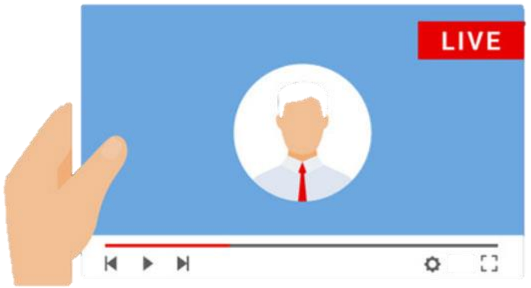


Welcome to GPAT "Live online class"

Lecture: Pharmaceutics Parenterals 1

Agenda

Today's Topic: **Sterilization**

- Introduction
- Types of sterilization
  - Heat Sterilization
  - Chemical sterilization
  - Gaseous sterilization
  - Filtration sterilization
  - Radiation sterilization
- Biological indicators for Sterilization

Solve **GPAT Questions**

Definition

- **Sterilization** *[Latin 'sterilis', unable to produce offspring or barren]*
  - It is the process by which all living cells, spores, and acellular entities (e.g., viruses, viroids, and prions) are either destroyed or removed from an object or habitat
- **Disinfection**
  - It is the killing, inhibition, or removal of microorganisms that may cause disease. Used only on inanimate objects
  - A disinfectant does not necessarily sterilize an object because viable spores and a few microorganisms may remain
- **Sanitization**
  - In sanitization, the microbial population is reduced to levels that are considered safe by public health standards
  - Eg:- sanitizers are used to clean eating utensils in restaurants

Definition

- **Antisepsis**
  - It is the prevention of infection or sepsis and is accomplished with antiseptics
  - These are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population
  - Because they must not destroy too much host tissue, antiseptics are generally not as toxic as disinfectants

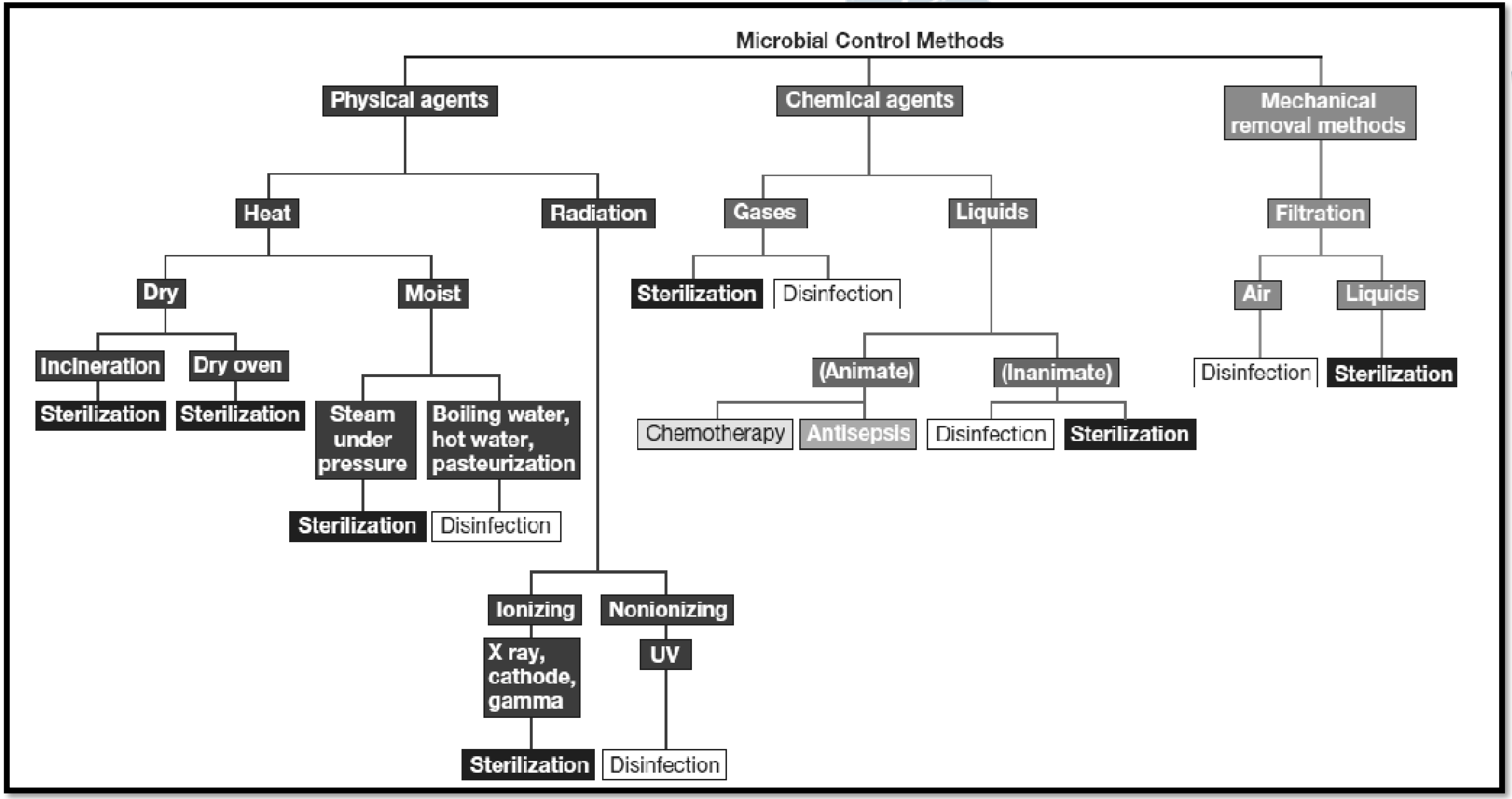
Key points

- **Substances that kill organisms often have the suffix –cide [Latin cida, to kill]**

  Eg:-- germicide, bactericide, fungicide, algicide, or viricide
- **Chemicals do not kill, but they do prevent growth. If these agents are removed, growth will resume. Their names end in -static [Greek statikos, causing to stand or stopping]**

  Eg:-- bacteriostatic and fungistatic
- **Decimal reduction time (D) or D value:** is the time required to kill 90% of the microorganisms or spores in a sample at a specified temperature
- **Thermal destruction value or Z value:** The z value is the increase in temperature required to reduce D to 1/10 its value
- **The F value is** the time in minutes at a specific temperature (usually 250°F or 121.1°C) needed to kill a population of cells or spores

Types of sterilization



Moist Heat

- Moist heat invariably kills microbes by the process known as **<u>'coagulation of proteins'</u>** that is caused by the specific cleavage of the H-bonds which critically retain the protein in its 3D-structure
- Moist heat sterilization may be achieved effectively by the following widely accepted known methods, such as :
  1. Boiling,
  2. Autoclaving, and
  3. Pasteurization

Moist Heat - Boiling

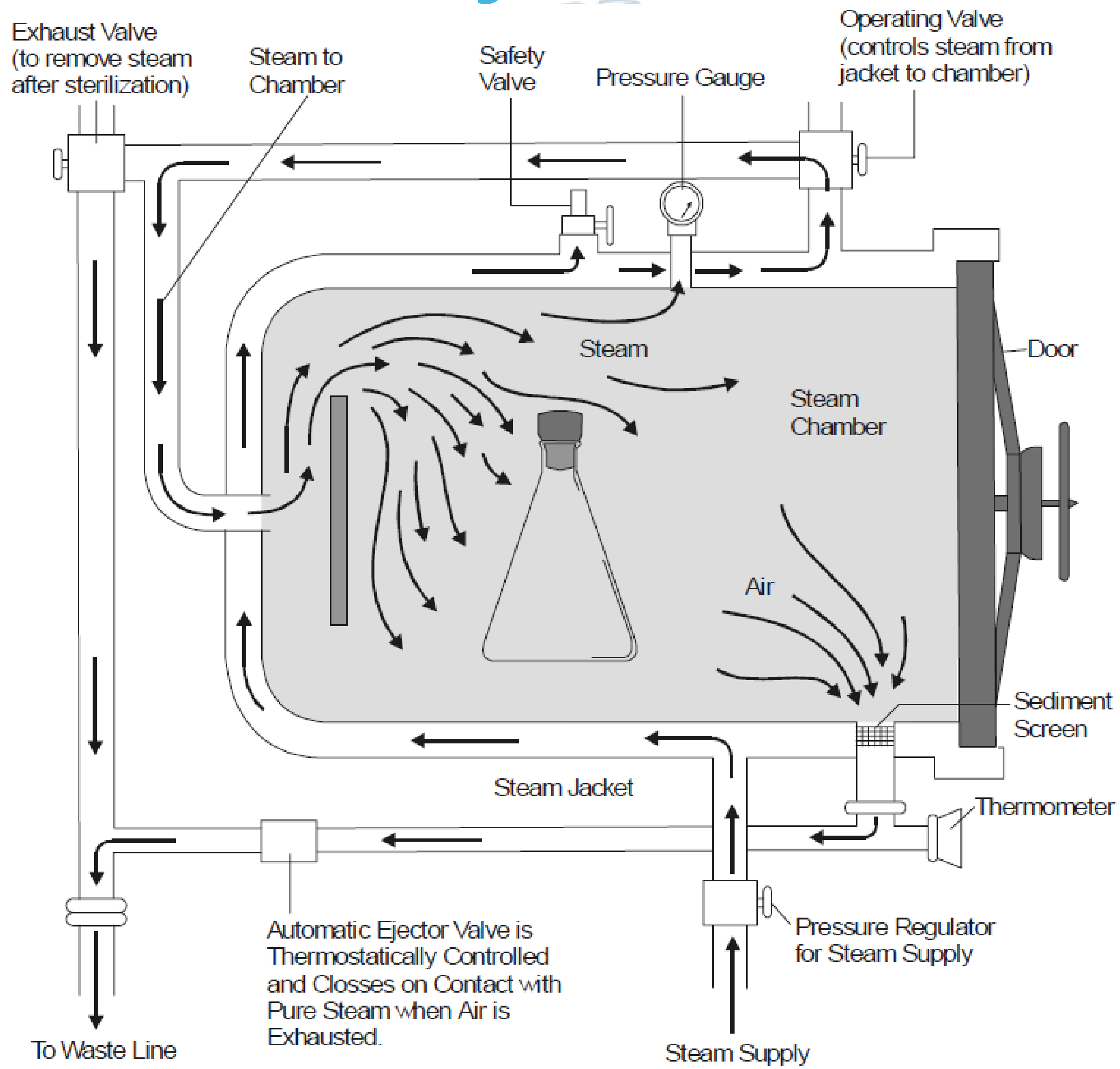
- Boiling at **100°C at 760 mm atmospheric pressure** is found to kill particularly several varieties of vegetative states of microbial strains, a good number of viruses and fungi; besides their 'spores' **within a span of 10 minutes only**
- The **endospores of certain viruses are evidently not destroyed** in such a short duration of 10 minutes

Moist Heat - Autoclaving

- Autoclaving is a widely used sterilization technique that employs high-pressure saturated steam to kill or inactivate microorganisms, including bacteria, viruses, and fungi.
- The autoclaving process involves placing the items to be sterilized, such as laboratory equipment, medical instruments, or media, into a chamber within the autoclave.
- The chamber is sealed to create a closed system. **Steam is then introduced into the chamber, raising the temperature and pressure inside.**
- **The heat and pressure work synergistically to destroy microorganisms by denaturing their proteins and disrupting their cell membranes**.
- The steam penetrates the items being sterilized, reaching all surfaces, crevices, and pores to ensure thorough sterilization

TEMPERATURE (°C)	PRESSURE (psig)	TIME (min.)
115	10	30
121	15	15
126	20	10
134	30	3

Moist Heat - Autoclaving



Moist Heat - Pasteurization

- Pasteurization refers to the process of heating of a fluid at a moderate temperature for a
- definite period of time to destroy undesirable microorganisms without changing to any extent the chemical composition
- **Louis Pasteur in the 19th century**

- Pasteurization : At 63°C for 30 minutes
- HTST-Treatment : At 72°C for 15 seconds
- UHT-Treatment : At 140°C for < 1 second

Key points – Moist Heat Sterilization

Methods	Mechanism of Action	Comment	Preferred Use
Boiling	Protein denaturation	Kills vegetative bacterial and fungal pathogens and almost all viruses within 10 min; less effective on endospores	Dishes, basins, pitchers, various equipment
Autoclaving (**Chamberland**)	Protein denaturation	Very effective method of sterilization; at about 15 PSI of pressure (121°C), all vegetative cells and their endospores are killed in about 15 min	Microbiological media, solutions, linens, utensils, dressings, equipment, and other items that can withstand temperature and pressure
Pasteurization (**Louis Pasteur**)	Protein denaturation	Heat treatment for milk (72°C for about 15 sec) that kills all pathogens and most non-pathogens	Milk, cream, and certain alcoholic beverages (beer and wine)

Dry Heat

- Microorganisms get killed by dry heat due to the **oxidation effects**

Dry Heat - Direct Flaming

- Direct flaming designates one of the **most-simple method** of dry-heat sterilization
- The dry-heat sterilization is mostly used in a 'microbiology laboratory' for the sterilization of the 'inoculating loops', which is duly accomplished by heating the loop wire to a **'redglow'**, and this is 100% effective in actual practice
- Likewise, the same principle is even extended to the process of **'inceneration'** to sterilize as well as dispose of heavily contaminated paper bags, cups, and used dressings

Dry Heat - Hot-Air Sterilization

- Various items need to be sterilized are duly kept in an electric oven, preferably with a stainless-steel chamber inside, and duly maintained at **170°C for a duration of approximately 2 hours (to ensure complete sterilization).**

Key points – Dry Heat Sterilization

Methods	Mechanism of Action	Comment	Preferred Use
Direct flaming	Burning contaminants to ashes	Very effective method of sterilization	Inoculating loops
Incineration	Burning to ashes	Very effective method of sterilization	Paper cups, contaminated dressings, animal carcasses, bags, and wipes
Hot-air sterilization	Oxidation	Very effective method of sterilization but requires temperature of 170°C for about 2 hr.	Empty glassware, instruments, needles, and glass syringes

Disinfection

Phenol and Phenolic compounds 0.3-5%

MOA- disruption of cell wall, precipitate of Proteins, Inactivation of enzyme, leakage of amino acids.

Alcohol-70- 95%

- Higher the alcohol (higher molecular weight) higher the activity. But immiscible with water so they can not be used.
- Methyl alcohol less effective and toxic-not used
- Ethyl alcohol and propyl alcohol only used

MOA- Protein denaturants, lipid dissolving (damages proteins and lipid complexes

Note: absolute alcohol and high percentage of alcohol is dehydrating agent. So it removes water from cell. So cell become dry. Then alcohol can not penetrate the dry cell. 100% alcohol is ineffective.

Disinfection

Halogens- (Iodine & Chlorine)

Iodine:- Tincture of Iodine (2% Iodine + 2% NaI in diluted alcohol)

(7% Iodine + 5% KI in 83% alcohol)

(5% Iodine + 10% KI in water)

Iodophores- Iodine + Surfactants (PVP commonly used)

VP-I complex slowly releases iodine. Prolonged effect.

MOA- oxidizing protein and other cell components. Halogenation of tyrosine amino acid in protein and enzymes.

Disinfection

Halogens- (Iodine & Chlorine)

Cholorine-

Hypochlorites & chloramines (1% personnel hygiene, 5-12% as household

Bleaches (water disinfection)

Hypochlorites: Calcium hypochlorite (Chlorinated Lime)

Chloramine- hydrogen atom in amino group of compound replaced with chlorine

monochloramine, chloramine-T, azochloramine

MOA:- combine with water releases HClO- which intern releases nascent oxygen [o]. That oxidizes various components of cell.

Disinfection

Heavy metal- mercury, silver and copper salts

- Low activity. Not commonly used.
- **MOA-** Readily binds with sulfhydryl groups in enzymes and proteins and inactivates.

Quaternary Ammonium Compounds

- Advantages high solubility, noncorrosive, low toxicity, detergent activity, much higher dilution also produces static activity. Floor cleaning, surfaces cleaning in hospitals and public places.
- **MOA-** Denature protein, interferences of glycolysis, membrane damage.

Evaluation of Disinfectants

- Carrier tests:- silk or catgut thread or a penicylinder dipped in test microbe culture and dried and dipped in test disinfectant and evaluated
- Suspension test- a sample of the bacterial culture is suspended into the disinfectant solution and after exposure it is verified by subculture whether this inoculum is killed or not.
- preferred over carrier tests as the bacteria are uniformly exposed to the disinfectant.
    - Phenol co-efficient method (Rideal walker method)
    - Chick martin method

Evaluation of Disinfectants

	Rideal -Walker	Chick-Martin
Volume medium	5.0 ml	10.0 ml
Diluent for test disinfectant	Distilled water	Water with yeast suspension or feces
Reaction temperature	17.5±0.5°C (20°C in some books)(cold condition)	30°C or (room temperature)
Organism	Salmonella typhi	Salmonella typhi, Staphylococcus aureus
Sampling times	2.5, 5.0, 7.5, 10.0 min.	30.0 min.
Calculation of coefficient	Dilution test killing in 7.5 mins divided by same for phenol	Mean concentration of phenol showing no growth after 30 min. divided by same for test

Key points - Gaseous sterilization

- For heat sensitive and moisture sensitive
- Most frequently used → ethylene oxide, propylene oxide, and formaldehyde
- Highly flammable in mixture with air → diluted with an inert gas such as carbon dioxide or a suitable fluorinated hydrocarbon
- Exposure temperature: 50°C to 60°C
- Exposure time: 4–16 hours
- Mechanism: Interfering with the metabolism of the bacterial cell

- ***Ethylene oxide gas has good penetrating power, which makes it very useful for sterilization of catheters, needles and plastic disposable syringes in their final plastic packaging before the shipment***

Gaseous sterilization

APOMIND

Ethylene oxide sterilization

- It is a colourless liquid with a boiling point of 10.8°C. Flammable gas
- Its mixture with **carbon dioxide or fluorinated hydrocarbons (freons)** in certain proportions makes ethylene oxide **non-inflammable**.
- Effect of ethylene oxide as a sterilizing agent depends on the concentration of gas, temperature, moisture, time, conditions and accessibility of the microorganism.
- The action of ethylene oxide is due to **its power of alkylating the amino, carboxyl, hydroxyl and sulphydryl groups in the enzymes and protein molecule.** It reacts with DNA and RNA, in this reaction, the ring in the ethylene oxide molecule splits and attaches itself where the hydrogen is present.
- Ethylene oxide is a powerful sterilizing agent for **heat and moisture sensitive materials.** It can be used for sterilization of medical and biological preparations, catgut, plastic types of equipment, antibiotics, plaster bandages, culture media, hospital bedding, foodstuff, heavy equipment, books, clothing and soil.

Gaseous sterilization

Ethylene oxide sterilization

Parameters	Conditions
Temperature	50-60°C
Relative humidity	60%
Time	4-16 hrs
Conc. Of EtO	300-450 mg/l
Content of EtO	10 ppm

Concentration (mg/lit)	Exposure time (hrs.)
44	24
88	10
442	4
884	2

Gaseous sterilization

Ethylene oxide sterilization

Residual Limit of Ethylene oxide and derivatives (Parts per million)

Drug Product	Ethylene Oxide	Ethylene Chlorohydrin	Ethylene Glycol
Ophthalmic (for topical use)	10	20	60
Injectable	10	10	20
Intrauterine device	5	10	10
Surgical scrub sponges	25	250	500
Hard Gelatin capsule shells	35	10	35

Gaseous sterilization

Formaldehyde sterilization

- Formaldehyde gas is produced by heating formalin (37%) solution of formaldehyde
- It combines readily with vital organic nitrogen compounds such as proteins and nucleic acids. It is a bactericidal agent with poor penetrating power.
- It kills **both vegetative cells and spores**. Formaldehyde in gaseous form can be used for disinfection and sterilization of enclosed areas such as operation theatres, hospital rooms, aseptic areas and microbiology laboratories.

Parameters	Conditions
Temperature	50-60°c
Relative humidity	60%
Time	4-8 hrs
Gas conc.	4 mg/l

Filtration sterilization

- Filtration may be defined as the process of removing particles from a solution by allowing the liquid position to pass through a membrane or other particle barrier

Materials	Name of the filter
Asbestos pad	Seitz filter
Diatomaceous earth	Berkefeld filter
Procelain	Chamberland-Pasteur filter
Sintered glass disks	Sintered glass filter
Cellulose	Membrane filter
Borosilicate glass fiber	HEPA filter
Clay, mud	Candle filter

- The pressure required to force air through the wet filter is called bubble point pressure of the membrane.
- **Bubble point pressure** (40-50 psig), is inversely proportional to size of largest pore in the membrane
- **Routine filtration** is done at pressure lower than the bubble point (15-20 psig)

Filtration sterilization

Membrane filters

- Membrane filters are the most common type of filters used for liquid sterilization in the microbiology laboratory.
- Membrane filters are composed of high tensile strength polymers such as **cellulose acetate, cellulose nitrate, or polysulfone.**
- Porosities of membrane filters range from 0.1µm to 10µm and the most commonly used membrane filter has the pore size of 0.22µm and 0.45µm

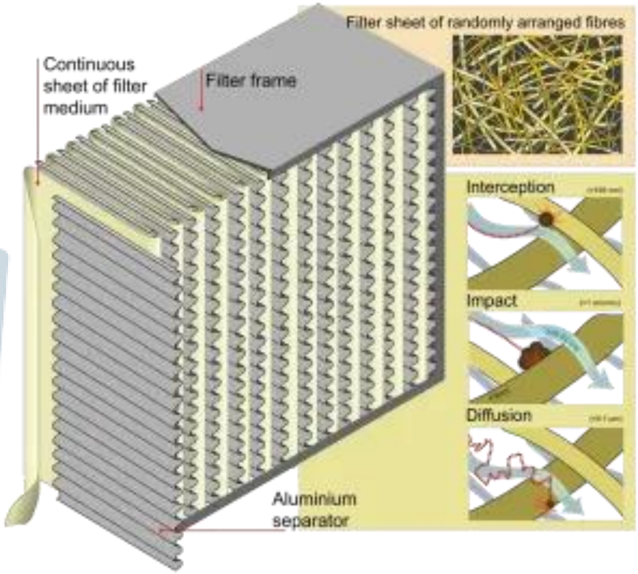
Uses of membrane filter

- Sterilization of fluid materials (pharmaceuticals, ophthalmic solutions, antibiotics, and other heat-sensitive solutions in laboratories and industries)
- Identification and enumeration of microorganisms

Filtration sterilization

HEPA filter

- A typical HEPA filter is a single sheet of borosilicate glass fiber that has been treated with a water-repellent binder.
- HEPA filters allows the construction of **“clean rooms”** and isolation rooms for quarantine, as well as specialized diagnostic/research laboratories.
- HEPA filters typically remove **0.3 µm** test particles with an efficiency of at least **99.97%** including most microorganisms, from the airstream.



Filtration sterilization

Advantages of Filtration Sterilization

- Less capital intensive
- Suitable for heat-sensitive liquids (infusions, vaccines, hormones, etc).
- Large volume of liquids can be filtered reasonably fast

Limitations of Filtration Sterilization

- Only liquids and gases can be sterilized by this process
- Filters are expensive to replace, especially nano-filters
- Inherent limitations of materials used in filters affect the efficacy of this process i.e, breakage of glass filters, rupture of the membrane filter and absorption of the filtrate by Sietz filter
- Clogging may occur

Radiation

- Radiation refers to — any form of radiant energy emission or divergence, as of energy in all directions from luminous bodies, radiographical tubes, particle accelerators, radioactive elements, and fluorescent substances

- The **ionizing radiation** normally possess a wavelength distinctly shorter in comparison to the nonionizing radiation (size < 1 nm) e.g., γ-rays, X-rays, or high-energy electron beams.
- **Nonionizing radiation** viz., UV-light has a wavelength ranging between 1–380 nm, where the visible spectrum commences

Key points - Radiation Sterilization

Methods	Mechanism of Action	Comment	Preferred Use
Ionizing	Destruction of DNA	Not widespread in routine sterilization	Sterilizing pharmaceuticals and medical and dental supplies
Nonionizing	Damage to DNA	Radiation is not very penetrating.	Control of closed environment with UV (germicidal) lamp

Indicators for Sterilization

Sterilization process	Biological indicator	Chemical indicators
Dry heat	Bacillus subtilis var. niger	Browne’s tubes/Bowie-Dick heat sensitive tapes
Moist heat	B. stearothermophilus (i.e., Clostridium sporogenes), B. Coagulans	Stripe papers
Radiation	Bacillus subtilis var. globigii, B. Pumulis	Dosimeters
Ethylene oxide	Bacillus subtilis var. niger	Royce sachet
Filtration	Serratia marscence (i.e., Chromobacter prodiogism)	Bubble point test

GPAT QUESTION

At the temperature 170 C for two hour, complete sterilization occurs in

A. Autoclave

B. Hot air oven

C. Incubator

D. Laminar flow

GPAT QUESTION

Correct sequence of true(T)/false(F) for the following statements can be?

1. Pasteurization kills all the microorganisms present in the milk
2. Moist heat and dry heat methods are physical methods of sterilization
3. Presence of microorganism in any food decreases the decay rate of the food
4. Parenteral and ophthalmic needed to be highly sterilized

A. TFFT

B. FFTT

C. FFFT

D. FTFT

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THANK YOU