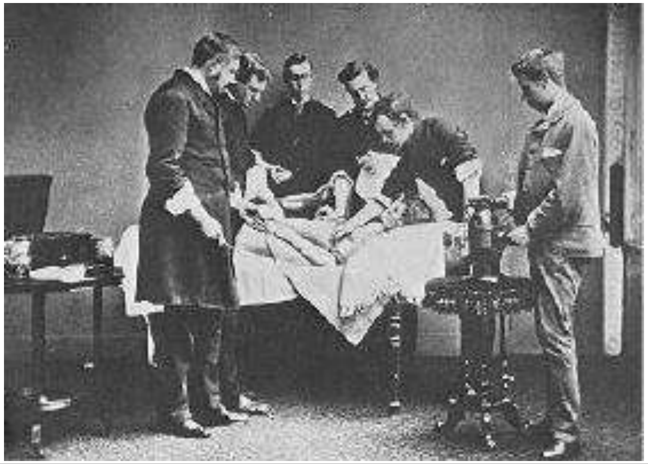
SNS COLLEGE OF PHARMACY AND HEALTH SCIENCES
Sathy Main Road, SNS Kalvi Nagar,
Saravanampatti Post, Coimbatore - 641 035,
Tamil Nadu.

Sterilization and Disinfection

- Early civilizations practiced salting, smoking, pickling, drying, and exposure of food and clothing to sunlight to control microbial growth.
- Use of spices in cooking was to mask taste of spoiled food. Some spices prevented spoilage.
- In mid 1800s Semmelweiss and Lister helped developed **aseptic techniques** to prevent contamination of surgical wounds. Before then:
  - Nosocomial infections caused death in 10% of surgeries.
  - Up to 25% mothers delivering in hospitals died due to infection



Antimicrobial Definitions

- Sterilization
  - To completely remove all kinds of microbes (bacteria, mycobacteria, viruses, & fungi) and by physical or chemical methods
  - Effective to kill "bacterium spores"
  - Sterilant: material or method used to remove or kill all microbes

Antimicrobial Definitions

- Disinfection
  - To reduce the number of pathogenic microorganisms to the point where they no longer cause diseases
  - Usually involves the removal of **vegetative** or **non-endospore forming** pathogens
  - May use physical or chemical methods
    - Disinfectant: An agent applied to inanimate objects.
    - Antiseptic: A substance applied to living tissue.
    - Degerming: Removal of most microbes in a limited area. Example: Alcohol swab on skin.
    - Sanitization: Use of chemical agent on food-handling equipment to meet public health standards and minimize chances of disease transmission. e.g.: Hot soap & water

Antimicrobial Definitions

- Bacteriostatic
  - prevents growth of bacteria
- Germicide
  - An agent that kills certain microorganisms.
    - Bactericide: An agent that kills bacteria. Most do not kill endospores.
    - Viricide: An agent that inactivates viruses.
    - Fungicide: An agent that kills fungi.
    - Sporicide: An agent that kills bacterial endospores of fungal spores.

Method of Control

- physical or chemical?
  - physical control includes heat, irradiation, filtration and mechanical removal
  - chemical control involves the use of antimicrobial chemicals
  - depends on the situation
  - degree of control required

air filters

antimicrobial chemicals

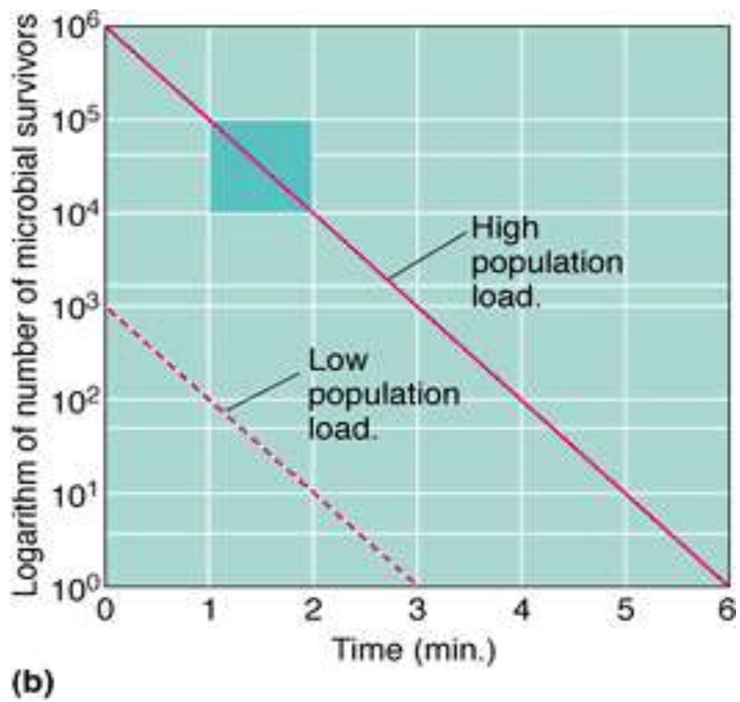
Factors influence the effectiveness of sterilization and disinfection

- **Nature of heat: Dry heat or moist heat**
- **Number of Microbes**: The more microbes present, the more time it takes to eliminate population.
- **Type of Microbes**: Endospores are very difficult to destroy. Vegetative pathogens vary widely in susceptibility to different methods of microbial control.
- **Environmental influences:** Presence of organic material (blood, feces, saliva, pH etc.) tends to inhibit antimicrobials.
- **Time of Exposure:** Chemical antimicrobials and radiation treatments are more effective at longer times. In heat treatments, longer exposure compensates for lower temperatures.

- Temperature and time:
  - Time required is inversely related to temp. of exposure
  - Thermal death point(TDT)- refers to the minimum time required to kill a suspension of organism at a predetermined temperature in a specified enviroment.

Rate of Microbial Death

When bacterial populations are heated or treated antimicrobial chemicals, they usually die at a constant rate.



Methods of Sterilization and Disinfectants

A. Physical Methods
   1. Sunlight
   2. Drying
   3. Heat
      a. Dry Heat
         i. Incineration
         ii. Red heat
         iii. Flaming
         iv. Hot air sterilizer
         v. Microwave oven
      b. Moist Heat
         i. Pateurization

        ii. Boiling

        iii. Steam under normal pressure

        iv. Steam under pressure

    2. Filtration
    3. Radiation
    4. Ultrasonic and sonic vibration

B. Chemical Methods

1. Agents that damage the cell membranes
    a. Surface active disinfectants
    b. Phenolic compounds
    c. Alcohols
2. Agents that damage proteins
    a. Acids and alkalies

3. Agents that modify functional groups of proteins and nucleic acids

a. Heavy Metals

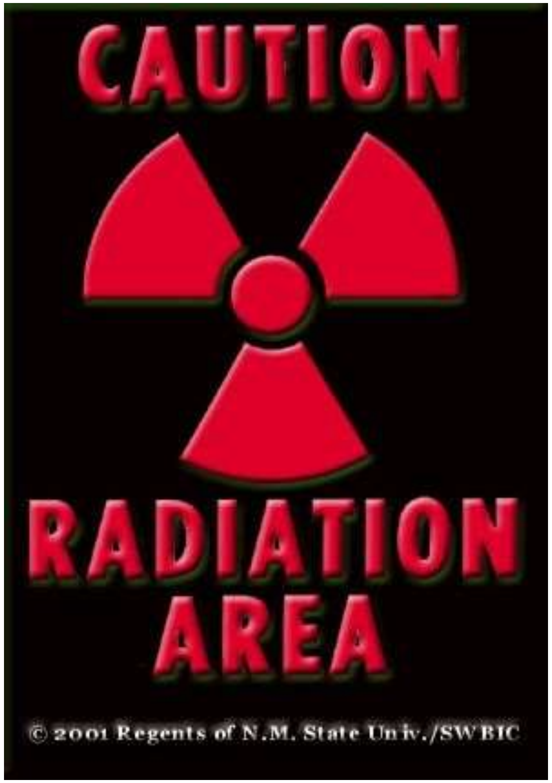
b. Oxidizing agents

c. Dyes

d. Alkalyting agents

Physical Methods of Microbial Control

- heat
- filtratio n
- r adiati on



- Sunligh
t
  - Bactericidal activity
  - Disinfectant action is due to ultraviolent rays and heat
  - Natural method of sterilization of water in tank, rivers ands lakes

- Drying
  - Water constitutes four-fifth of the weight of bacteria
  - Drying in air has deleterious effect
  - Spores are not effected

- Heat
  - Kills microorganisms by denaturing their enzymes and other proteins. Heat resistance varies widely among microbes.
  - fast, reliable, inexpensive
  - does not introduce potential toxic substances

- Types of heat
  - Dry heat
  - Moist heat

- Mechanism of Action
  - Dry Heat-kills by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes
  - Moist Heat- kills by coagulation, denaturation of enzymes and structural proteins

APPLICATION OF HEAT

- Thermal death time (TDT) is the length of time required to kill all bacteria in a liquid culture at a given temperature.
- Thermal death point (TDP) is the lowest temperature at which all bacteria in a liquid culture will be killed in 10 minutes.
- Decimal reduction time (DRT) is the length of time in which 90% of a bacterial population will be killed at a given temperature (especially useful in canning industry).

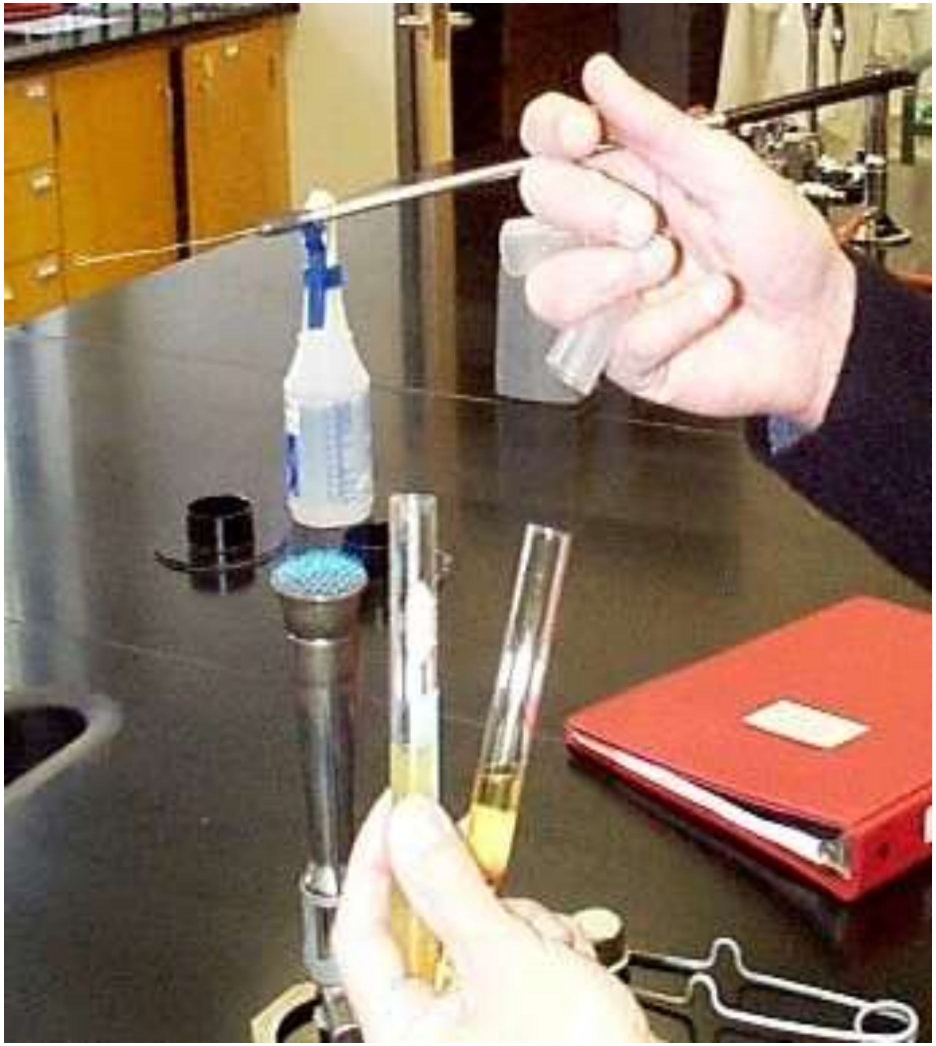
Dry heat:

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

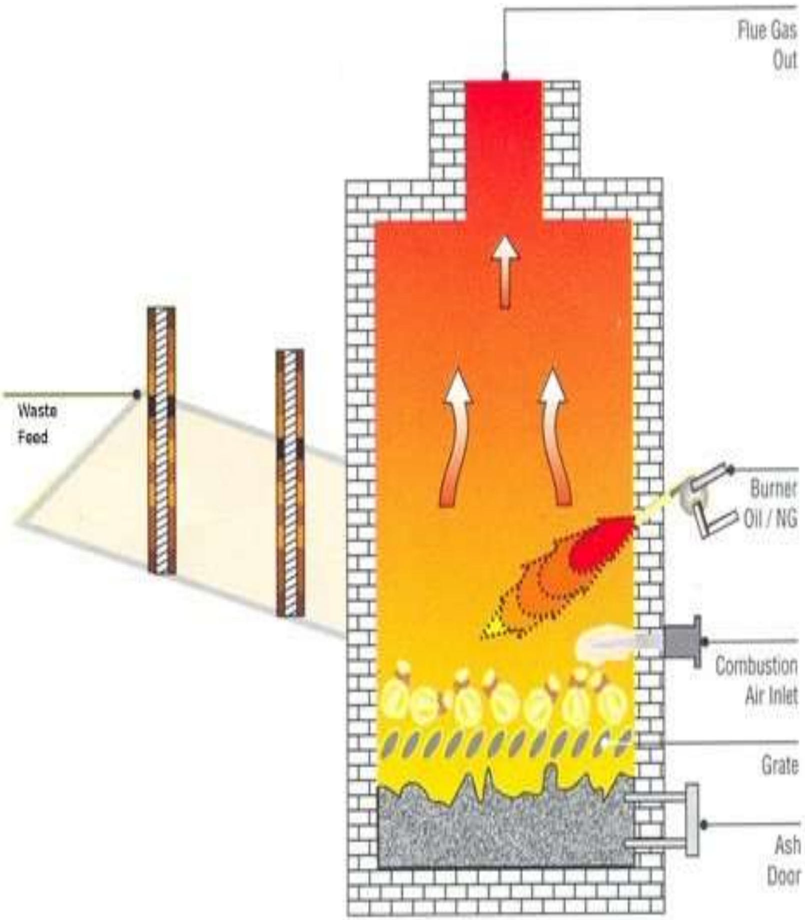
Dry heat:

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**



3. Incineration:



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

Hot air oven

- 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:
  - 160^0C for 2 hours.
  - 170^0C for 1 hour
  - 180^0C for 30 minutes.

Fig. 5.1: Hot air oven

Uses

- **Sterilization of**
  1. Glassware like glass syringes, petri dishes, pipettes and test tubes.
  2. Surgical instruments like scalpels, scissors, forceps etc.
  3. Chemicals like liquid paraffin, fats etc.

Precautions

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

Sterilisation controls

- Sterilisation controls
  1. Spores of ***Bacillus subtilis subsp. niger***
  2. Thermocouples
  3. Browne's tube

Physical Methods of Microbial Control

Moist Heat: Kills microorganisms by **coagulating** their proteins and denaturation of their enzymes and structural proteins

A. Sterilization at Temp. below 100°C

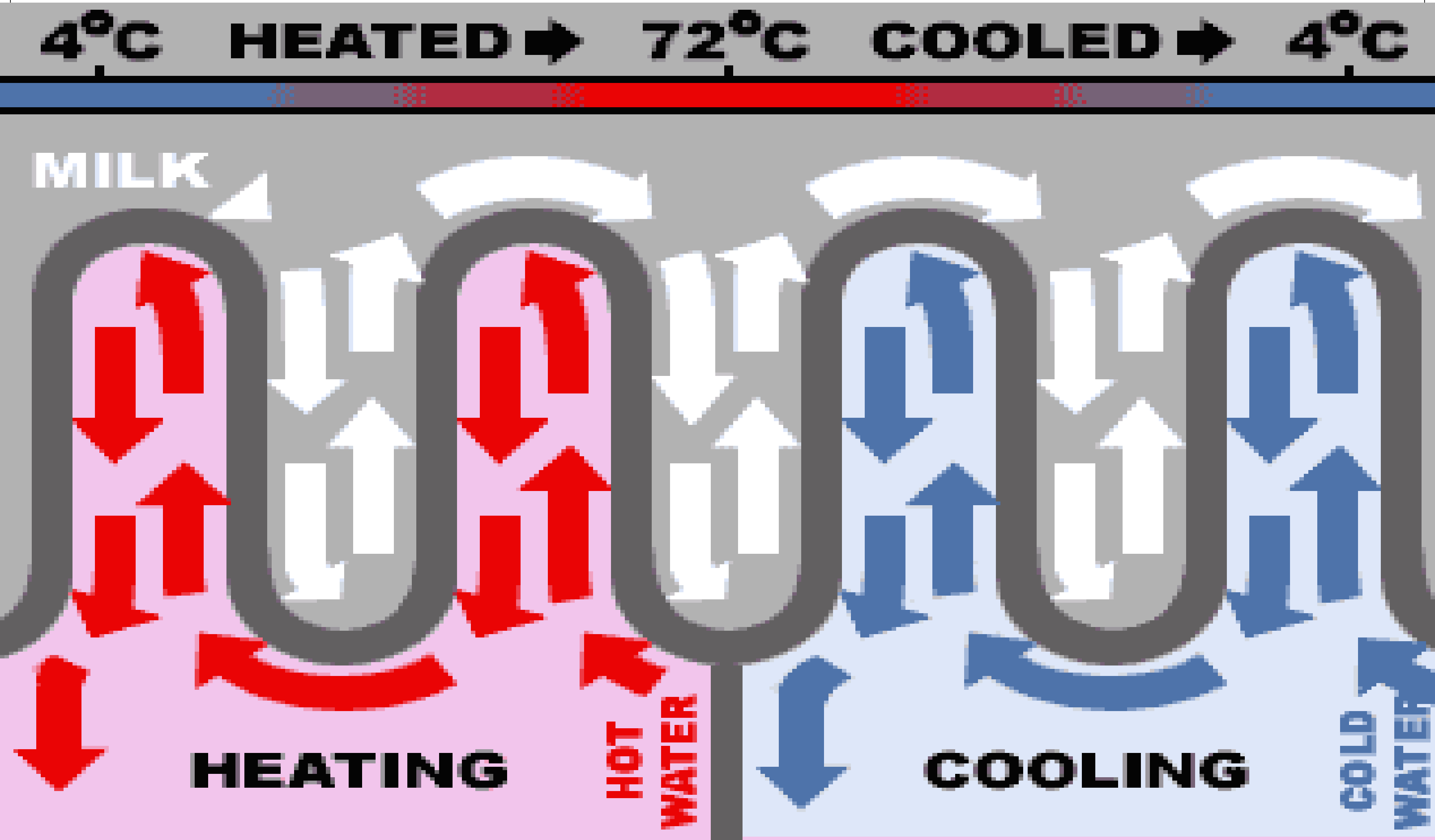
1. Pasteurization: Developed by Louis Pasteur to prevent the spoilage of beverages. Used to reduce microbes responsible for spoilage of beer, milk, wine, juices, etc.

- **Classic Method of Pasteurization**: Milk was exposed to 65°C for 30 minutes. (Holder Method)

- **High Temperature Short Time Pasteurization (HTST):** Used today. Milk is exposed to 72°C for 15 -20 seconds.(Holders method)
- Ultra High Temperature Sterilization (UHT):

  Heated to 140-150C for 1-3 seconds

Principle of Pasteurization



2. Inspissation:

- Heating at 80-85°C for half an hour daily on three consecutive days
- Lowenstein –Jensen and Loflller's Serum media are sterilised

3. Vaccine bath:

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

Waterbath

Inspissator

B . Temperature at 100°C

1 Boiling

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

Addition of 2% sodium bicarbonate may promote

Uses: For the disinfection of surgical instruments

2. Tyndallisation/intermittent sterilization :

- Steam at 100°C for 20 minutes on three successive days
- Vegetative cells and some spore are killed 1st day and more resistant spores susequently germinate and are killed during 2nd or 3rd day.
- Used for egg , serum and sugar containing media.

3. Steam sterilizer :

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

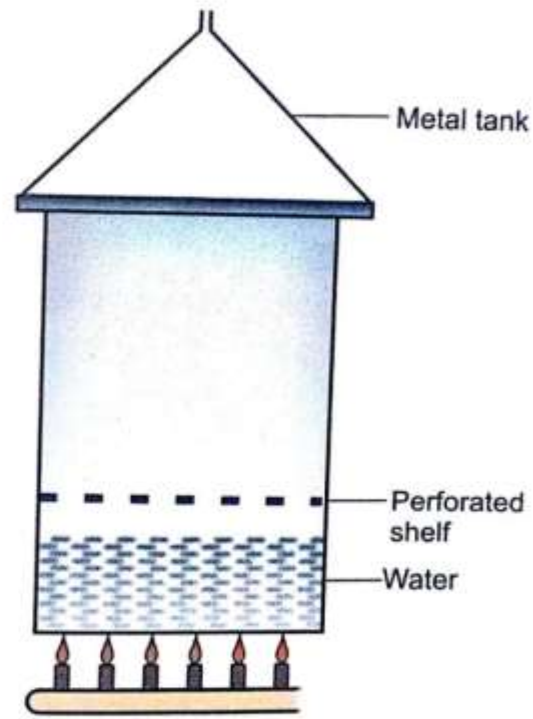


Fig. 5.2: Steamer

C. Temperatures above 100°C

temperature above 100°C

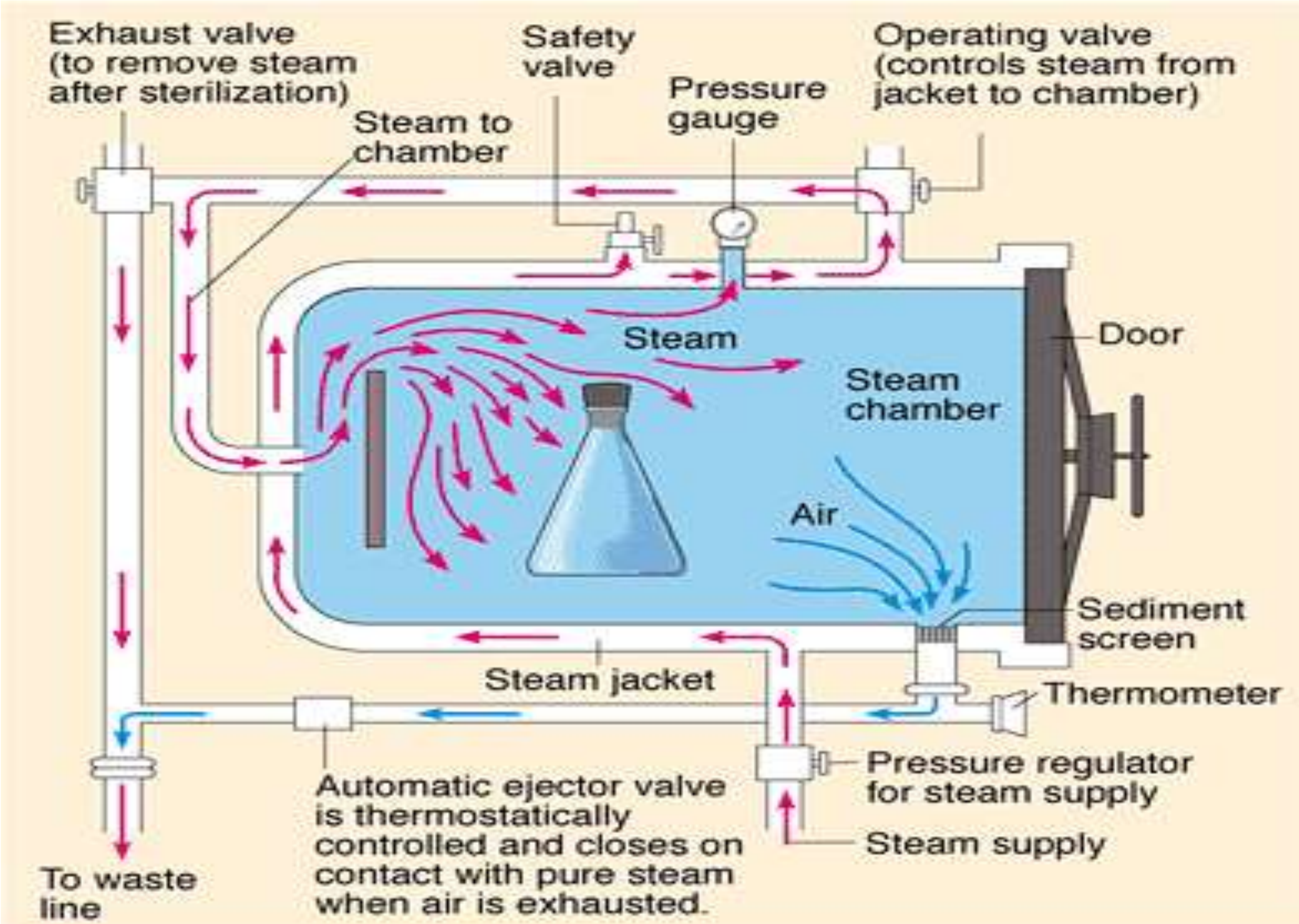
Autoclave :

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

-Bacteria are more susceptible to moist heat.

- **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.
  - Heating is done by gas or electricity.

- Principle
  - Water boils when its vapour pressure equals that of surrounding atmosphere
  - Pressure is directly proportional to temperature
  - Steam condense to water and gives up its latent heat and comes contact with cooler surface

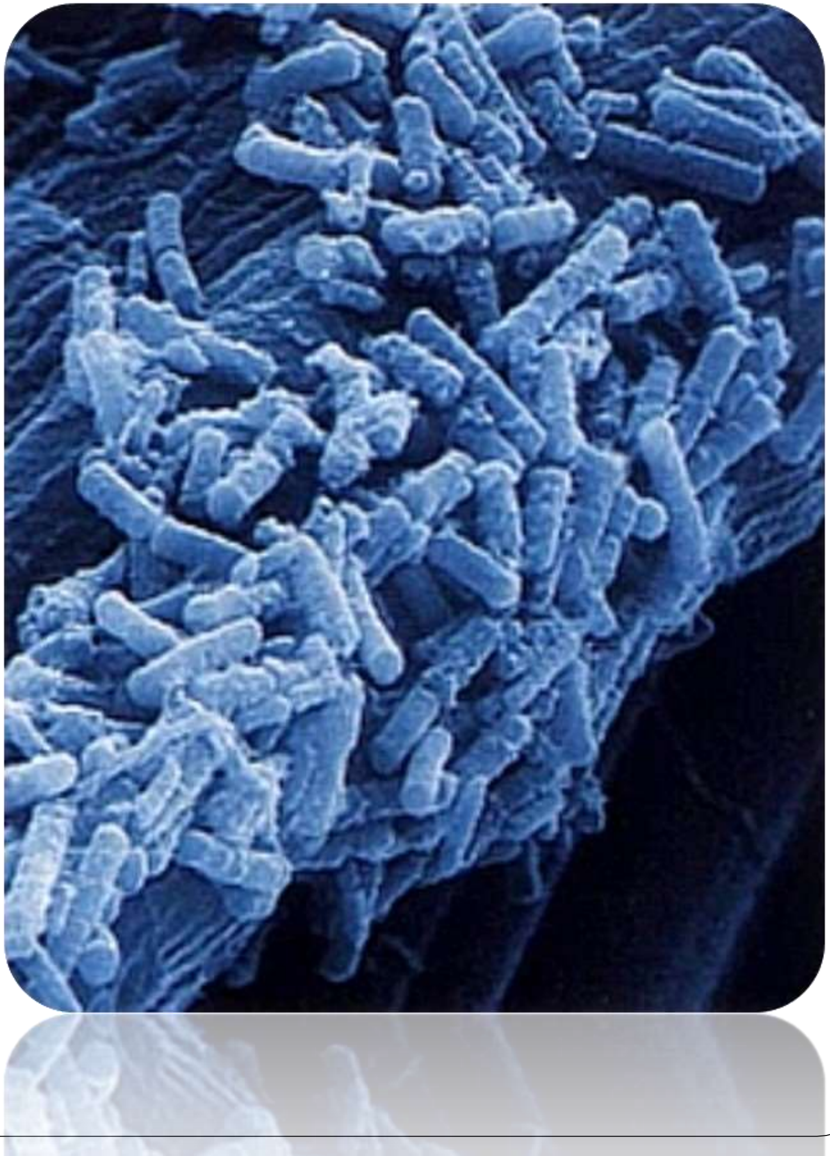

Exhaust valve (to remove steam after sterilization)
Safety valve
Pressure gauge
Operating valve (controls steam from jacket to chamber)
Steam to chamber
Steam
Door
Steam chamber
Air
Sediment screen
Steam jacket
Thermometer
Pressure regulator for steam supply
Automatic ejector valve is thermostatically controlled and closes on contact with pure steam when air is exhausted.
Steam supply
To waste line


TECHMECH
GROUP

- Sterilization conditions:
  - Temperature - at 121°C
  - Chamber pressure - 15 lb per square inch.
  - Holding time – 15 minutes
  - Others :
    - 126°C for 10 minutes
    - 133°C for 3 minutes
- Uses
  1. Useful for materials which can not withstand high temp.
  2. To sterilize culture media, rubber material, gowns, dressings, gloves etc.

Sterilisation controls:

- Sterilisation controls:
  1. Thermocouples
  2. Bacterial spores- ***Bacillus stearothermophils***
  3. Browne's tube
  4. Autoclave tapes



Filtration:

Removal of microbes by passage of a liquid or gas through a screen like material with small pores. Used to sterilize heat sensitive materials like vaccines, enzymes, antibiotics, and some culture media

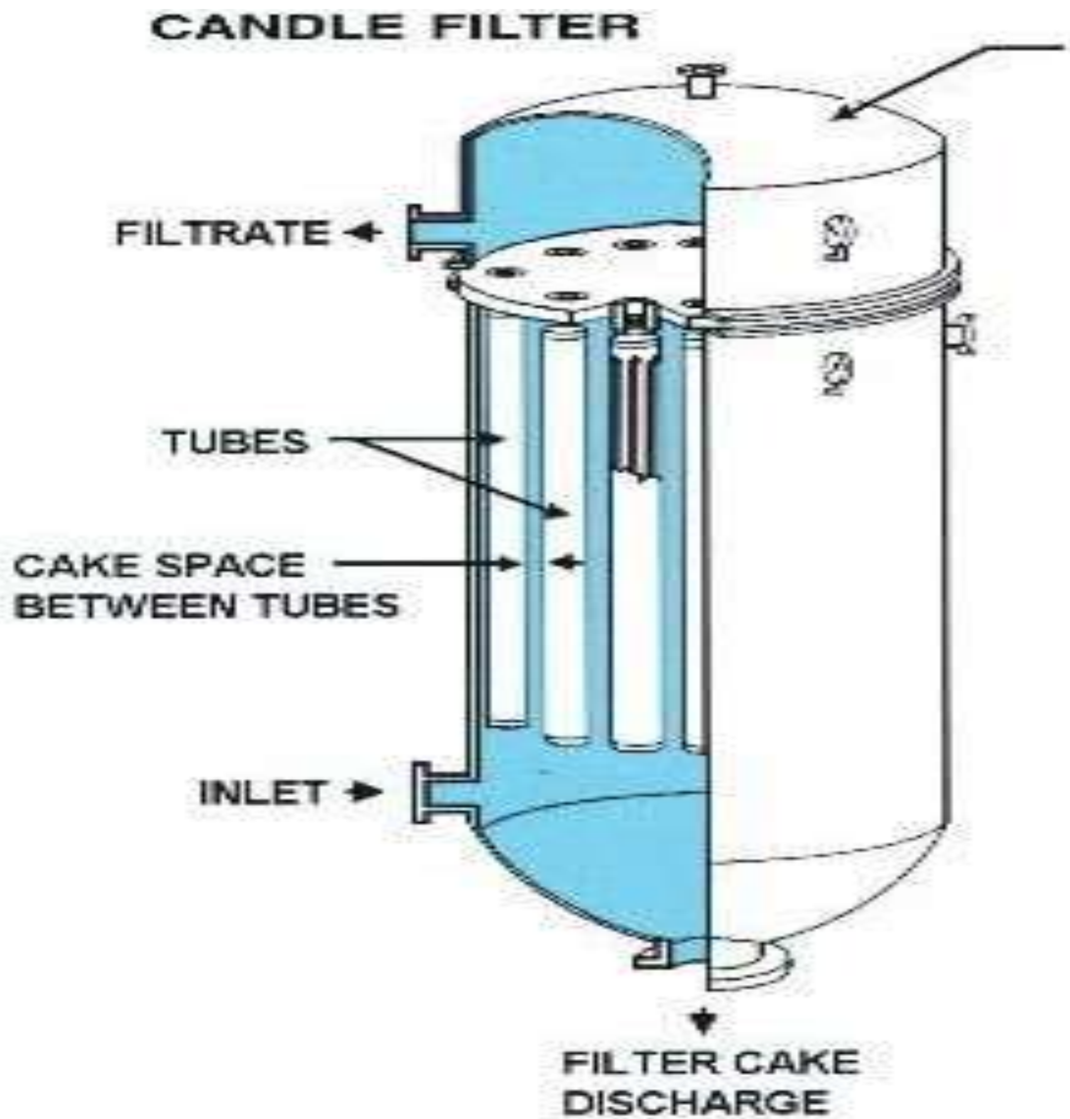
- **Application**
  - Useful for substances which get damaged by heat.
  - To sterilize sera, sugars and antibiotic solutions.
  - To obtain bacteria free filtrates of clinical samples.
  - Purification of water.

Types of Filters

- Types of filters:
  1. Earthware filters
  2. Asbestos disc filters
  3. Sintered glass filters
  4. Membrane filters
  5. Air filters
  6. Syringe filters
  7. Vacuum and in-line filters
  8. Pressure filtration
  9. Air filters

- Earthware Filters/Candle filter
  - Have different grade of porosity
  - Widley used for purification of water
  - Fluid to be sterilized is forced by suction or pressure, sterilized by scrubbing with stiff brush followed by boiling or autoclaving
  - Types

a. Unglazed ceramic filters-eg Chamberland and doulton filters
b. Compressed diatomaceous earth filters-eg Berkfeld and mandler filters


CANDLE FILTER
FILTRATE
TUBES
CAKE SPACE
BETWEEN TUBES
INLET
FILTER CAKE
DISCHARGE

- Asbestos filters/Seitz filter
  - Made up of disk of asbestos(magnesium trisilicate)
  - Fitted on to a sterile flask through silicon rubber bung
  - Fluid to be sterilized is passed through funnel
  - Eg. Seitz filter, carlson and sterimat filters

- **Sintered glass filters**
  - Prepared by size grading powdered glass followed by heating
  - Pore size can be controlled
- **Membrane filters**
  - Made up of cellulose nitrate, cellulose diacetate, polycarbonate and polyester
  - Diameter from 13 to 293 mm with pore sixe from 0.015 to 12µm
  - 0.22µm pore size is most widely used
- **Uses**
  - Water analysis and purification
  - Sterilization and sterility testing
  - For preparation sterile solution for parenteral use
  - Bacterial count of water

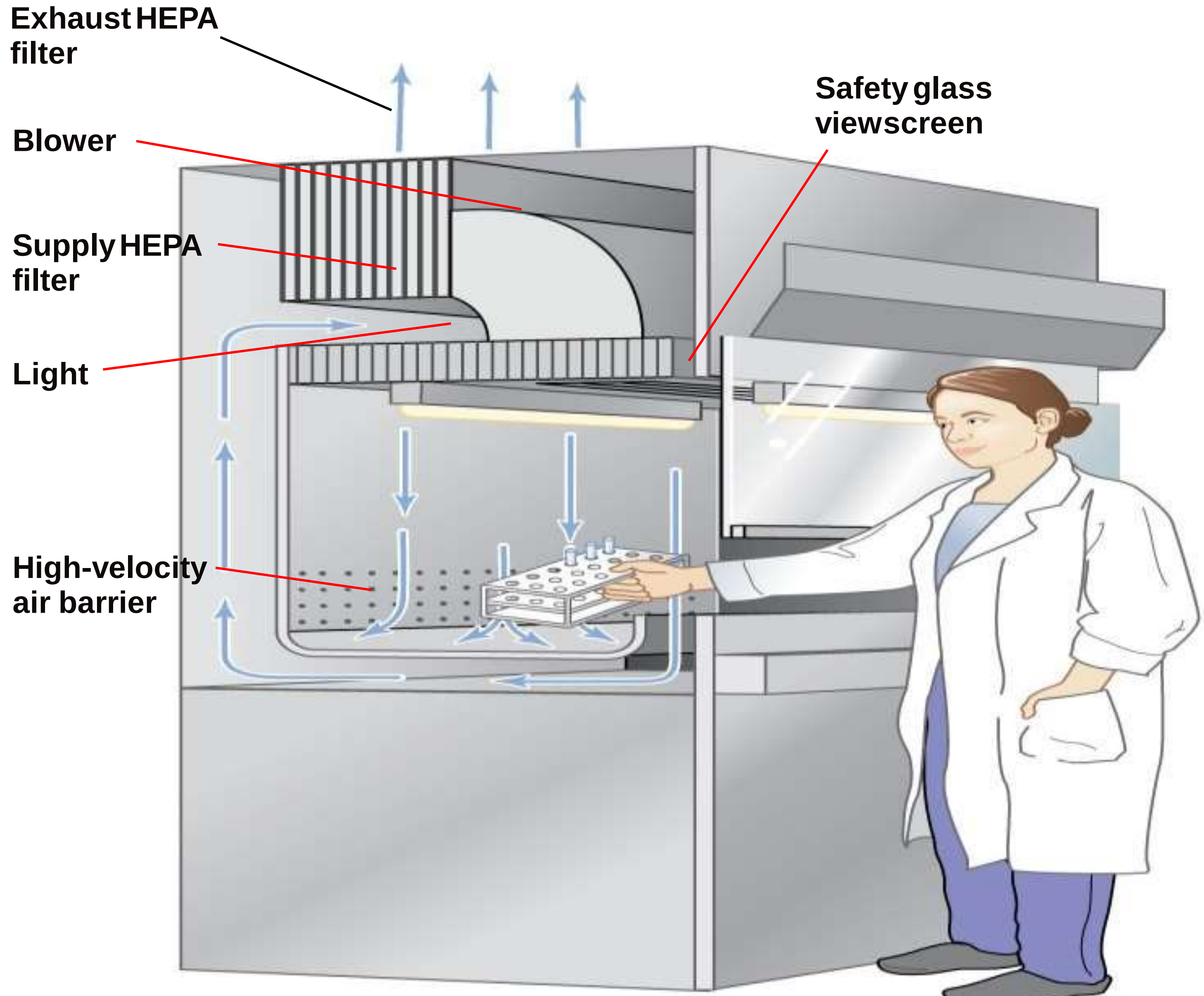
- **Syringe filters**
  - Membrane of 13-25 mm diameter
  - fitted in syringe

- **Vaccum and in-line filters**
  - Membrane of 25-45 mm diameter are used
  - Fitted either with inline holder or vaccume holder
  - Used for sterilization or disinfection of large volumes of liquid or air

- **Pressure filtration**
  - Large membrane of 100-293 mm diameter
  - Fitted with Teflon filter
  - Used for production of pure water for lab

- Air filters
  - High efficiency particulate air (HEPA) filter is used
  - Used for filtration of large volume of air
  - HEPA can remove particles of 0.3 µm
  - Incorporated Laminar Air Flow

The roles of HEPA filters in biological flow safety cabinets



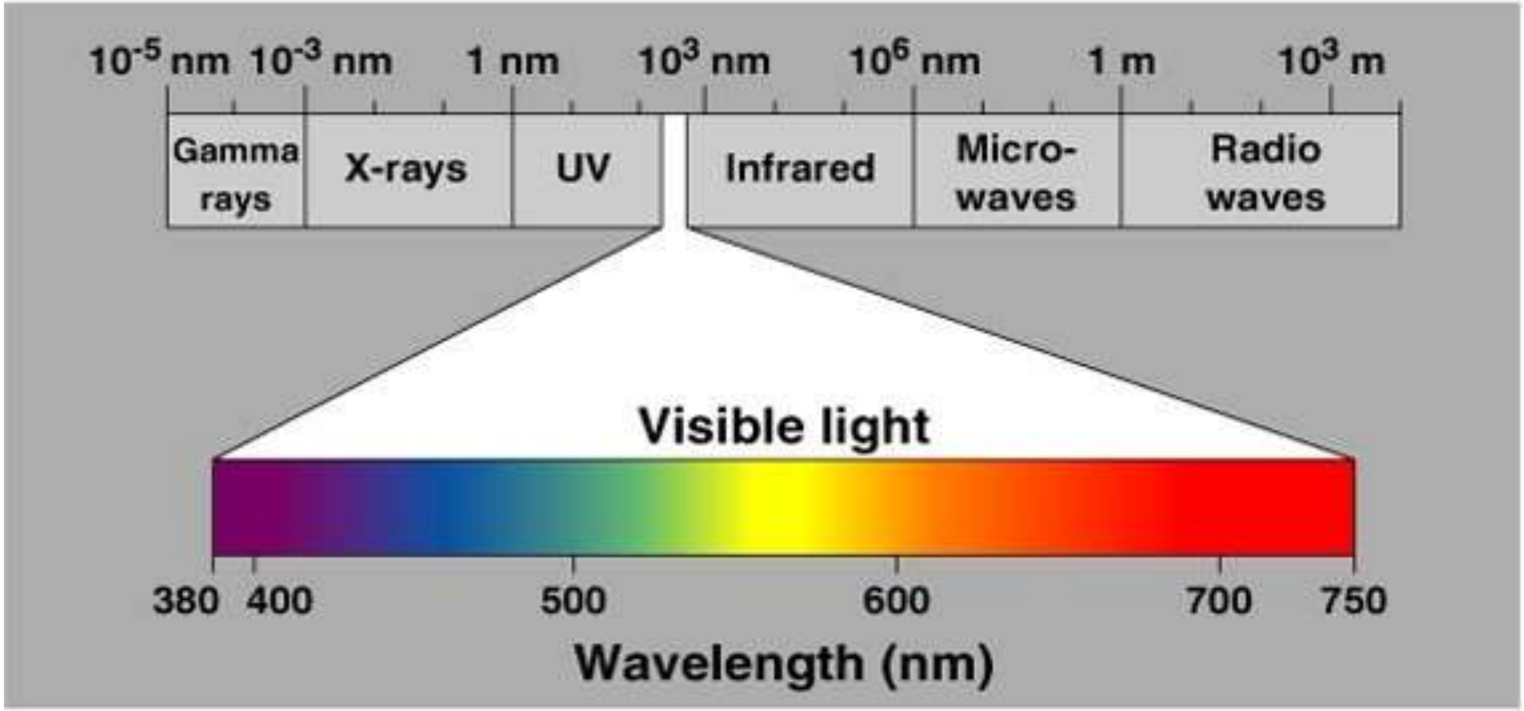
Radiation: Three types of radiation kill microbes:

1. Ionizing Radiation:

- Gamma rays, X rays, electron beams, or higher energy rays.
- Have short wavelengths (less than 1 nanometer).
- Damage DNA and inhibit DNA synthesis
- Used to sterilize pharmaceuticals, disposable medical supplies and plastic syringes, intravenous lines, catheters and gloves that are unable to withstand heat

- **Disadvantages**: Penetrates human tissues. May cause genetic mutations in humans.

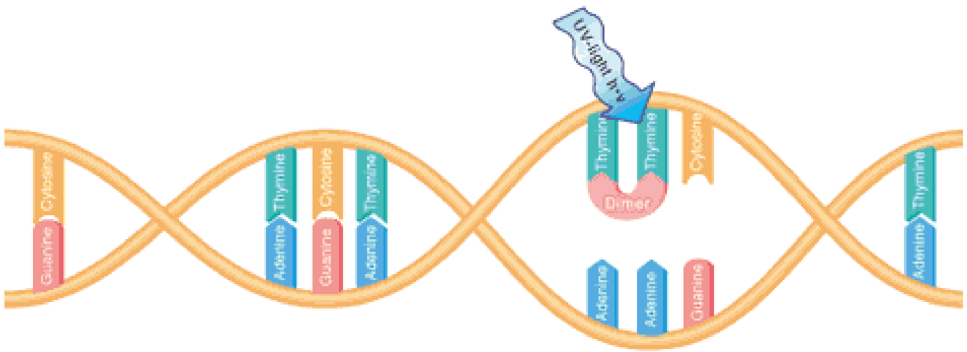
Forms of Radiation



2. Nonionizing Radiation:

- Infrared and **Ultraviolet light**
- Wavelength is longer (Bactericidal wavelenght is 240nm-260nm)
- Damages DNA by producing thymine dimers, which cause mutations.
- Used to disinfect operating rooms, nurseries, cafeterias.

- **Disadvantages**: Damages skin, eyes. Doesn't penetrate paper, glass, and cloth.



3. Microwave Radiation:

- Wavelength ranges from 1 millimeter to 1 meter.
- Heat is absorbed by water molecules.
- May kill vegetative cells in moist foods.
- Bacterial endospores, which do not contain water, are not damaged by microwave radiation.
- Solid foods are unevenly penetrated by microwaves.

Disinfection

- To reduce the number of pathogenic microorganisms to the point where they no longer cause diseases
- Usually involves the removal of **vegetative** or **non-endospore forming** pathogens
- Chemical used for disinfection is called as disinfectant

- Characteristics of disinfectant
  - Have wide spectrum
  - Be active at high dilution
  - Be active in acidic as well as basic
  - Have speedy action
  - Have high penetration power
  - Be stable
  - Be compatible with other compounds

- Not corrode metals
- Not cause local irritation
- Not interfere with healing
- Not be toxic
- Be cheap and easily available
- Be safe and easy to use

- Factors that determine Potency of Disinfectants
  - Concentration and stability of agent
  - Nature of organism
  - Time of action
  - PH
  - Temperature
  - Presence of organic materials
  - Nature of items to be disinfected

Classification of disinfectants

1. Based on consistency

a. Liquid (E.g.Alcohols, Phenols)

b. Gaseous (Formaldehyde vapor, Ethylene oxide)

2. Based on spectrum of activity

a. High level

b. Intermediate level

c. Low level

3. Based on mechanism of action

a. Agents that damage cell membrane

b. Agents that Denature Proteins

c. Agents that Modify Functional Groups of proteins and

4. Sporicidal Agents

a) Glutaraldehyde
b) Formaldehyde
c) Chlorine
d) Iodine and iodophores
e) Ethylene oxide
f) **β**-propiolactone

- Based on Spectrum of activity

1. High Level Disinfection

- Used for items involved with invasive procedure that cannot withstand sterilization procedure, like endoscopes, surgical instrument
- eg. Glutaraldehyde, Hydrogen peroxide, Peracetic acid, Chlorine dioxide etc…

2. Intermediate –level

- Used to clean surfaces or instruments in which contamination of bacterial spores and highly resistant organism are not present
- instruments like Fiberoptic endoscopes, laryngoscopes, anaesthesia breathing circuits etc….
- Eg. Alcohols, iodophhor compounds, phenolic

3. Low level Disinfection

- Used to treat noncritical instruments such as blood pressure cuffs, ECG electrodes, stethoscopes etc…
- Eg. Quaternary ammonium compounds

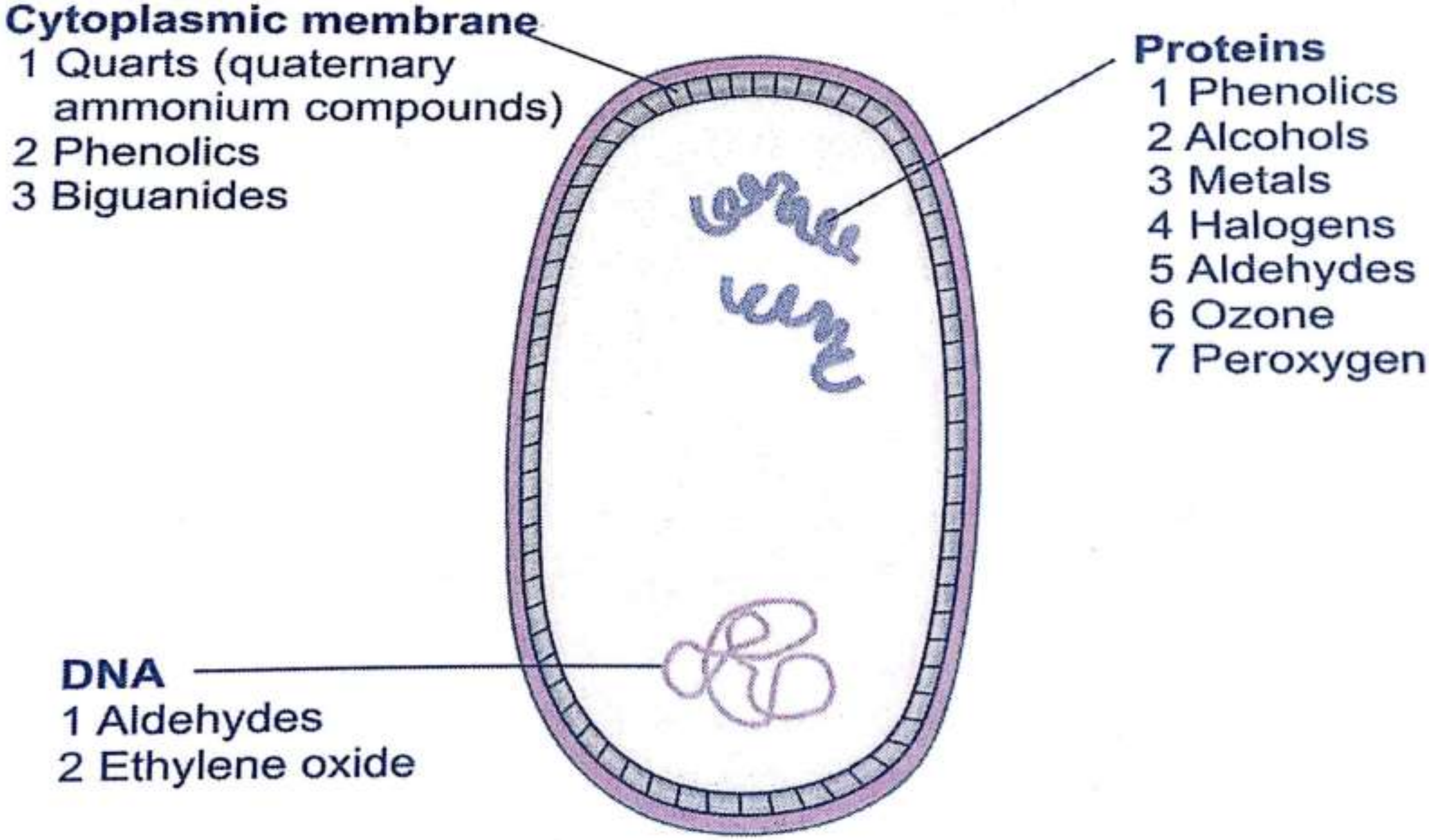


Fig. 5.4: Sites of action of chemical agents

- On the basis of Mechanism of Action

A. Agents that damage the cell membrane

1. Surface active agents

– Surfactant that alter energy relationship, producing a reduction of surface

– Hydrophobic

– Hydrophilic

Classification

a. Cationic agents

θ Act on phosphate groups of cell membrane phospholipids

θ lead to loss of cell membrane semi permeability and leakage of nitrogen and phosphorous

θ denature proteins

θ Eg. Cetrimide, Benzalkonium chloride, Cetylprimidium chloride

- Uses
  - Active against GPC, non-sporing
  - Lethal to GNB if high concentration used
  - Fungistatic
  - Active against enveloped viruses

- Anionic agents
  - Include soap and fatty acids
  - active at acidic PH
  - Active against GPC

- Amhollytic(amphoteric) compounds
  - Known as Tego
  - Both properties of anionic and cationic
  - Effective against GPC and GNB

2. Phenols and Phenolics:

Phenol (carbolic acid) was first used by Lister as a disinfectant in 1867

Destroy plasma membranes and denature proteins

Rarely used today because it is a skin irritant and has strong odor.

1% of concentration act as bactericidal

Phenolics are chemical derivatives of phenol

- **Cresols (Lysol)**:
  - Derived from coal tar
  - Uses for contaminated glassware, cleaning floors, disinfection of excreta

- Chlorhexidine –comercially savlon
  - Member of biguanide group
  - Widely used in wounds, preoperative disinfection of skin

- Chloroxyleno
l
  - Active ingredient of dettlo
  - Less toxic and less irritant

- Hexachlorophene
  - Biphenols
  - Used for surgical and hospital microbial control
  - Effective against gram-positive staphylococci and streptococci.
  - Excessive use in infants may cause neurological damage

Advantages: Stable, persist for long times after applied, and remain active in the presence of organic compounds.

- Uses
  - Use in hospitals
  - Active against GPC and GNB
  - Sporicidal activity
  - Disinfection of pus, saliva and feaces
  - Used for discarded culture, contaminated materials
  - Used for preservation of sera and vaccines at a concentration of 0.5%

B. Agents that denature proteins

1. Acids and Alkalies

- θ antibacterial activity by free H+ and OH-
- θ by altering the PH
- θ Used in food and pharmacy industry
- θ eg. Sodium hydroxide, sodium carbonate

2. Alcohols

Kill bacteria, fungi, but not endospores or naked viruses.

Act by denaturing proteins and disrupting cell membranes.

Used to mechanically wipe microbes off skin before injections or blood drawing.

Not good for open wounds, because cause proteins to coagulate.

Ethanol: Drinking alcohol. Optimum concentration is 70%.

Isopropanol: Rubbing alcohol. Better disinfectant than ethanol. Also cheaper and less volatile.

Methyl alcohol- Effective against fungal spores so used for treating cabinets and incubators, Toxic and inflammable

C. Agents that modify Functional groups of proteins and nucleic acids

a. Heavy metals

- The salts of silver, copper and mercury are used as disinfectants.
- Act by coagulating proteins
- Marked bacteriostatic, weak bactericidal and limited fungicidal activity
- Mercuric chloride and silver nitrate are commonly used

- Uses
  - Bactericidal for gonococcus
  - Prophylaxis of opthalmic neonatorum in newborn
  - To prevent infection of burn

b. Oxidizing agents

i. Halogens:

- θ chlorine and iodine
- θ bactericidal and sporicidal

A. Iodine:

Iodine tincture (alcohol solution) was one of first antiseptics used, iodophors (povidine-iodine) for wound infection

B. Chlorine:

When mixed in water forms **hypochlorous acid**:

$$Cl_2 + H_2O \longrightarrow H^+ + Cl^- + HOCl$$

- θ Used to disinfect drinking water, pools, and sewage.

- Hypochlorites is commonly used and have bactericidal, fungicidal, virucidal and sporicidal

Uses

  - For sanitizing food and dairy industries
  - As sanitizers for house and hospital
  - As bleaching powder

ii. Hydrogen peroxide

  - 3-6 % concentration is used
  - Sporicidal
  - Used to disinfect plastic implants, contact lenses, surgical prostheses

iii. Dyes

a. Aniline dyes

- θ derivatives of triphenylmethane
- θ eg. Brilliant green, malachite green, crystal violet
- θ active against GPC than GNB
- θ used in preparation of culture media

b. Acridine dyes

- θ flavins-yellow colour
- θ bacteristatic and bacteriocidal
- θ active against GPC than GNB
- θ eg. Proflavine and acriflavine
- θ used in wound infection

4. Alkylating Agents

a. Formaldehyde

θDenaturation of proteins

θBactericidal, sporicidal, viricidal, fungistic

θUsed in liquid and gaseous phase

θ37% formaldehyde (formalin)

θToxic and irritant

b. Gluteraldehyde

θDenaturation of proteins

θBactericidal, viricidal, fungistic but less effect in spor

θ2 % of gluteraldehyde

θUsed as cold sterilant for disinfection surgical instruments and endoscopes

Vapor- phase disinfectants

1. Ethylene oxide

- Action is due to its alkylating amino, carboxyl, hydroxyl and sulfhydryl groups in amino acids
- Has high penetration power
- Colourless liquid but used by mixing with inert gases like CO_2, N_2 to a concentration of 10%

Used

- for sterilization of article liable to damage by heat
- Sterilization of materials like glass, metal, paper, clothing's, etc

Disadvantage

- Irritant, toxic, inflammable, mutagenicity and carcinogenicity

2. Formaldehyde Gas

- θ Used for fumigation of heat sensitive equipment, anesthetic machine, incubators, laboratory, operation theaters
- θ Irritant and has unpleasant smell

3. Betapropiolactone

- θ Condensed product of ketane and formaldehyde
- θ Concentration of 0.2% is used
- θ Has less penetration power
- θ Carcinogenic
- θ Used for sterilization of vaccines and seras

Testing of Disinfectant

1. Phenol coefficient test

a. Rideal Walker test

Prrocedure

- Series of dilution of phenol and experimental disinfectant are inoculated with test bacteria
- Concentration and time of action is noted

Result – dilution of test diinfectant which sterilizes in a given time divided by dilution of phenol is known as phenol coefficient (phenol=1)

Interpretation- Phenol coefficient=1, it equal to effectiveness as phenol

<1.0, less effective, >1.0 more effective

b. Chick-Martin test

- Modification of Rideal- Walker test
- Keep organic matter to stimulate natural situations

2. Minimum inhibitory concentration (MIC)

- Lowest concentration of disinfectant that inhibites growth of organism
- Depend upon concentration and time of exposure

3. Kelsey-Sykes test (Capacity test)

- In specific concentration of disinfectant addition of organism in increments
- Used to see capacity of disinfectant to cope with succesive bacteria

Procedure

- Test organisms are added to disinfectant in 3 successive lots at 0, 10 and 20 minutes
- Time of exposure is noted like 8, 18 and 28 minutes
- Subcultured on routinely used media

4. In-use test

- Test should perform to confirm the chosen disinfectant is effective
- Eg. In hospitals disinfectant jar in which discarded materials or contaminated materials are kept
- Kept for overnight
- Subculture and compared with known standard strains