STERILIZATION

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WHAT IS STERILIZATION:

Sterilization can be defined as any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses and prions) from a surface, equipment, foods, medications, or biological culture medium.

METHODS OF STERILIZATION

- The various methods of sterilization are:
- 1. **Physical Method**
  - a. Thermal (Heat) methods
  - b. Radiation method
  - c. Filtration method
- 2. **Chemical Method**
  - a. Gaseous method

PHYSICAL METHODS:

1. HEAT STERILIZATION:

- Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents.
- This method of sterilization can be applied only to the THERMO STABLE PRODUCTS and MOISTURE-SENSITIVE MATERIALS.

i) Dry Heat (160-1800˚C)

Sterilization for thermo stable products

ii) Moist heat (121-1340 ˚C)

sterilization is used for moisture- resistant materials.

- The efficiency with which heat is able to inactivate microorganisms is dependent upon
  - i) the degree of heat, the exposure time and
  - ii) the presence of water.
- The action of heat will be due to induction of lethal chemical events mediated through the action of water and oxygen.
- In the presence of water much lower temperature time exposures are required to kill microbe than in the absence of water.

THERMAL (HEAT) METHODS

Thermal methods includes:

i) Dry Heat Sterilization

Ex:1. Incineration

2. Red heat

3. Flaming

4. Hot air oven

ii) Moist Heat Sterilization

1.Dry saturated steam – Autoclaving

2. Boiling water/ steam at atmospheric pressure

3. Hot water below boiling point

Dry Heat Sterilization

- 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. It is also used for sterilizing non-aqueous thermo stable liquids and thermo stable 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

Physical methods for Sterilization

Sunlight:

- The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it.
- It is responsible for spontaneous sterilization in natural conditions.
- In tropical countries, the sunlight is more effective in killing germs due to combination of ultraviolet rays and heat.
- By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes.

Heat:

- Heat is considered to be most reliable method of sterilization of articles that can withstand heat.
- Heat acts by oxidative effects as well as denaturation and coagulation of proteins.
- Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure.

Factors affecting sterilization by heat:

- **Nature of heat**: Moist heat is more effective than dry heat
- **Temperature and time**: temperature and time are inversely proportional. As temperature increases the time taken decreases.
- **Number of microorganisms:** More the number of microorganisms, higher the temperature or longer the duration required.
- **Nature of microorganism:** Depends on species and strain of microorganism, sensitivity to heat may vary. Spores are highly resistant to heat.
- **Type of material**: Articles that are heavily contaminated require higher temperature or prolonged exposure. Certain heat sensitive articles must be sterilized at lower temperature.
- **Presence of organic material**: Organic materials such as protein, sugars, oils and fats increase the time required.

Action of heat:

- Dry heat acts by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes.
- The moist heat acts by coagulation and denaturation of proteins.
- Moist heat is superior to dry heat in action.
- Temperature required to kill microbe by dry heat is more than the moist heat.
- **Thermal death time** is the minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.

DRY HEAT STERILIZATION

Red heat

- Articles such as bacteriological loops, straight wires, tips of forceps and searing spatulas are sterilized by holding them in Bunsen flame till they become red hot.
- This is a simple method for effective sterilization of such articles, but is limited to those articles that can be heated to redness in flame.

Flaming

- This is a method of passing the article over a Bunsen flame, but not heating it to redness.
- Articles such as scalpels, mouth of test tubes, flasks, glass slides and cover slips are passed through the flame a few times.
- Even though most vegetative cells are killed, there is no guarantee that spores too would die on such short exposure.
- This method too is limited to those articles that can be exposed to flame. Cracking of the glassware may occur.

Incineration:

- This is a method of destroying contaminated material by burning them in incinerator.
- Articles such as soiled dressings; animal carcasses, pathological material and bedding etc. should be subjected to incineration.
- This technique results in the loss of the article, hence is suitable only for those articles that have to be disposed.
- Burning of polystyrene materials emits dense smoke, and hence they should not be incinerated.

Hot air oven:

Hot air oven:

- This method was introduced by Louis Pasteur.
- Articles to be sterilized are exposed to high temperature (160°C) for duration of one hour in an electrically heated oven.
- Since air is poor conductor of heat, even distribution of heat throughout the chamber is achieved by a fan.
- The heat is transferred to the article by radiation, conduction and convection.
- The oven should be fitted with a thermostat control, temperature indicator, meshed shelves and must have adequate insulation.

Hot air oven:

- **Articles sterilized:**

Metallic instruments (like forceps, scalpels, scissors), glass wares (such as petri-dishes, pipettes, flasks, all-glass syringes), swabs, oils, grease, petroleum jelly and some pharmaceutical products.

- **Sterilization process:**
- Articles to be sterilized must be perfectly dry before placing them inside to avoid breakage.
- Articles must be placed at sufficient distance so as to allow free circulation of air in between.
- Mouths of flasks, test tubes and both ends of pipettes must be plugged with cotton wool.
- Articles such as petri dishes and pipettes may be arranged inside metal canisters and then placed.
- Individual glass articles must be wrapped in Kraft paper or aluminum foils.

Hot air oven:

- **Sterilization cycle:**
- This takes into consideration the time taken for the articles to reach the sterilizing temperature, maintenance of the sterilizing temperature for a defined period (holding time) and the time taken for the articles to cool down.
- Different temperature-time relations for holding time are
  - 60 minutes at 160°C,
  - 40 minutes at 170°C and
  - 20 minutes at 180°C.
- Increasing temperature by 10 degrees shortens the sterilizing time by 50%.
- The hot air oven must not be opened until the temperature inside has fallen below 60°C to prevent breakage of glassware.

Hot air oven:

- **Sterilization control:**
- Three methods exist to check the efficacy of sterilization process, namely physical, chemical and biological.
  - Physical: Temperature chart recorder and thermocouple.
  - Chemical: Browne's tube No.3

  (green spot, color changes from red to green)
  - Biological: 106 spores of *Bacillus subtilis* var niger or *Clostridium tetani* on paper strips are placed inside envelopes and then placed inside the hot air oven.
- Upon completion of sterilization cycle, the strips are removed and inoculated into thioglycollate broth or cooked meat medium and incubated at 37°C for 3-5 days.
- Proper sterilization should kill the spores and there should not be any growth.

Hot air oven:

- **Advantages:**
- It is an effective method of sterilization of heat stable articles.
- The articles remain dry after sterilization.
- This is the only method of sterilizing oils and powders.
- **Disadvantages:**
- Since air is poor conductor of heat, hot air has poor penetration.
- Cotton wool and paper may get slightly charred.
- Glasses may become smoky.
- Takes longer time compared to autoclave.

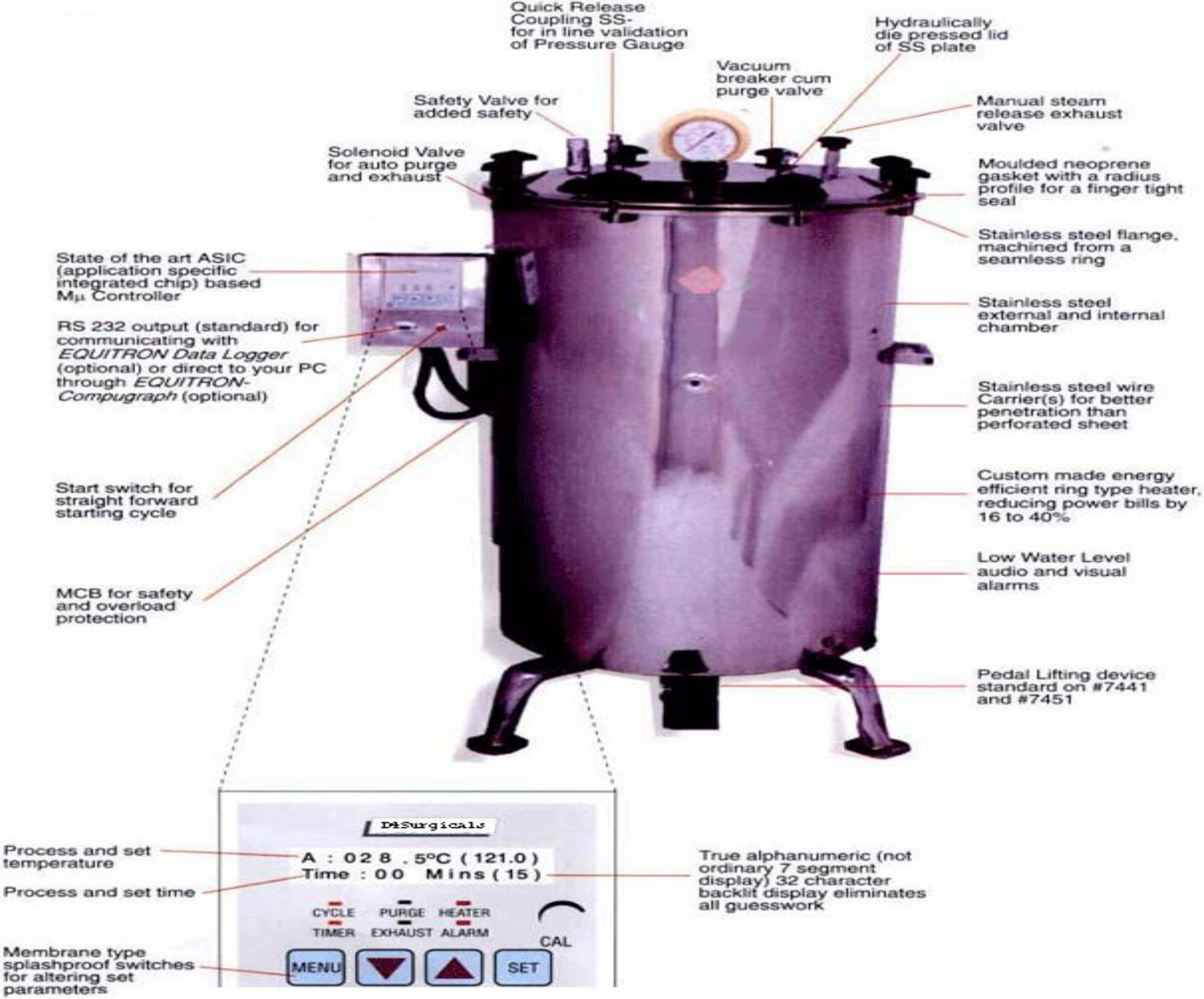
MOIST HEAT STERILIZATION

Moist Heat Sterilization

- 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.

Autoclave

- Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber.
- Generally the conditions employed are Temperature upto121-134°C for 15-20 min under 15 lbs pressure,based on type of metiral used.



Quick Release Coupling SS- for in line validation of Pressure Gauge
Hydraulically die pressed lid of SS plate
Vacuum breaker cum purge valve
Safety Valve for added safety
Manual steam release exhaust valve
Solenoid Valve for auto purge and exhaust
Moulded neoprene gasket with a radius profile for a finger tight seal
Stainless steel flange, machined from a seamless ring
State of the art ASIC (application specific integrated chip) based Mμ Controller
Stainless steel external and internal chamber
RS 232 output (standard) for communicating with *EQUITRON Data Logger* (optional) or direct to your PC through *EQUITRON-Compugraph* (optional)
Stainless steel wire Carrier(s) for better penetration than perforated sheet
Custom made energy efficient ring type heater, reducing power bills by 16 to 40%
Start switch for straight forward starting cycle
Low Water Level audio and visual alarms
MCB for safety and overload protection
Pedal Lifting device standard on #7441 and #7451
D4Surgicals
Process and set temperature
A : 028 .5ºC (121.0)
Time : 00 Mins (15)
Process and set time
True alphanumeric (not ordinary 7 segment display) 32 character backlit display eliminates all guesswork
CYCLE
PURGE
HEATER
TIMER
EXHAUST
ALARM
CAL
MENU
SET
Membrane type splashproof switches for altering set parameters

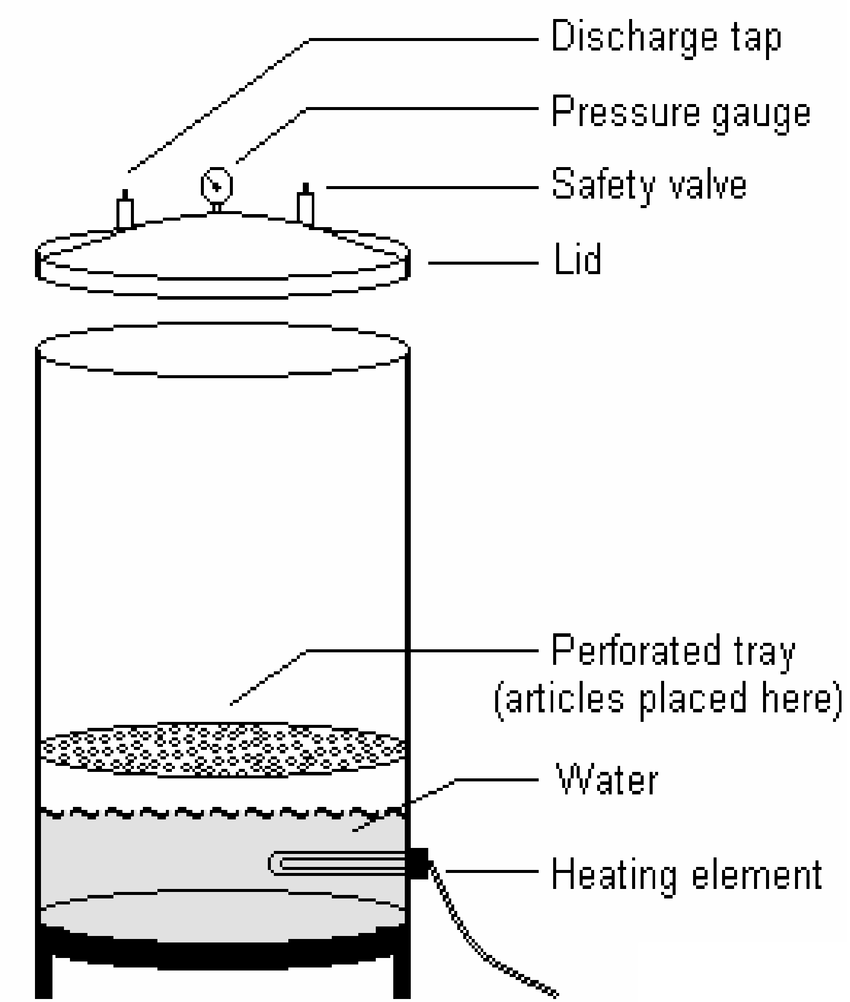
Autoclave

- Sterilization can be effectively achieved at a temperature above 100°C using an autoclave.
- Water boils at 100°C at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases.
- In an autoclave the water is boiled in a closed chamber. As the pressure rises, the boiling point of water also raises.
- At a pressure of 15 lbs inside the autoclave, the temperature is said to be 121°C.
- Exposure of articles to this temperature for 15 minutes sterilizes them.
- To destroy the infective agents associated with spongiform encephalopathies (prions), higher temperatures or longer times are used; 135°C or 121°C for at least one hour are recommended.

Autoclave

- **Advantages of steam:**
- It has more penetrative power than dry air, it moistens the spores (moisture is essential for coagulation of proteins), condensation of steam on cooler surface releases latent heat, condensation of steam draws in fresh steam.
- **Different types of autoclave:**
  - Simple "pressure-cooker type" laboratory autoclave,
  - Steam jacketed downward displacement laboratory autoclave
  - High pressure pre-vacuum autoclave

Construction And Operation Of Autoclave:



Construction And Operation Of Autoclave:

- A simple autoclave has vertical or horizontal cylindrical body with a heating element, a perforated try to keep the articles, a lid that can be fastened by screw clamps, a pressure gauge, a safety valve and a discharge tap.
- The articles to be sterilized must not be tightly packed.
- The screw caps and cotton plugs must be loosely fitted.
- The lid is closed but the discharge tap is kept open and the water is heated.
- As the water starts boiling, the steam drives air out of the discharge tap.
- When all the air is displaced and steam start appearing through the discharge tap, the tap is closed.
- The pressure inside is allowed to rise up to 15 lbs per square inch.
- At this pressure the articles are held for 15 minutes, after which the heating is stopped and the autoclave is allowed to cool.
- Once the pressure gauge shows the pressure equal to atmospheric pressure, the discharge tap is opened to let the air in.
- The lid is then opened and articles removed.

- **Articles sterilized:**
- Culture media, dressings, certain equipment, linen etc.
- **Precautions:**
  - Articles should not be tightly packed, the autoclave must not be overloaded.
  - Air discharge must be complete and there should not be any residual air trapped inside
  - Caps of bottles and flasks should not be tight.
  - Autoclave must not be opened until the pressure has fallen or else the contents will boil over.
  - Articles must be wrapped in paper to prevent drenching, bottles must not be overfilled.
- **Advantage:** Very effective way of sterilization, quicker than hot air oven.
- **Disadvantages:**
  - Drenching and wetting or articles may occur,
  - trapped air may reduce the efficacy,
  - takes long time to cool

Radiation Sterilization

- Many types of radiation are used for sterilization like electromagnetic radiation (e.g. gamma rays and UV light), particulate radiation (e.g. accelerated electrons).The major target for these radiation is microbial DNA.
- Radiation sterilization with high energy gamma rays or accelerated electrons has proven to be a useful method for the industrial sterilization of heat sensitive products.

- Two types of radiation are used, ionizing and non-ionizing.
- Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power. Since radiation does not generate heat, it is termed "cold sterilization".
- In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent.

Non-ionizing rays:

- Rays of wavelength longer than the visible light are non-ionizing. Microbicidal wavelength of UV rays lie in the range of 200-280 nm, with 260 nm being most effective.
- UV rays are generated using a high-pressure mercury vapor lamp. It is at this wavelength that the absorption by the microorganisms is at its maximum, which results in the germicidal effect.
- UV rays induce formation of thymine-thymine dimers, which ultimately inhibits DNA replication.
- UV readily induces mutations in cells irradiated with a non-lethal dose.
- Microorganisms such as bacteria, viruses, yeast, etc. that are exposed to the effective UV radiation are inactivated within seconds.

Non-ionizing rays:

- Since UV rays don’t kill spores, they are considered to be of use in surface disinfection.
- UV rays are employed to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc.
- Disadvantages of using uv rays include low penetrative power, limited life of the uv bulb, some bacteria have DNA repair enzymes that can overcome damage caused by uv rays, organic matter and dust prevents its reach, rays are harmful to skin and eyes.
- It doesn't penetrate glass, paper or plastic.

Ionizing rays:

- Ionizing rays are of two types, particulate and electromagnetic rays.
- Electron beams are particulate in nature while gamma rays are electromagnetic in nature.
- High speed electrons are produced by a linear accelerator from a heated cathode.
- Electron beams are employed to sterilize articles like syringes, gloves, dressing packs, foods and pharmaceuticals.
- Sterilization is accomplished in few seconds. Unlike electromagnetic rays, the instruments can be switched off.

Ionizing rays:

- Disadvantage includes poor penetrative power and requirement of sophisticated equipment.
- Electromagnetic rays such as gamma rays emanate from nuclear disintegration of certain radioactive isotopes (Co60, Cs137).
- They have more penetrative power than electron beam but require longer time of exposure. These high-energy radiations damage the nucleic acid of the microorganism.
- A dosage of 2.5 megarads kills all bacteria, fungi, viruses and spores. It is used commercially to sterilize disposable petri dishes, plastic syringes, antibiotics, vitamins, hormones, glasswares and fabrics.
- Disadvantages include; unlike electron beams, they can't be switched off, glasswares tend to become brownish, loss of tensile strength in fabric.
- Gamma irradiation impairs the flavour of certain foods. Bacillus pumilus E601 is used to evaluate sterilization process.

FILTRATION STERILIZATION

- Filtration does not kill microbes, it separates them out.
- Membrane filters with pore sizes between 0.2-0.45 µm are commonly used to remove particles from solutions that can't be autoclaved.
- It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution.
- Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and purifying hydrated fluid.
- Filtration is aided by using either positive or negative pressure using vacuum pumps.
- The older filters made of earthenware or asbestos are called depth filters.

Different types of filters are:

Earthenware filters:

These filters are made up of diatomaceous earth or porcelain. They are usually baked into the shape of candle.

Different types of earthenware filters are:

a. Pasteur-Chamberland filter:

These candle filters are from France and are made up of porcelain (sand and kaolin).

Similar filter from Britain is Doulton. Chamberland filters are made with various porosities, which are graded as L1, L1a, L2, L3, L5, L7, L9 and L11.

Doulton filters are P2, P5 and P11.

b. Berkefeld filter:

These are made of Kieselguhr, a fossilized diatomaceous earth found in Germany.

They are available in three grades depending on their porosity (pore size); they are V (veil), N (normal) and W (wenig).

Quality of V grade filter is checked using culture suspension of Serrtia marcescens (0.75 µm).

c. Mandler filter:

This filter from America is made of kieselguhr, asbestos and plaster of Paris.

Asbestos filters:

- These filters are made from chrysotile type of asbestos, chemically composed of magnesium silicate.
- They are pressed to form disc, which are to be used only once.
- The disc is held inside a metal mount, which is sterilized by autoclaving.
- They are available in following grades; HP/PYR (for removal of pyrogens), HP/EKS (for absolute sterility) and HP/EK (for clarifying).

- **Sintered glass filters:**
- These are made from finely ground glass that are fused sufficiently to make small particles adhere to each other.
- They are usually available in the form of disc fused into a glass funnel.
- Filters of Grade 5 have average pore diameter of 1-1.5 µm.
- They are washed in running water in reverse direction and cleaned with warm concentrated H_2SO_4 and sterilized by autoclaving.

Membrane filters:

- These filters are made from a variety of polymeric materials such as cellulose nitrate, cellulose diacetate, polycarbonate and polyester.
- The older type of membrane, called gradocol (graded colloidion) membrane was composed of cellulose nitrate.
- Gradocol membranes have average pore diameter of 3-10 µm.
- The newer ones are composed of cellulose diacetate. These membranes have a pore diameter ranging from 0.015 µm to 12 µm.
- These filters are sterilized by autoclaving.

- Membrane filters are made in two ways, the capillary pore membranes have pores produced by radiation while the labyrinthine pore membranes are produced by forced evaporation of solvents from cellulose esters.
- The disadvantages of depth filters are migration of filter material into the filtrate, absorption or retention of certain volume of liquid by the filters, pore sizes are not definite and viruses and mycoplasma could pass through.
- The advantages of membrane filters are known porosity, no retention of fluids, reusable after autoclaving and compatible with many chemicals. However, membrane filters have little loading capacity and are fragile.

Air Filters:

- Air can be filtered using HEPA (High Efficiency Particle Air) filters.
- They are usually used in biological safety cabinets.
- HEPA filters are at least 99.97% efficient for removing particles >0.3 µm in diameter.
- Examples of areas where HEPA filters are used include rooms housing severely neutropenic patients and those operating rooms designated for orthopedic implant procedures.
- HEPA filter efficiency is monitored with the dioctylphthalate (DOP) particle test using particles that are 0.3 µm in diameter.

SONIC AND ULTRASONIC VIBRATIONS:

- Sound waves of frequency >20,000 cycle/second kills bacteria and some viruses on exposing for one hour.
- Microwaves are not particularly antimicrobial in themselves, rather the killing effect of microwaves are largely due to the heat that they generate.
- High frequency sound waves disrupt cells.
- They are used to clean and disinfect instruments as well as to reduce microbial load.
- This method is not reliable since many viruses and phages are not affected by these waves.

Filtration Sterilization

- Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non viable particles.
- The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material.
- Ex:HEPA FILTERS

- Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas. They are also used in industry as part of the venting systems on fermentors, centrifuges, autoclaves and freeze driers. Membrane filters are used for sterility testing

- There are two types of filters used in filtration sterilization:

(a) Depth filters:

(b) Membrane filters: These are porous membrane about 0.1 mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate, and polyvinylidene fluoride, or some other synthetic material.

CHEMICAL STERILIZATION METHOD

GASEOUS METHOD

- The chemically reactive gases such as formaldehyde, (methanol, H.CHO) and ethylene oxide (CH2)2O possess biocidal activity. Ethylene oxide is a colorless, odorless, and flammable gas.
- The mechanism of antimicrobial action of the two gases is assumed to be through alkylations of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids.

- The concentration ranges (weight of gas per unit chamber volume) are usually in range of 800-1200 mg/L for ethylene oxide and 15-100 mg/L for formaldehyde with operating temperatures of 45-63°C and 70-75°C respectively.
- Both of these gases being alkylating agents are potentially mutagenic and carcinogenic. They also produce acute toxicity including irritation of the skin, conjunctiva and nasal mucosa

MERITS, DEMERITS AND APPLICATIONS OF DIFFERENT METHODS OF STERILIZATION

S.no	METHOD	MECHANISM	MERITS	DEMERITS	APPLICATIONS
1	Heat sterilization	Destroys bacterial endo toxins	Most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents	Can be applied only to the thermo stable products	Dry heat is applicable for sterilizing glass wares and metal surgical instruments and moist heat is the most dependable method for decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents.

S.no	METHOD	MECHANISM	MERITS	DEMERITS	APPLICATIONS
1	Gaseous sterilization	Alkylation	Penetrating ability of gases.	Gases being alkylating agents are potentially mutagenic and carcinogenic.	Ethylene oxide gas has been used widely to process heat-sensitive devices.
2	Radiation sterilization	Ionization of nucleic acids	It is a useful method for the industrial sterilization of heat sensitive products	Undesirable changes occur in irradiated products,an example is aqueous solution where radiolysis of water occurs.	Radiation sterilization is generally applied to articles in the dry state; including surgical instruments, sutures, prostheses, unit dose ointments, plastics

S.no	METHOD	MECHANISM	MERITS	DEMERITS	APPLICATIONS
1	Filtration sterilization	Does not destroy but removes the microorganisms	It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non viable particles	Does not differentiate between viable and non viable particles	This method is Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas