerate dry heat

Hot air oven:

- **Hot air ovens are electrical devices used in sterilization.**
- **The oven uses dry heat to sterilize articles.**
- **Generally, they can be operated from 50 to 300C .There is a thermostat controlling the temperature.**
- **This is the most widely used method of sterilization by dry heat.**

Items: glassware, forceps, scissors, scalpels, all-glass syringes, swabs, liquid paraffin, dusting powder, fats, grease.

(Materials should be properly arranged to allow free circulation of air)

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Precautions:

- **Glass wares should be dry.**
- **Oven should not be over loaded.**
- **Articles are to be arranged in a manner to allow free circular of air.**
- **Door of the Oven should be opened after it cools down (2Hours).**

Temerature (c)	Holding time(in minutes
160	45
170	18
180	7.5
190	1.5

Advantages & Disadvantages:

- They do not require water and there is not much pressure build up within the oven, unlike an autoclave, making them safer to work with.
- Suitable to be use in a laboratory environment.
- They are much smaller than autoclaves but can still be as effective.
- As they use dry heat instead of moist heat, some organisms like prions, may not be killed by them every time.

Moist heat:

Moist heat can be categorized into 3 groups:

- **Temperature below 100 C**
- **Temperature at 100 C**
- **Temperature above 100 C**

Below 100

◆**Pasteurization:** Developed by Louis Pasteur to prevent the spoilage of beverages. Used to reduce microbes responsible for spoilage of beer, milk, wine, juices, etc.

◆**Classic Method of Pasteurization**: Milk was exposed to 65^{o}C for 30 minutes.

◆**High Temperature Short Time Pasteurization (HTST):** Used today. Milk is exposed to 72^{o}C for 15 seconds.

- **Below 100-** Moist heat

  Pasteurization-63 for 30minutes

  – Used for milk, ice cream, yogurt, and fruit juices

    » Heat-tolerant microbes survive

  – **Pasteurization of milk**

    » Holder method with cooling 13

    » Flash pasteurization 72 for 15-20secs with quick cooling

    Non sporing pathogens- mycobacterium, brucelle ,salmonellae

Inspissation:

Inspissation: Fractional sterilization

Water jacketed copper box
Eg. Lowenstein Jansen medium

- Heating at 80-85°C for half an hour daily on three consecutive days
- Serum or egg media are sterilized
- Mesophilic non sporing bacteria
- Replaced by tyndallisation

Vaccine bath:

- Heating at 60°C for an hour daily in vaccine bath for several successive days.
- Serum or body fluids can be sterilized by heating at 56°C for an hour daily for several successive days.

Washer disinfectors—71 for 3min.

A temperature at 100°C

1. **Boiling**
2. **Tyndallisation**
3. **Steam sterilisation**

1. Boiling for 10 – 30 minutes may kill most of vegetative forms but spores with stand boiling.

2.Tyndallization : Intermittened sterilization: John Tyndall

- Steam at 100C for 30 minutes on three successive days
- Used for egg , serum and sugar containing media.

3.Steam sterilizer : Arnold sterilizer-Streaming steam

- Steam at 100°C for 90 minutes.
- Used for media which are decomposed at high temperature.

Temperatures above 100°C

Autoclave : steam under pressure

Temperature increases with pressure

In a closed system

-Steam above 100°C has a better killing power/penetrating power than dry heat.

-Bacteria are more susceptible to moist heat.

Components of autoclave:

- **Components of autoclave:**
  - Consists of vertical or horizontal cylinder of

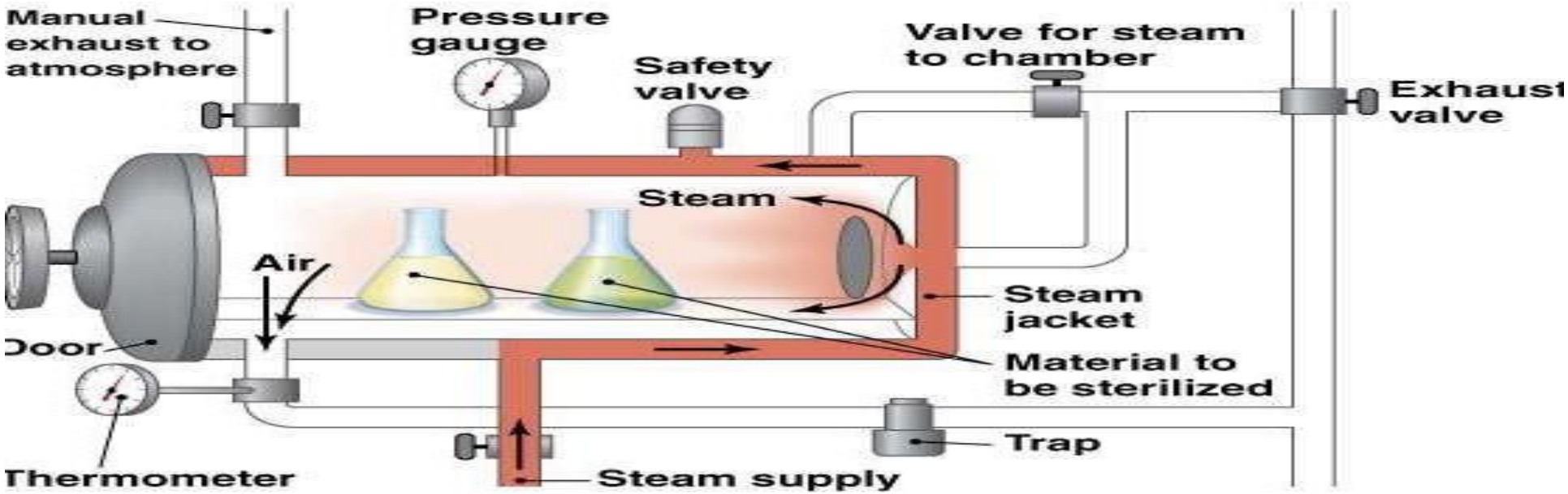
gunmetal or stainless steel.

  - Lid is fastened by screw clamps and

rendered air tight by an asbestos washer.

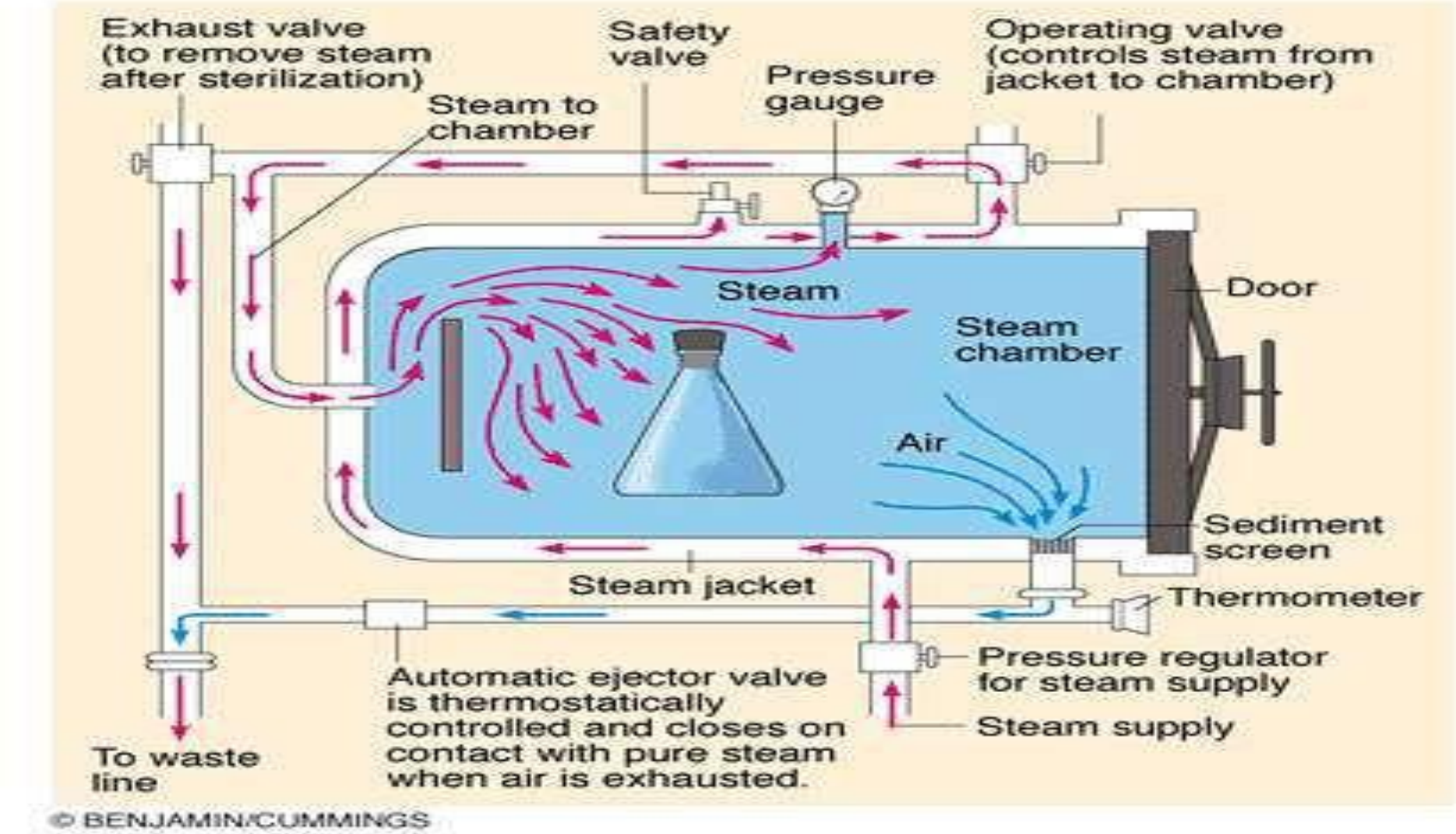
  - Lid bears a discharge tap for air and steam, a pressure gauge and a safety valve.

(a)



(b)

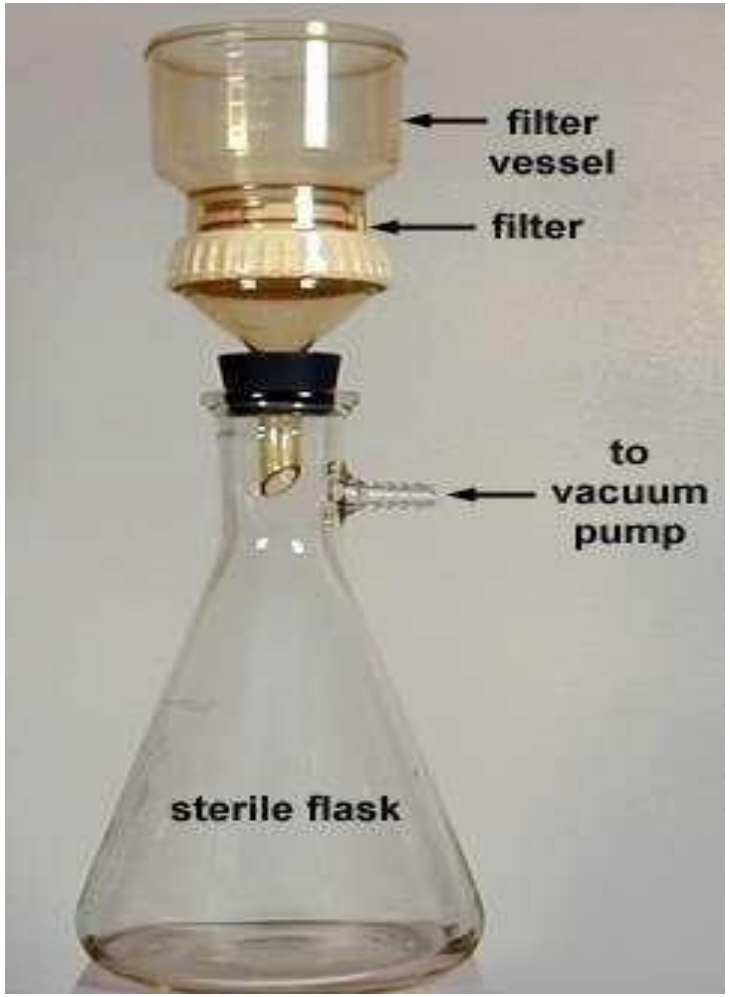
Autoclave: Closed Chamber with High Temperature and




TECHMECH
GROUP

Filtration:

- Useful for substances which get
- damaged by heat.
- To sterilize sera, sugars and antibiotic
- solutions.
- To obtain bacteria free filtrates of
- clinical samples.
- Purification of water.


filter
vessel
filter
to
vacuum
pump
sterile flask

Several Types of Filters

Types of filters:

1. **Candle filters**
2. **Asbestos disc filters**
3. **Sintered glass filters**
4. **Membrane filters**
5. **Air filters**
6. **Syringe filters**

The filtering Depends on Pore Size

The pore size for filtering bacteria, yeasts, and fungi is in the range of 0.22-0.45 µm (filtration membranes are most popular for this purpose).

Candle filters

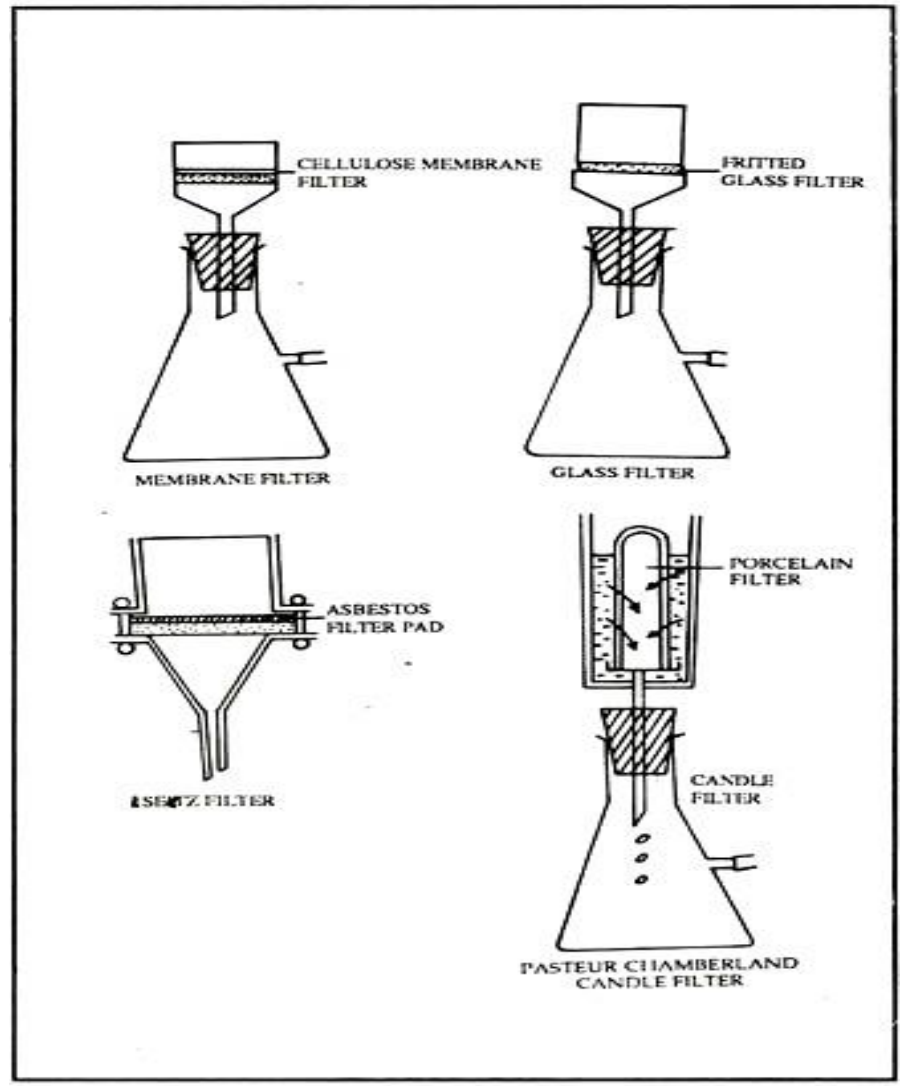

CELLULOSE MEMBRANE FILTER
MEMBRANE FILTER
FRITTED GLASS FILTER
GLASS FILTER
ASBESTOS FILTER PAD
SEITZ FILTER
PORCELAIN FILTER
CANDLE FILTER
PASTEUR CHAMBERLAND CANDLE FILTER

Fig. 1.3.

Berkefeld Filter:Germany

This is made up of diatomaceous earth pressed in the shape of a candle. On the basis of porosity they are variously classified into V (veal) N (normal), W (dense) etc.

Mandle filters; American

Diatomaceous earth,asbestos and plaster of Paris

Chambered or porcelain filters (French)

This type of filter is made of unglazed porcelain. There are different types of this filter depending on their pore size. The grades are L_1, L_{1a}, L_2, L_3, L_5, L_7, L_{11}, and L_{13}. L_1 is the coarser and L_{13} is the finest in the order. These filters are used for removing organisms from fluid to obtain bacterial toxin.

2.Asbestos Filters

Inorganic Filter: It consists of pad of porcelain or ground glass mounted in filter flask. It is used in sterilization of serum during media preparation.
Eg: Seitz Filter -disposible

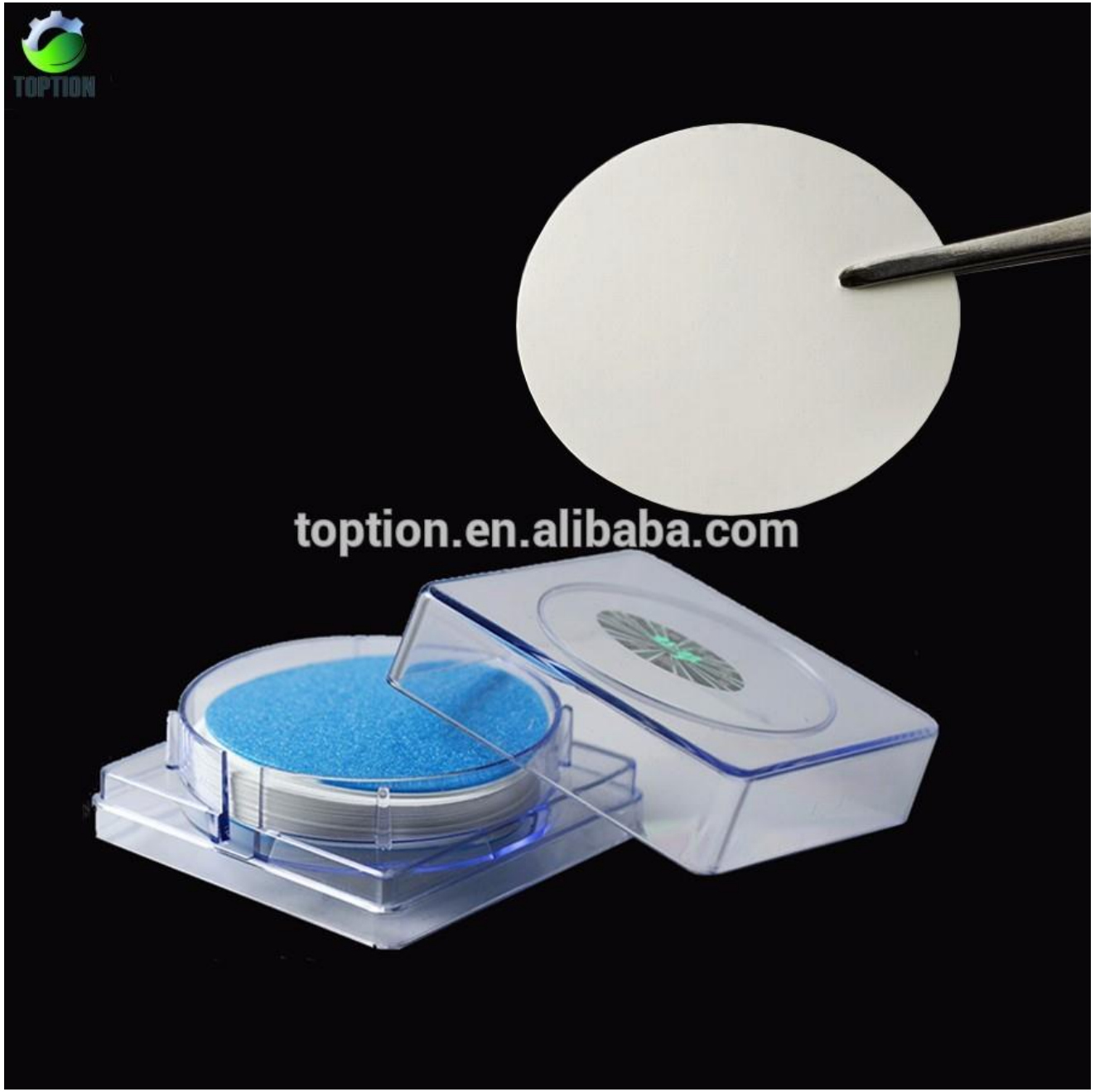

EQUITRON
SEITZ FILTER

4. Sintered Glass Filter: Fritted glass filters

This is made up of finely ground glass which is subsequently fused to make the small particles adhere to each other. This filter is sterilised after use, avoiding temperature extremes.

Membrane filters

They are composed of inert cellulose acetate or poly carbonate. This filters are used in lab & industries for sterilization of fluids. In Microbiology lab, it is used for isolation, enumeration & identification of bacteria from water sample.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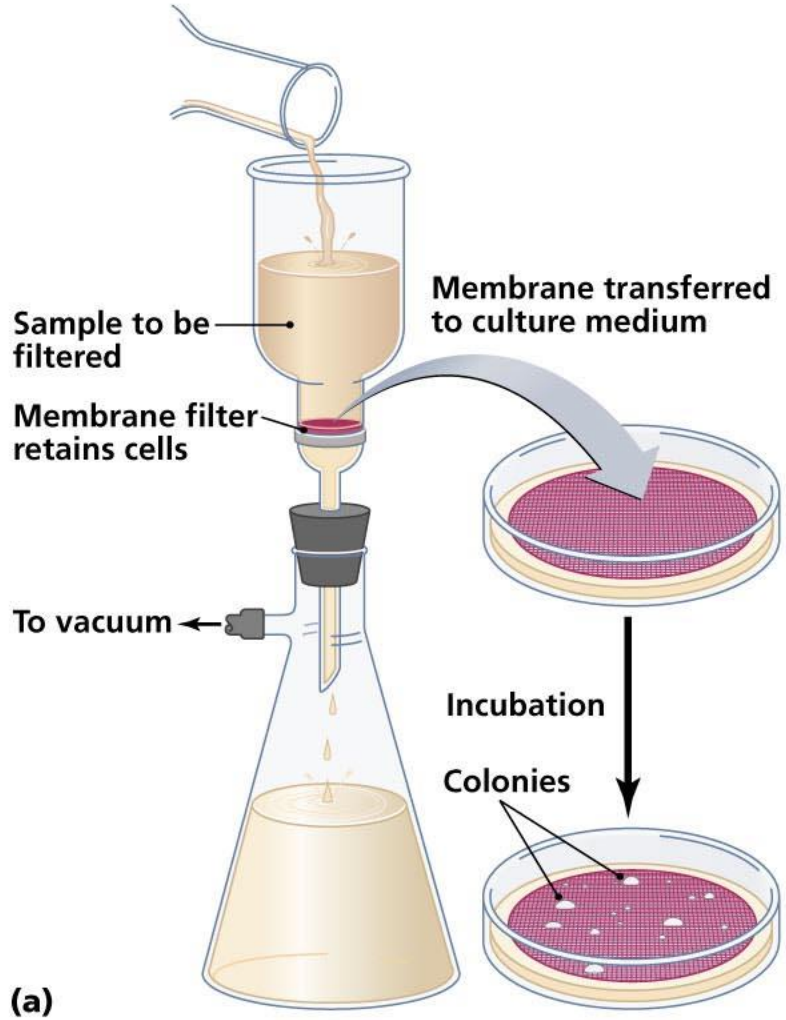
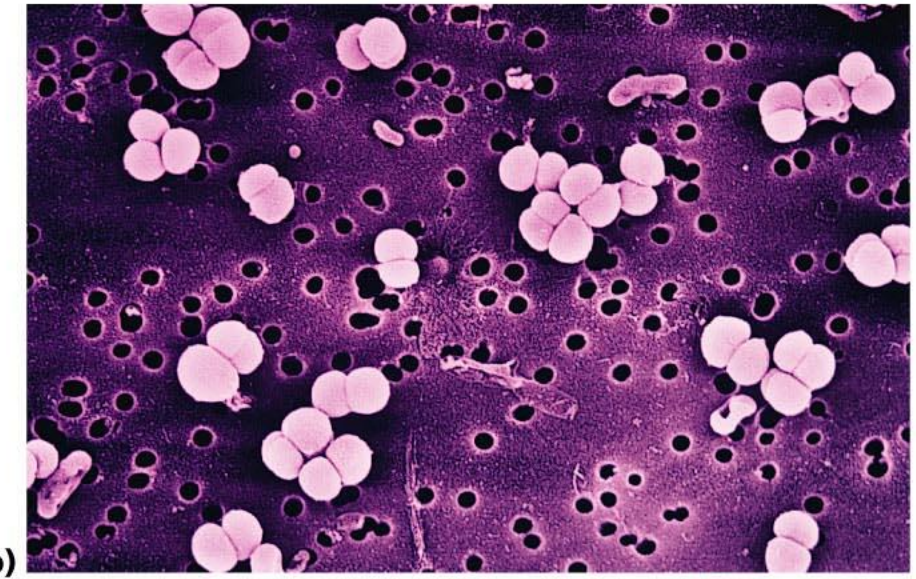
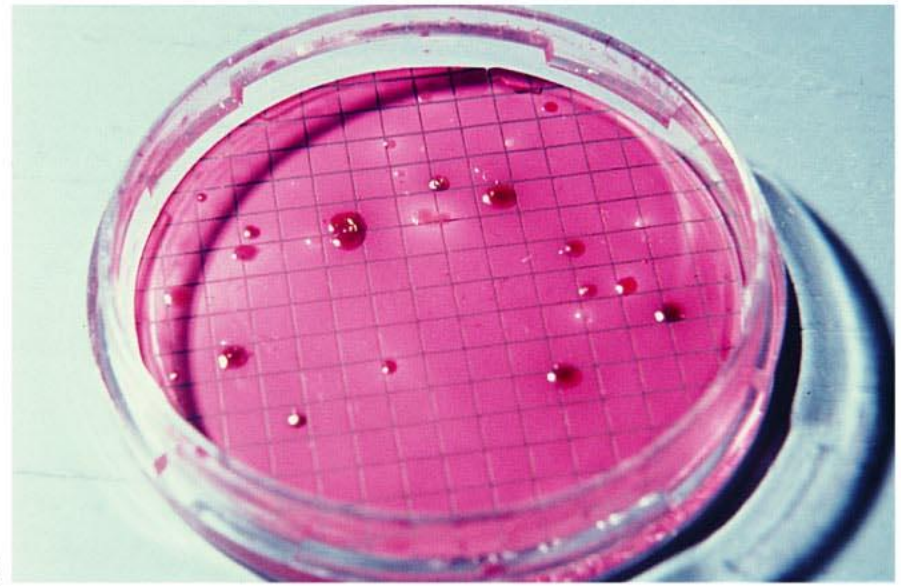
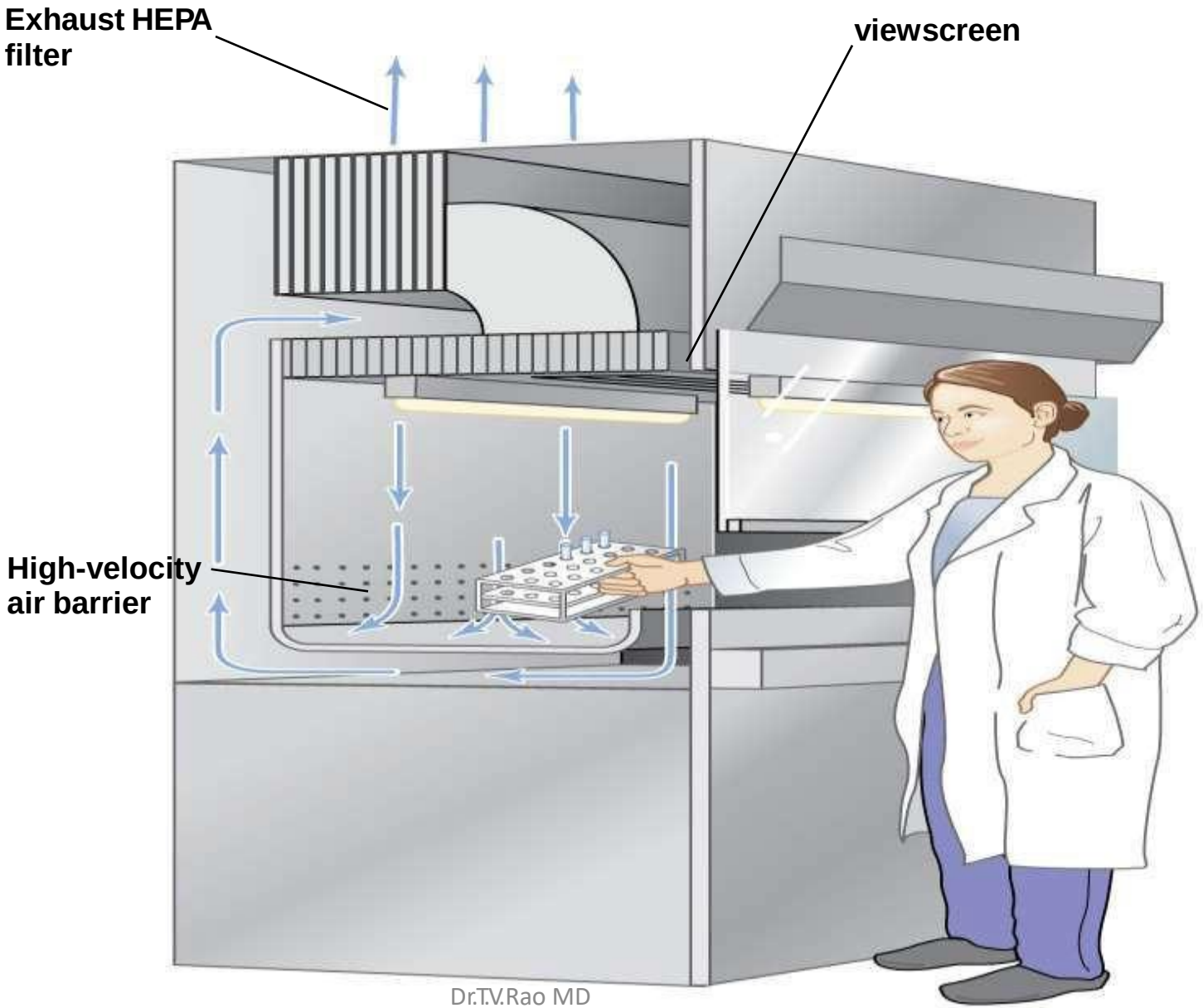






- HEPA filter are used with laminar flow to produce dust & bacterial free air. Similarly, they are used to treat air entering surgical units, pharmaceutical fillings rooms.



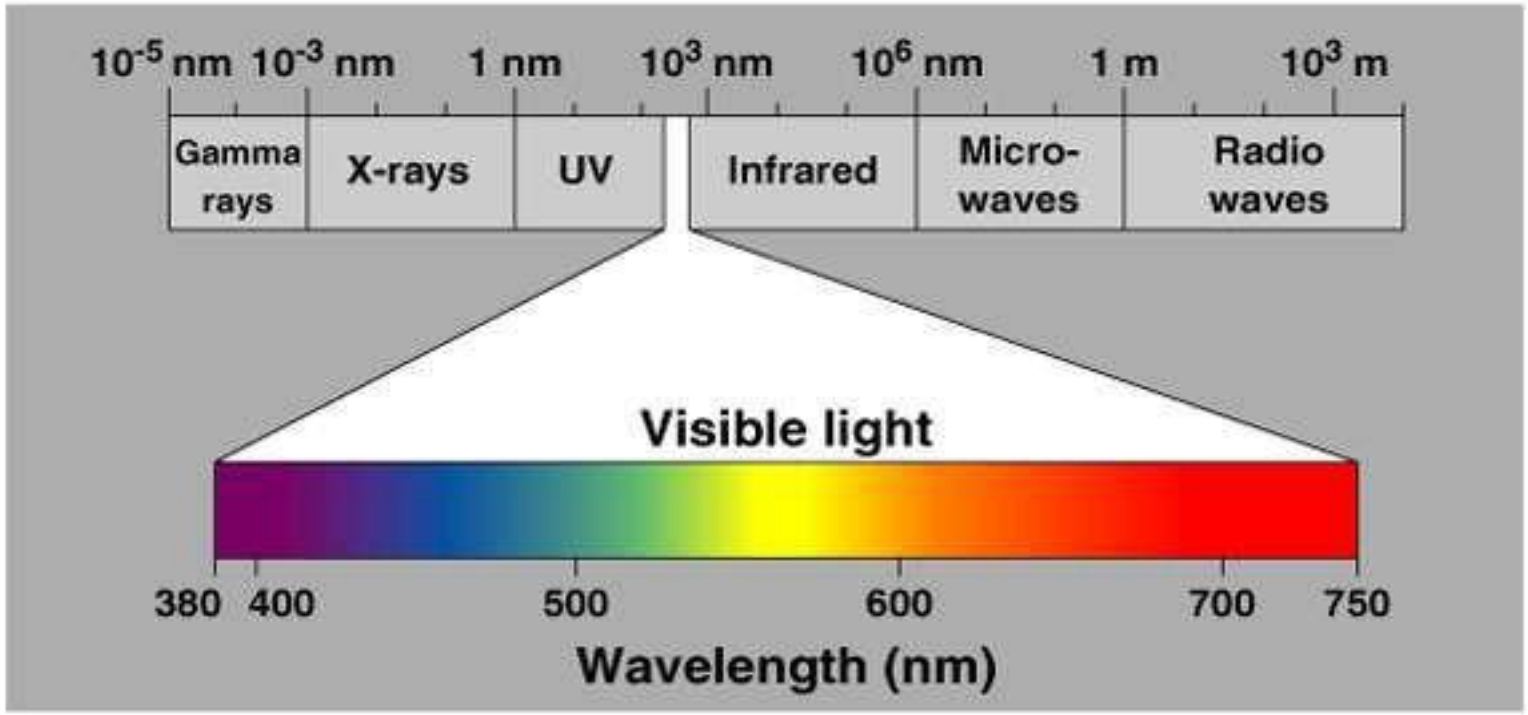
Radiations :

- **Radiations :**

•Ionizing radiations

•Non - Ionizing radiations

Forms of Radiation



- Nonionizing radiation
  - Wavelengths greater than 1 nm
  - Excites electrons, causing them to make new covalent bonds
- Affects 3-D structure of proteins and nucleic acids
  - UV light causes pyrimidine dimers in DNA
  - UV light does not penetrate well
  - Suitable for disinfecting air, transparent fluids, and surfaces of objects

Non-Ionising radiation:

1. **Infra red rays**
2. **Ultraviolet (UV) rays**

– Infra red is used for rapid mass sterilisation of
syringes and catheters.

– Ultraviolet radiation is used for disinfecting enclosed areas such as bacterial laboratory, inoculation hood, laminar flow and operation theatres.

Ionising radiations:

- **Ionizing radiations:**
  1. X rays
2. Gamma rays
3. Cosmic rays

- Gamma radiation are commercially used for sterilisation of disposable items. (cold sterilisation)

Ultrasonic vibrations

- High frequency Sounds beyond the range of human ear
- When propagated in fluids ,cause the formation of microscopic bubbles or cavities. Need liquid medium
- Appears to boil
- Cavities collapse and send out shock waves.
- Cavitation and Cavitron Device.