Sterilization techniques

Microbiology I

Introduction

- Microorganisms capable of causing infection are constantly present in the external environment and on the human body.

- Microorganisms are responsible for contamination and infection.

- The aim of sterilization is to **remove** or **destroy** them from materials or from surfaces.

Sterilization:

- It is a process by which an article, surface or medium is made free of all microorganisms either in vegetative or spore form.

Disinfection:

- Destruction of all pathogens or organisms capable of 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.

Antiseptics :

- Chemical disinfectants which can safely applied to living tissues and are used to prevent infection by inhibiting the growth of microorganisms.

Asepsis :

- Technique by which the occurrence of infection into an uninfected tissue is prevented.

Effect of sterilization on microorganisms:

- Denaturation of proteins (e.g. wet heat, ethylene oxide)
- Oxidation (e.g. dry heat, hydrogen peroxide)
- Interruption of DNA synthesis/repair (e.g. radiation)
- Interference with protein synthesis (e.g. bleach)
- Disruption of cell membranes (e.g. phenols)

Factors that influence efficacy of disinfection/sterilization:

- Contact time
- Physico-chemical environment (e.g. pH)
- Presence of organic material
- Temperature
- Type of microorganism
- Number of microorganisms
- Material composition

Relative Resistance of Microbial Forms

High resistance	**Moderate resistance**	**Least resistance**
Bacterial endospore (*Bacillus* & *Clostridium*)	Protozoan cyst, Some fungal spores, some naked virus, vegetative bacteria that have higher resistance (*M. tuberculosis*, *S. aureus*, *Pseudomonas*)	Most bacterial vegetative cells, Ordinary fungal spores & hypae, Enveloped virus, Yeasts, Trophozoites

- **Uses of sterilization:**

1. Sterilization of materials, instruments used in surgical and diagnostic procedures.
2. Sterilization of Media and reagents used in the microbiology laboratory.
3. Food and drug manufacturing to ensure safety from contaminating organisms.

Ideal sterilization/disinfection process:

- Highly efficacious
- Fast
- Good penetrability
- Compatible with all materials
- Non-toxic
- Effective despite presence of organic material
- Difficult to make significant mistakes in process
- Easily monitored

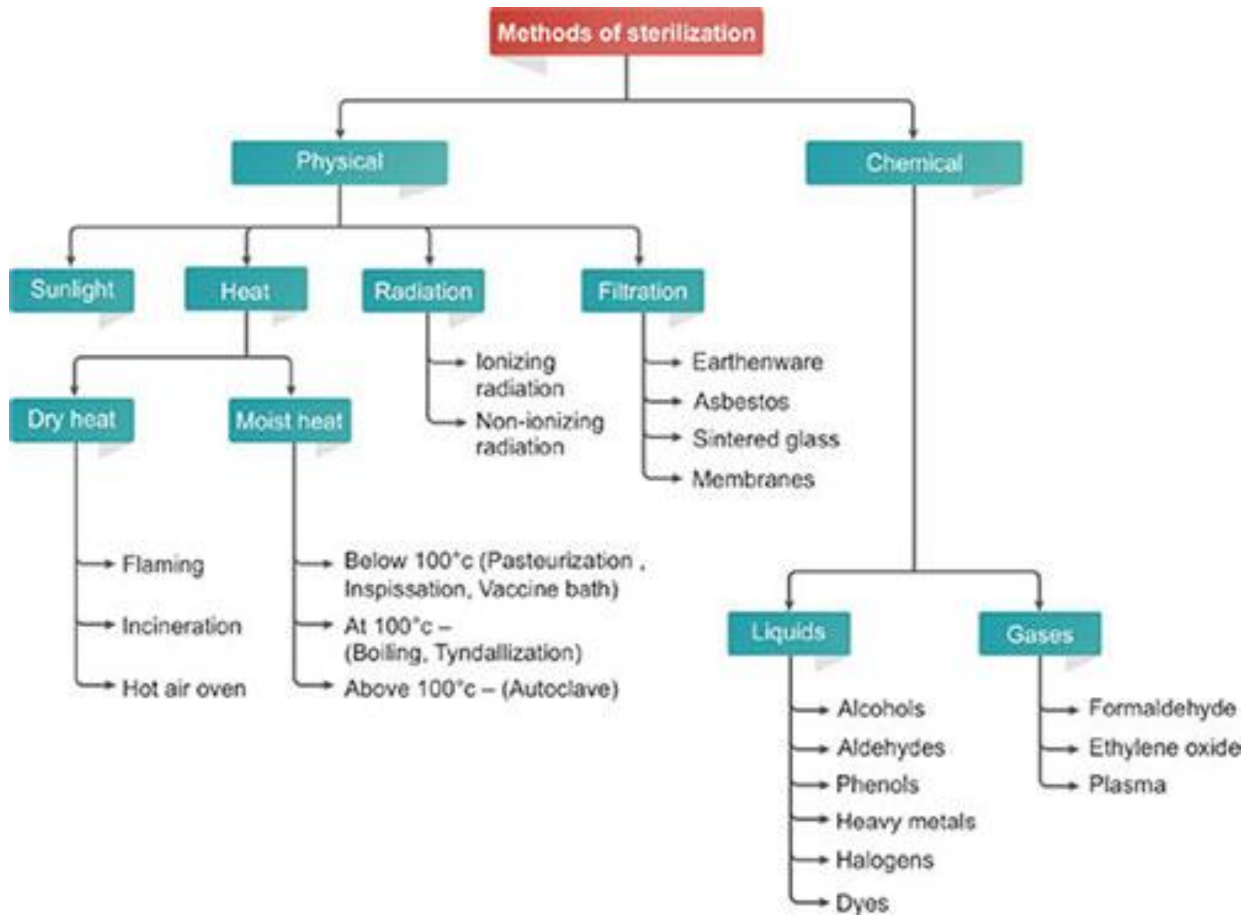
Methods of sterilization:

Physical sterilization:

- Sunlight
- Heat
  - Dry heat
  - Moist heat
- Filtration
- Radiation

Chemical sterilization:

- Alcohols
- Aldehydes
- Phenolics
- Oxidizing agents
- Quaternary ammonium compounds
- Ethylene oxide gas



Methods of sterilization
Physical
Chemical
Sunlight
Heat
Radiation
Filtration
Dry heat
Moist heat
Ionizing radiation
Non-ionizing radiation
Earthenware
Asbestos
Sintered glass
Membranes
Flaming
Incineration
Hot air oven
Below 100°c (Pasteurization , Inspissation, Vaccine bath)
At 100°c – (Boiling, Tyndallization)
Above 100°c – (Autoclave)
Liquids
Gases
Alcohols
Aldehydes
Phenols
Heavy metals
Halogens
Dyes
Formaldehyde
Ethylene oxide
Plasma

Physical methods:

1. Sun light:

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

2. Heat:

- Factors influencing sterilization:
  - Nature of heat
  - Temperature and duration
  - Characteristic of organism and spores
  - Type of material

- Dry heat kills the organism by:
  - Denaturation of the bacterial proteins,
  - Oxidative damage
  - Toxic effect of elevated levels of electrolytes.

Dry heat sterilization methods includes:

1.Red heat

2.Flaming

3.Incineration

4.Hot air oven

Red heat:

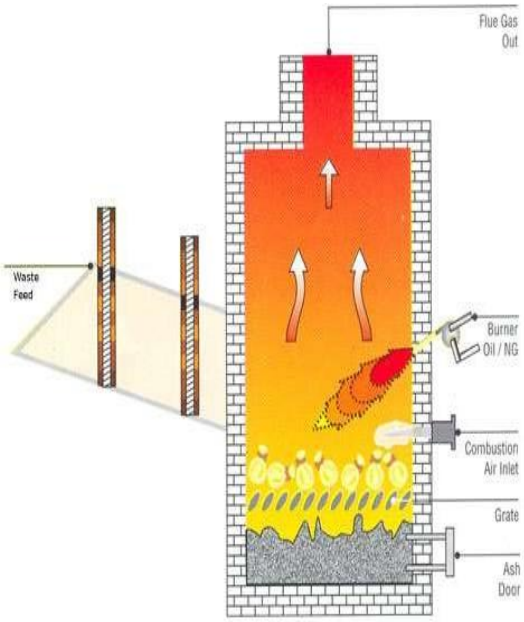
- Materials are held in the flame of a bunsen burner till they become red hot.
- Inoculating wires or loops
- Tips of forceps
- Needles

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

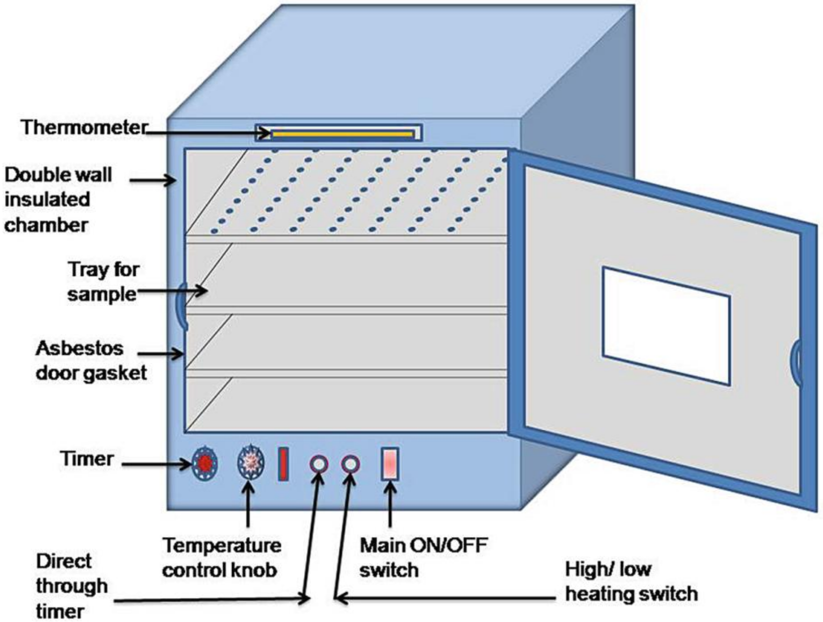


Hot Air Oven:

- It's an electrical device which use dry heat to sterilize. Most widely used method.
- Electrically heated and fitted with a fan to even distribution of air in the chamber.
- Fitted with a thermostat that maintains the chamber air at a chosen temperature.

Temperature and time:

- 160° C for 2 hours.
- 170° C for 1 hour
- 180° C for 30 minutes.



Uses of Hot air oven:

- Glassware like glass syringes, petridishes, pipettes and test tubes.
- Surgical instruments like scalpels, scissors, forceps etc.
- Chemicals like liquid paraffin, fats etc.

Glass petriplates

Scalpels

Forceps, scissors, surgical instruments

Liquid paraffin

Glassware kept in Hot Air Oven

Precautions:

- Should not be overloaded
- Arranged in a manner which allows free circulation of air
- Material to be sterilized should be perfectly **dry**.
- Test tubes, flasks etc. should be fitted with **cotton plugs**.
- Petridishes and pipettes should be wrapped in paper.
- **Rubber materials** and inflammable materials should not be kept inside.
- The oven must be allowed to cool for two hours before opening, since glass ware may crack by sudden cooling.

Disadvantages:

- Less reliable than autoclaving
- Large temperature difference may arise within device.
- Sharp instruments get dulled
- Many materials do not tolerate dry heat

Moist Heat Sterilization:

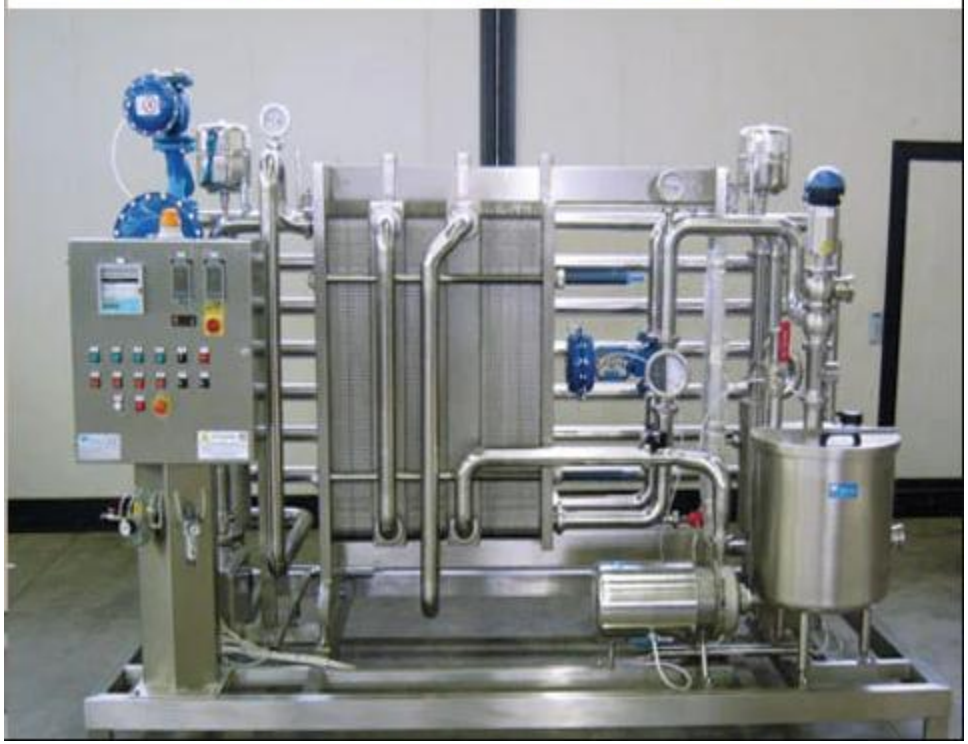
Sterilizing below 100°C:

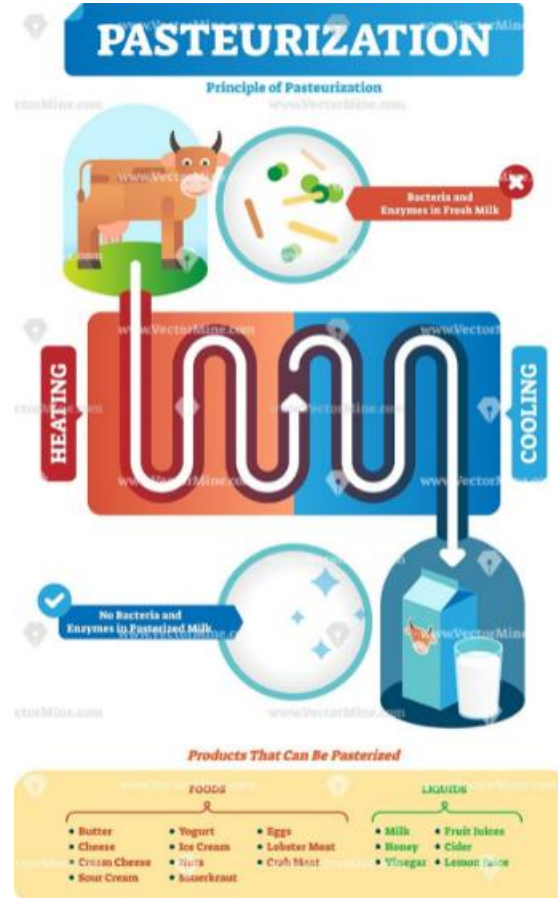
- Pasteurization
- Inspissation
- Vaccine bath

Pasteurization:

- It is a process of treating food with mild heat, which is less than 100°C , to eliminate harmful bacteria & extend shelf life.
- 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 is exposed to 65°C for 30 minutes.
- High Temperature Short Time Pasteurization (HTST): Used today.
- Milk is exposed to 72°C for 15 seconds.

Milk Pasteurization





Inspissation:

- It’s a process of heating an article at 80-85°C for 30 mins for 3 consecutive days by a special instrument called inspissator.
- It is also called fractional sterilization.
- Serum or egg media are sterilized.
- **Principle:** first heating kills the vegetative forms, and in the intervals between heating the spores germinate into vegetative forms which are then killed on subsequent heating

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.

Inspissator

Vaccine bath

Temperature at 100°C

- Boiling
- Tyndallization
- Steam sterilization

Boiling:

- Boiling for 10 – 30 minutes may kill most of vegetative forms but spores withstand boiling.

Tyndallization:

- Steam at 100°C for 20 minutes on three successive days
- Used for egg , serum and sugar containing media.

Steam sterilizer:

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

Temperatures above 100°C

- **Autoclave:**
- Steam above 100°C has a better killing power than dry heat.
- Bacteria are more susceptible to moist heat.

Sterilization conditions:

- Temperature: 121 °C
- Chamber pressure: 15 lbs per square inch.
- Holding time: 15 minutes
- Others :
- 126°C for 10 minutes
- 133°C for 3 mins

Moist heat:

Autoclaving – Standard sterilization method in hospitals.

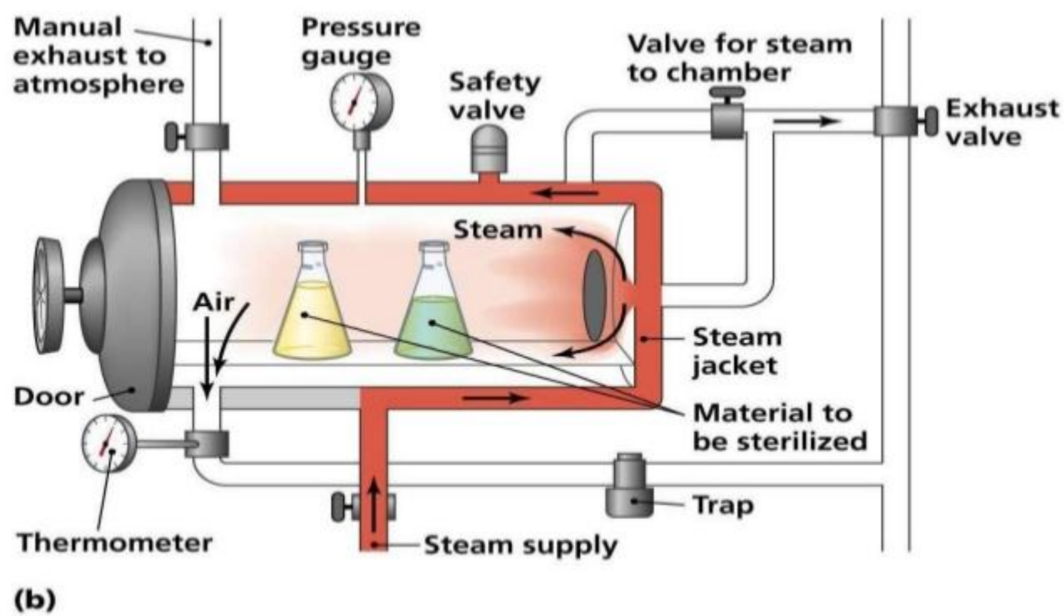
- The Autoclave works under the same principle as the pressure cooker where water boils at increased atmospheric pressure *i.e.* because of increased pressure the boiling point of water is >100°C.

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

Components of autoclave:

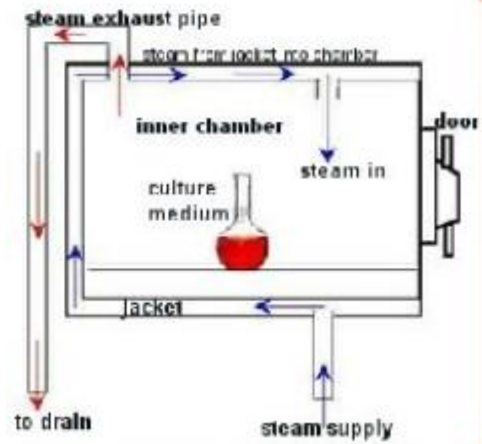
- Consists of vertical or horizontal cylinder of gunmetal or stainless steel.
- Lid is fastened by screw clamps and rendered air tight by an asbestos washer.
- Lid bears a discharge tap for air and steam, a pressure gauge and a safety valve.

Autoclave



Autoclave: working principle

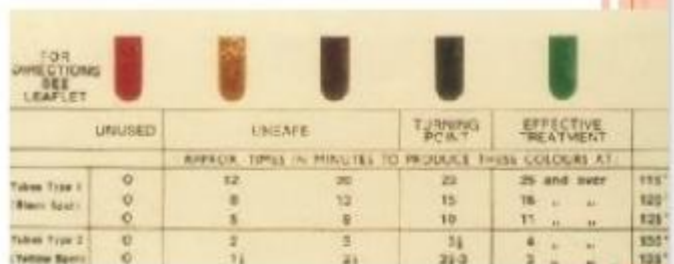
- The air in the chamber is evacuated and filled with saturated steam. The chamber is closed tightly the steam keeps on filling into it and the pressure gradually increases.
- The items to be sterilized get completely surrounded by saturated steam (moist heat) which on contact with the surface of material 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.



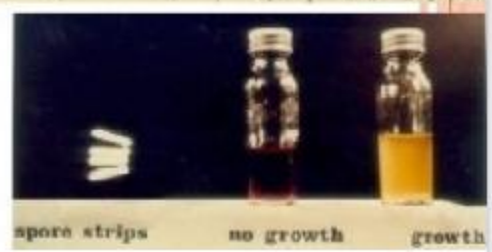
Monitoring of autoclaves

- 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.



Instrument Packing:

- Often instruments are packed for sterilization to be stored and handled without being contaminated.
- Packing depend on the intended shelf life after sterilization.
- The available packing options are:
- Textile has shelf life of 1 month
- Paper has shelf life of 1 – 6 months
- Nylon, glass, and metal have shelf life of 1 year if tightly closed.

Uses of Autoclave:

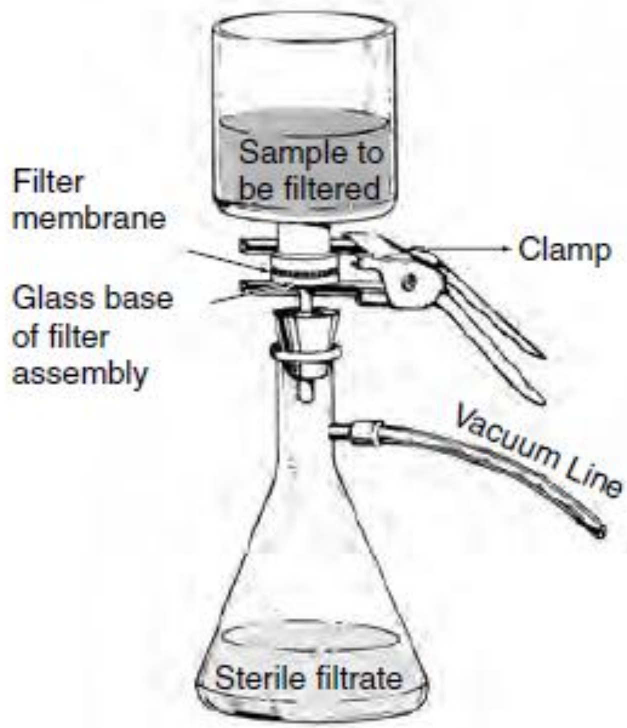
- Useful for materials which can not withstand high temp.
- To sterilize culture media, rubber material, gowns, dressings, gloves etc.

Filtration sterilization:

- It is used to reduce the microbial population in air and in **heat-sensitive** solutions.
- Ex. some culture media, oils, enzymes, vaccines, antibiotic solutions and other pharmaceuticals.
- Such solutions are passed through **porous filters** enough to <u>retain</u> microorganisms.

Membrane filter sterilization of liquids:

- The solution is forced through the filter with a vacuum or with pressure from a syringe peristaltic pump and collected in previously sterilized containers
- Filters used to remove microorganisms are composed of **cellulose acetate**, cellulose nitrate, polycarbonate, teflon or other suitable synthetic materials.
- These filters are only 0.1 mm thick and may consist of **variable pore size**.



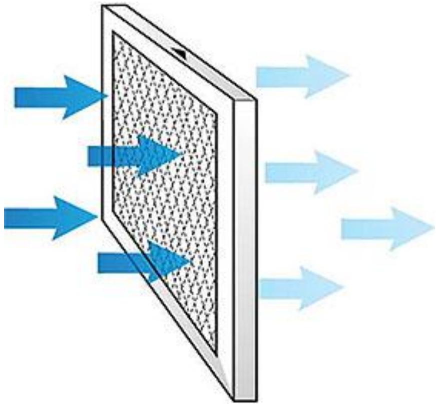
(a)

Pore size	Microbes/Particles retained
0.01 µm	All viruses
0.2 µm	All bacteria
0.45 µm	All coliforms
0.8 µm	Fungal cells, air borne particles
1.2 µm	All non-living particles
5.0 µm	All significant cells from body fluids for analysis

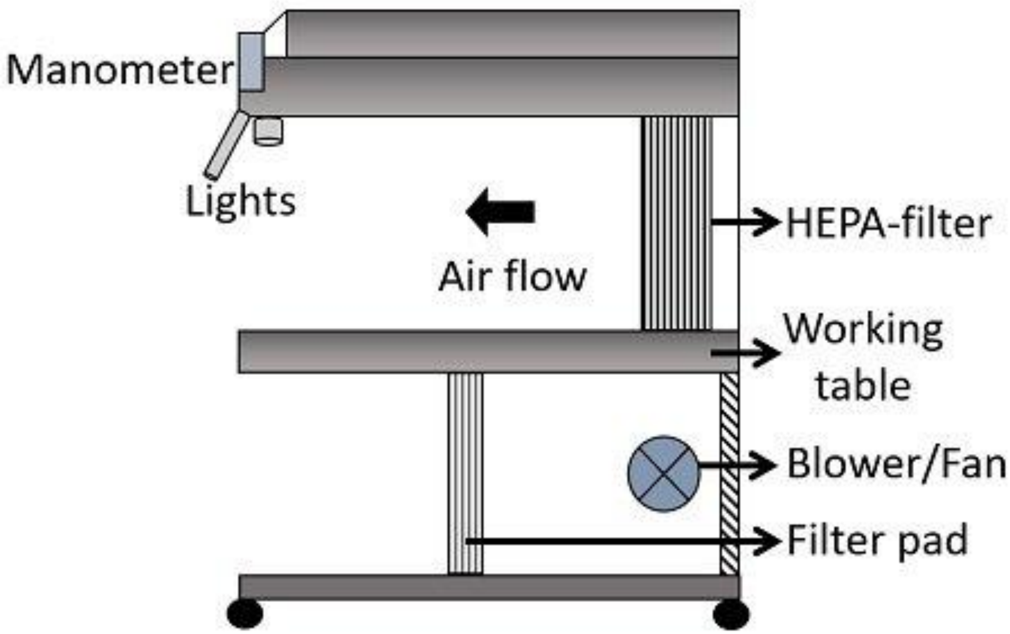
(b)

HEPA filter sterilization of air:

- Air filtration is frequently done to reduce microbial contaminants, especially in the hospital operating rooms and microbiology laboratories.
- The air of such places is sterilized by passing through **high-efficiency particulate air (HEPA) filters.**
- It removes 99.97% of microorganisms larger than about 0.3 m in diameter.
- HEPA filters are one of the most important air filtration systems.
- In microbiology laboratories and industries, the air in laminar flow hoods/laminar flow biological safe cabinets is sterilized by being passed through HEPA filters.
- This protects a worker from microorganisms being handled within the cabinet and prevents room contamination.

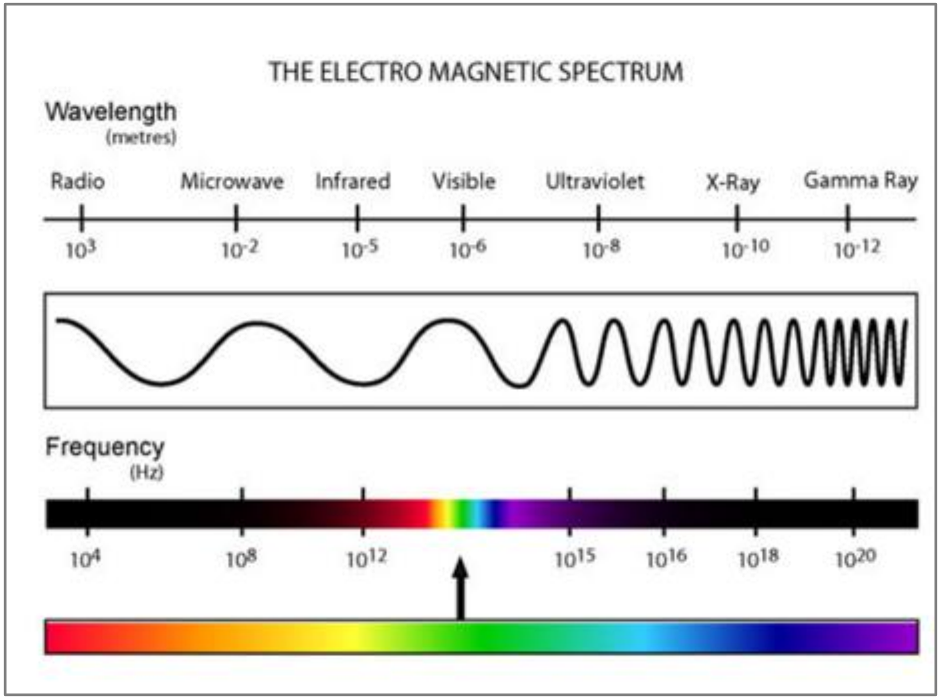


Horizontal Laminar Flow Cabinet



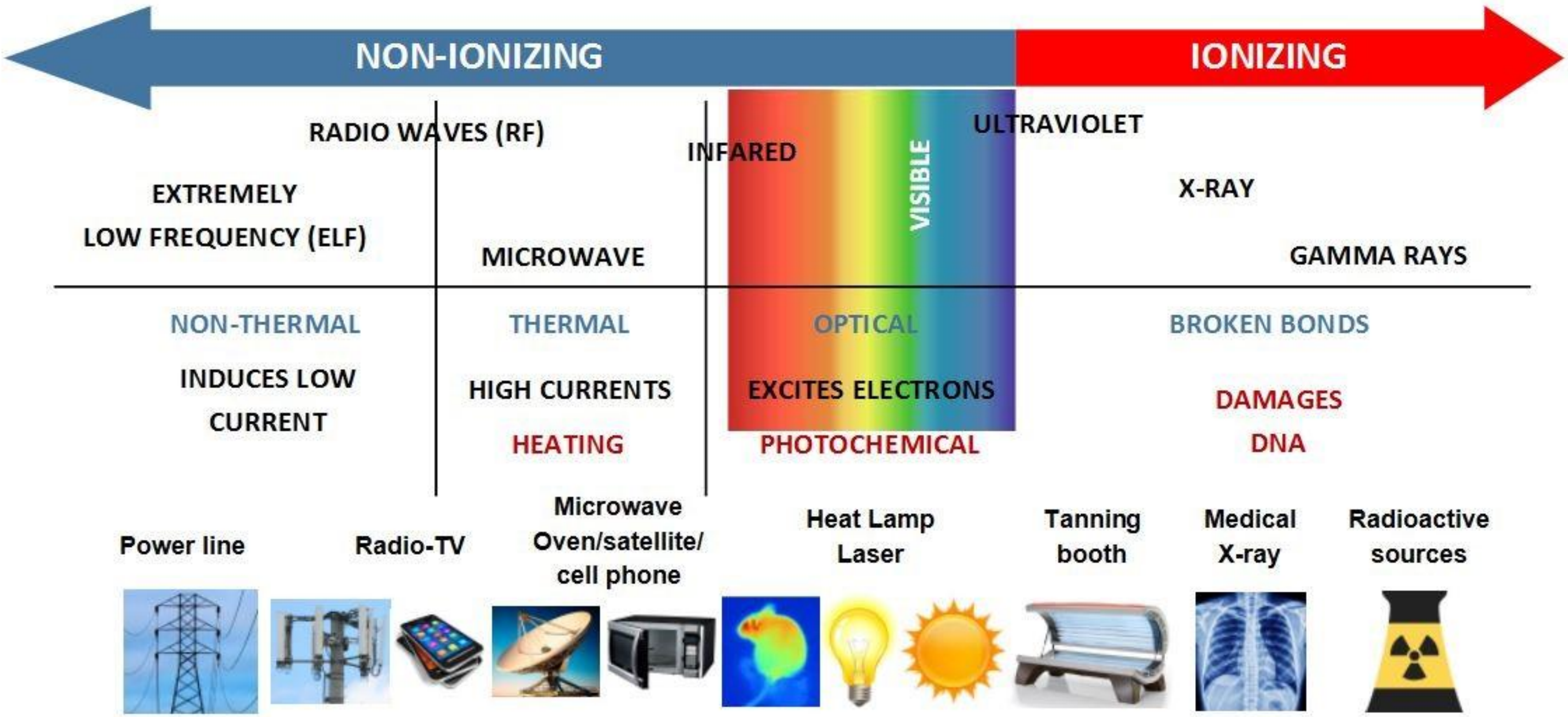
Radiation

- Electromagnetic radiation are of various types.
- As the **wavelength** of electromagnetic radiation **decreases**, the **energy** of the radiation **increases**.
- All microorganisms including viruses are sensitive to exposure to electromagnetic radiation.
- The effects of radiation on cells is dependent on its wavelength, intensity and duration.



Radiations are of two types:

- Ionizing radiation
- Non – ionizing radiation


NON-IONIZING
IONIZING
RADIO WAVES (RF)
INFARED
VISIBLE
ULTRAVIOLET
EXTREMELY
LOW FREQUENCY (ELF)
MICROWAVE
X-RAY
GAMMA RAYS
NON-THERMAL
THERMAL
OPTICAL
BROKEN BONDS
INDUCES LOW
CURRENT
HIGH CURRENTS
EXCITES ELECTRONS
DAMAGES
DNA
HEATING
PHOTOCHEMICAL
Power line
Radio-TV
Microwave
Oven/satellite/
cell phone
Heat Lamp
Laser
Tanning
booth
Medical
X-ray
Radioactive
sources

Ionizing radiation:

- It is an excellent sterilizing agent and **penetrates deep** into the object.
- Ex. **Gamma rays** and **X – rays**.
- Have shorter wavelength <**1nm**.
- These high-energy, short-wavelength forms of radiation are able to **kill** microorganisms within a sample, by **ionization of water**.

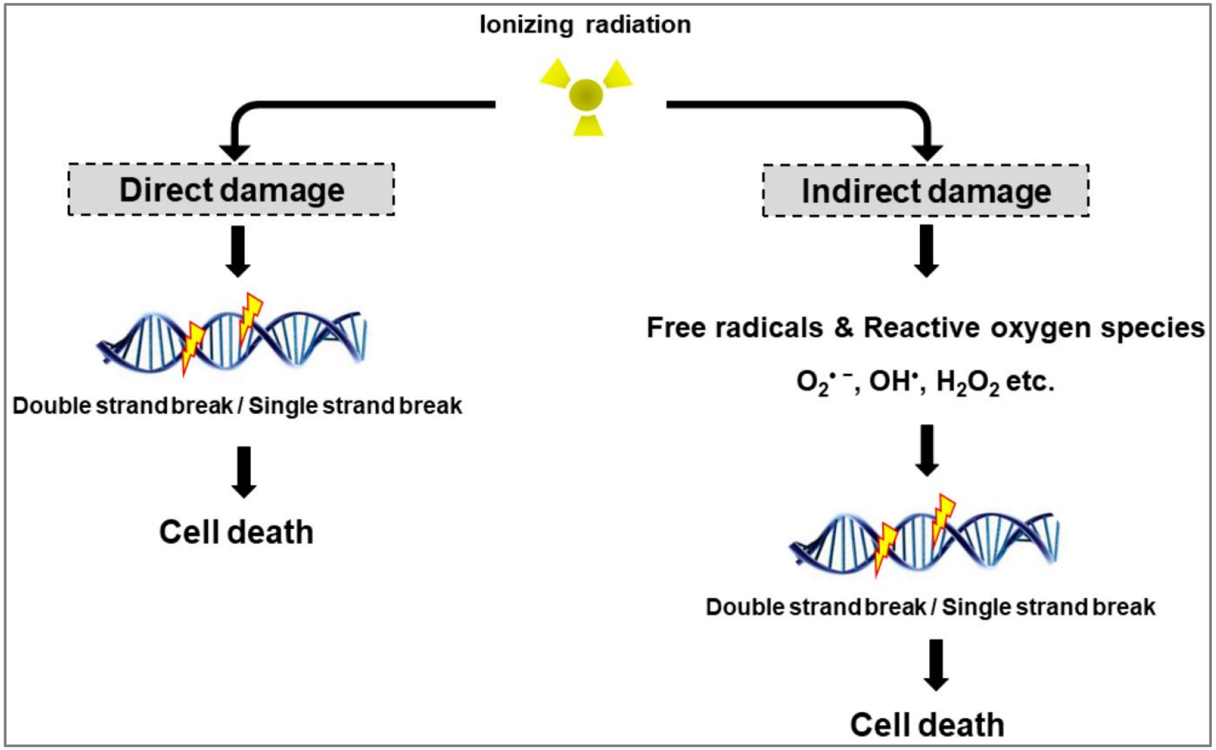
- Ionized water interacts with non-ionized water to produce a **hydroxyl radical** (**OH**):

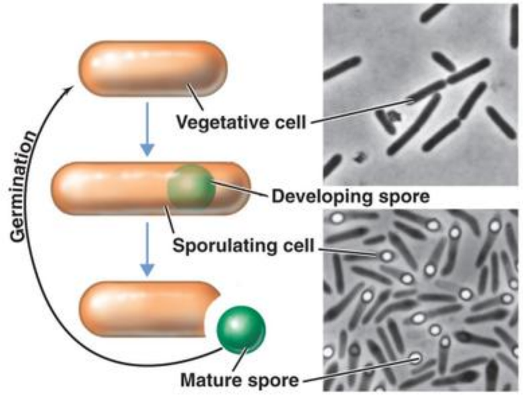
$$H_2O \xrightarrow{\text{Radiation}} H_2O^+ + e^-$$

$$H_2O^+ + H_2O \longrightarrow OH^- + H_3O$$

- The hydroxyl radical is a strong **oxidizing agent** that reacts with **DNA** (or RNA in some viruses), causing **breaks** in the **nucleotide chain**.

- All the microorganisms vary in their sensitivity towards ionizing radiation
- Bacterial **endospores** are more **resistant** than the vegetative cells.





Bacteria: Vegetative cell & Endospores

Ionization radiation Uses:

- **Ionizing radiation** is increasingly used for the sterilization of **pharmaceuticals** and disposable dental, medical and microbiology laboratory supplies such as petri-plates, plastic syringes, surgical gloves, suturing materials and catheters.

- Exposure to ionizing radiation can also be used for food preservation.

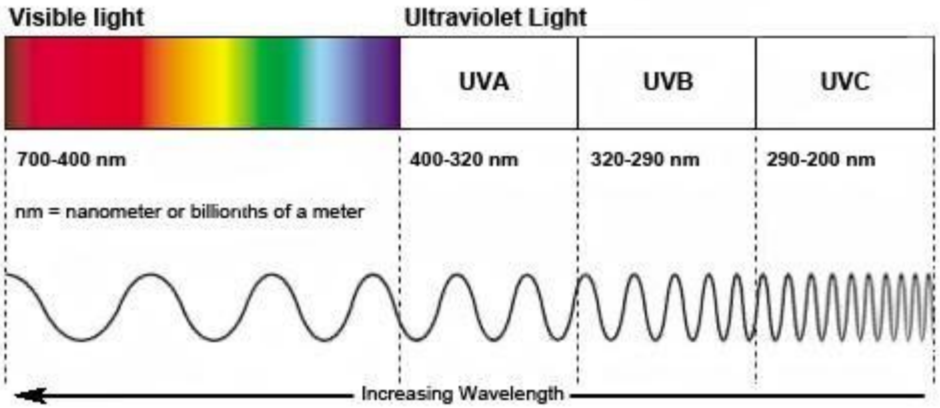


Medical & microbiology lab supplies

Non-ionizing radiation:

- It has a wavelength **longer** than that of ionizing radiation, usually >**1 nm**.
- Ex: **Ultraviolet** (UV) radiation.
- Wavelengths of **260 nm** are absorbed by the purine and pyrimidine components of **nucleic acids**, as well as certain aromatic amino acids in **proteins**.
- The absorbed energy causes a **rupture** of the **chemical bonds**, so that normal cellular function of the exposed cells is impaired.

UV radiation



Non-ionizing radiation uses:

- UV light is also sometimes used to sterilize vaccines, serum and **drinking water**.

- Commercial UV units are available for water treatment in which pathogens and other microorganisms are destroyed when a **thin layer** of water is passed under the lamps.

Disadvantages:

- UV radiation has very poor penetrating power and does not penetrate glass, dirt films, water and other substances very effectively.

- UV light can damage the eyes and prolonged exposures can even cause skin burns and **cancer**.

Chemical Sterilization

- Chemical Sterilization is the process of removal of microorganisms by the use of chemical bactericidal agents.
- Physical methods of sterilization is not suitable for heat-sensitive materials like plastics, fiber optics, and biological specimens.

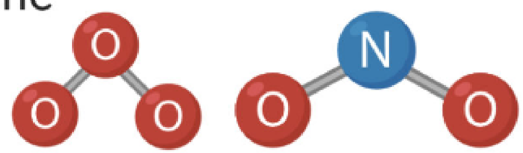
- Under such conditions, chemical either in liquid or gaseous state can be used for sterilization.
- It is crucial to ensure that the materials undergoing sterilization are compatible with the chemical being used.

- Besides, it is important to adopt safety rules in the workplace safety during the use of chemical agents.
- The chemical method of sterilization can be categorized as **liquid** and **gaseous sterilization**.

Chemical Sterilization

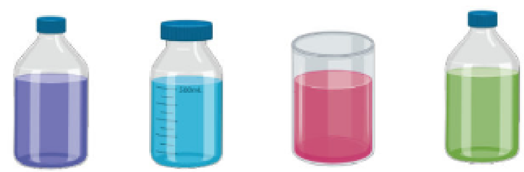
Gaseous Sterilization

- Ethylene oxide
- Formaldehyde
- Nitrogen dioxide (NO_2)
- Ozone



Liquid Sterilization

- Hydrogen peroxide
- Glutaraldehyde
- Hypochlorite



Alcohols
Aldehydes
Phenolics
Oxidizing agents
Quaternary ammonium compounds

1. Gaseous Sterilization

- Gaseous sterilization involves the process of exposing equipment or devices to different gases in a closed **heated** or **pressurized chamber**.

- Gaseous sterilization is a more effective technique as gases can pass through a **tiny orifice** and provide more effective results.

- Besides, gases are commonly used along with heat treatment which also facilitates the functioning of the gases.

- However, there is an issue of release of some toxic gases during the process which needs to be removed regularly from the system.

- The mechanism of action is different for different types of gases.

Ethylene oxide:

- It is a common gas used to sterilize different types of equipment and surfaces because of its wide range of compatibility with different materials.

- The mechanism of antimicrobial action of this gas is assumed to be through the **alkylation** of sulphydryl, amino, hydroxyl, and carboxyl groups on **proteins** and imino groups of **nucleic acids**.

- Ethylene oxide kills all known microorganisms, such as bacteria (including spores), viruses, and fungi (including yeasts and molds), and is compatible with almost all materials even when repeatedly applied.

Disadvantages:

- As the level of gas in the sterilizer goes on decreasing due to absorption, and the treated articles need to undergo a process of desorption to remove the toxic residual wastes.

Formaldehyde

- Formaldehyde is another important highly reactive gas which is used for sterilization.

- This gas is obtained by heating formalin (37%w/v) to a temperature of 70-80°C.

- It possesses broad-spectrum **biocidal** activity and has found application in the sterilization of **reusable** surgical instruments, specific medical, diagnostic and electrical equipment, and the **surface sterilization** of powders.

- Formaldehyde doesn’t have the same penetrating power of ethylene oxide but works on the same principle of modification of **protein** and **nucleic acid**.

Nitrogen dioxide (NO_2)

- Nitrogen dioxide is a rapid and effective sterilant that can be used for the removal of common bacteria, fungi, and even spores.

- NO_2 has a low boiling point (20°C) which allows a **high vapor pressure** at standard temperature.
- This property of NO_2 enables the use of the gas at standard temperature and pressure.

- The biocidal action of this gas involves the **degradation** of **DNA** by the nitration of phosphate backbone, which results in lethal effects on the exposed organism as it absorbs NO_2.

- An advantage of this gas is that **no condensation** of the gas occurs on the surface of the devices because of the low level of gas used and the high vapor pressure.
- This avoids the need for direct aeration after the process of sterilization.

Ozone

- Ozone is a highly reactive industrial gas that is commonly used to sterilize **air** and **water** and as a disinfectant for **surfaces**.

- Ozone has a potent **oxidizing** property that is capable of destroying a wide range of organisms including prions, without the use of hazardous chemicals as ozone is usually generated from medical-grade oxygen.

- Similarly, the high reactivity of ozone allows the removal of waste ozone by converting the ozone into oxygen by passing it through a simple catalyst.

- However, because ozone is an unstable and reactive gas, it has to be produced on-site, which limits the use of ozone in different settings.

- It is also very hazardous and thus only be used at a concentration of **5ppm**, which is 160 times less than that of ethylene oxide.

2. Liquid Sterilization

- Liquid sterilization is the process of sterilization which involves the **submerging** of equipment in the liquid sterilant to kill all viable microorganisms and their spores.

- Although liquid sterilization is not as effective as gaseous sterilization, it is appropriate in conditions where a **low** level of **contamination** is present.

Chemical agents used are:

- Alcohols
- Aldehydes
- Dyes
- Halogens
- Phenolic compounds
- Quaternary ammonium compounds

Alcohols

- **Ethyl alcohol**, isopropyl alcohol, and n-propanol exhibit rapid, **broad spectrum** antimicrobial activity against vegetative bacteria, viruses, and fungi but are not sporicidal.
- Activity is **optimal** when they are diluted to a concentration of **60–90%** with water.
- **Protein** slows its action whereas 1% mineral acid or alkali enhances the action.

Aldehydes

- **Formaldehyde** is bactericidal, sporicidal, and virucidal.
- It is active against the amino group in the **protein** molecule.
- Formaldehyde gas is used for sterilizing instruments and heat sensitive catheters and for fumigating wards, sick rooms and laboratories.

- **Glutaraldehyde** is used for low-temperature disinfection and sterilization of endoscopes and surgical equipment.
- It is normally used as a **2% solution** to achieve **sporicidal** activity.

Dyes

Two groups of dyes are there:

1. Aniline dyes

- Ex: brilliant green, malachite green, crystal violet

2. Acridine dyes

- Ex: proflavine, acriflavine, euflavine and aminacrine.
- They are used as skin and wound **antiseptics**.

- They are more active against **Gram positive** organisms than Gram negative organisms.

Halogens

- **Iodine** is an effective disinfectant that acts by iodinating or oxidizing essential components of the microbial cell.

- Iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal.

- 2% iodine in 50% alcohol (tincture), which kills more rapidly and effectively than alcohol alone.

- Iodophors (eg, povidone-iodine) are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active I_2.

- The most important types of chlorine-releasing agents are sodium hypochlorite, chlorine dioxide, and sodium dichloro-isocyanurate, which are oxidizing agents that destroy the cellular activity of proteins.

Phenolic compounds:

- The use of phenolic compounds are introduced by the Lister.
- The mode of action is due to their capacity to **disrupt** the **cell membrane**.

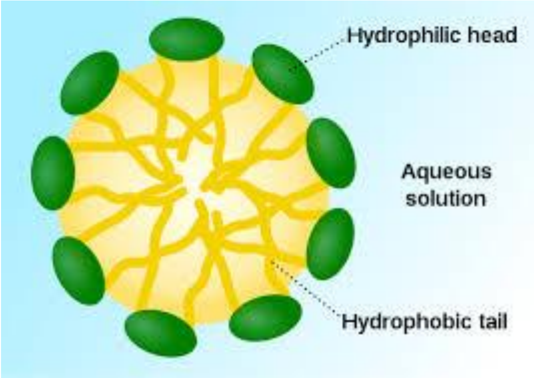
- Phenol (carbolic acid) is a potent protein denaturant and bactericidal agent.
- It is used as **disinfectants** for various hospital purposes.

- **Cresol** and Lysol are active against a wide range of organisms but they are toxic and corrosive.
- They are used to disinfect surgical instruments, contaminated materials and floors.

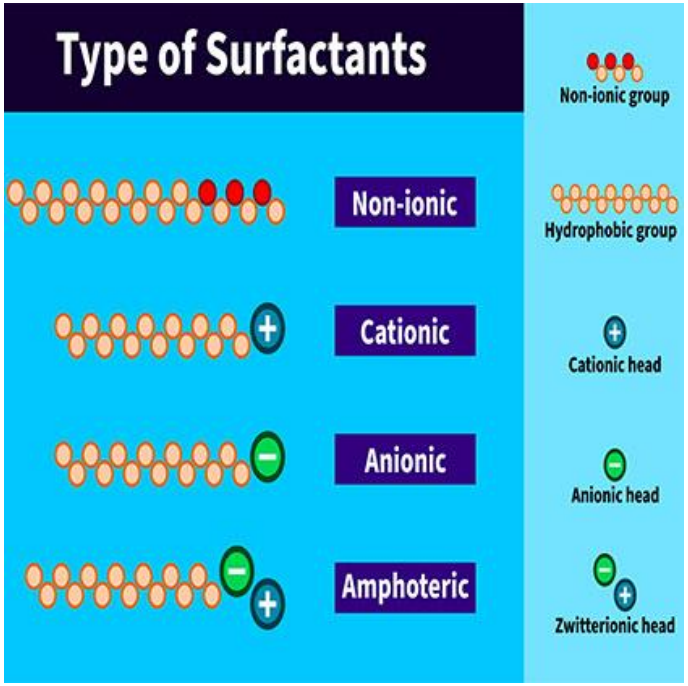
- Hexachlorophene is toxic and should be handled with care.
- **Cholrhexidene** is nontoxic, hence used as skin antiseptic which is more effective against Gram positive organisms than Gram negative organisms.

Surface active agents

- These compounds have **two regions** in their molecular structures:
  - 1. Water-repelling (**hydrophobic**) group and
  - 2. water-attracting (**hydrophilic**) group.



- They are classified into four groups:
  1. Anionic (- ve charge)
  2. Cationic (+ ve charge)
  3. Nonionic (no charge)
  4. Amphoteric (both charges)



They are mainly used as:

- Wetting agents
- Emulsifiers and
- Detergents.

Cationic detergents:

- Cationic detergents, particularly the **quaternary ammonium compounds** (QACs) are highly **bactericidal**.
- Ex: Benzalkonium chloride, acetyl trimethyl ammonium bromide.
- They are effective against **Gram +ve** organisms than Gram –ve organisms.
- They are more active in **alkaline pH**.
- They are inactive against spores and viruses, tubercle bacilli.

- **Anionic detergents:** Ex: common **soap.**

- **Amphoteric** or ampholytic compounds or tego compounds are active against **Gram+ve** organisms than Gram –ve organisms.

Metallic salts

- Salts of **mercury**, copper and silver are used as disinfectant.

- Mercuric chloride is **highly toxic**.
- Hence organic compounds such as:
  - mercurochrom
  - phenyl mercury nitrate and
  - thiomersal
- are used as **antiseptics** (less toxic).

- Copper salts are used as **fungicides**.

- Silver salts in aqueous solution have a limited use.

Hydrogen peroxide

- Hydrogen peroxide is a liquid chemical sterilizing agent which is a **strong oxidant** and can destroy a wide range of microorganisms.

- It is useful in the sterilization of heat or temperature-sensitive equipment like endoscopes. In medical applications, a higher concentration (**35-90%**) is used.

- H_2O_2 has a short sterilization cycle time as these cycles are as short as **28 mins** where ethylene oxide has cycles that as long as 10-12 hours.

- Vaporized hydrogen peroxide (VHP) is used to sterilize largely enclosed and sealed areas, such as entire rooms and aircraft interiors.

Drawbacks:

- H_2O_2 has like low material compatibility, lower capacity of penetration, and associated health risks.

Hypochlorite

- Hypochlorite solution, which is also called **liquid bleach**, is another liquid chemical that can be used as a **disinfectant**, even though sterilization is difficult to obtain with this chemical.

- **Submerging** devices for a short period in liquid bleach might kill some pathogenic organisms but to reach sterilization submersion for **20-24 h** is required.

- It is an **oxidizing agent** and thus acts by oxidizing organic compounds which results in the modification of **proteins** in microbes which might ultimately lead to **death**.

- Appropriate concentrations of hypochlorite can be used for the disinfection of workstations and even surfaces to clean blood spills and other liquids.