Chapter Three

Disinfection and Sterilization

- Disinfection and sterilization are now the most basic necessities in clinical practice and laboratory procedures.
- Some of the important terms include:
- **Sterilization** is the destruction or complete removal (by filtration) of all forms of microorganisms including their spores.
- **Disinfection** is the destruction of many microorganisms, but not the bacterial spores.

- **Antisepsis** is the destruction or inhibition of microorganisms in living tissues, there by limiting or preventing the harmful effect of infection.
- A **static** agent is the agent which only inhibit the growth of microorganisms (bacterostatic, fungistatic, sporostatic)
- A **cidal** agent is the agent which kill the microorganisms (bactericidal, virucidal, fungicidal)
- **Sterilants** are the chemicals which under controlled conditions can kill sporing bacteria or fungi.

The pattern of microbial death

- Microorganisms are **not killed immediately** when exposed to a lethal agent, their number is decreased at an exponential rate.
- Microorganism is considered dead when it is unable to grow.

The pattern of microbial death

- The effectiveness of a disinfectant or sterilizing agent is influenced by:

1. Population size.
2. Species.
3. Stage of growth.
4. Concentration or intensity of the agent.
5. Nature of the environment.

Properties of an ideal disinfectant

- Several qualities should be considered in deciding which disinfectant to use.
- An ideal disinfectant should:

1. Be fast acting even in the presence of organic substances, such as those in body fluids.
2. Be effective against all types of infectious agents without destroying tissues or acting as a poison if ingested

3. Easily penetrate material to be disinfected without damaging or discoloring the material.

4. Be easy to prepare and stable even when exposed to light, heat, or other environmental factors.

5. Be inexpensive and easy to obtain and use.

6. Not have an unpleasant odor.

- No disinfectant is likely to satisfy all these criteria, so the agent that meets the greatest number of criteria for the task at hand is chosen.
- In practice, many agents are tested in a wide range of situations and are recommended for use where they are most effective.
- The agents used for sterilization and disinfection are divided into 2 groups:
  - **Physical agents**
  - **Chemical agents**

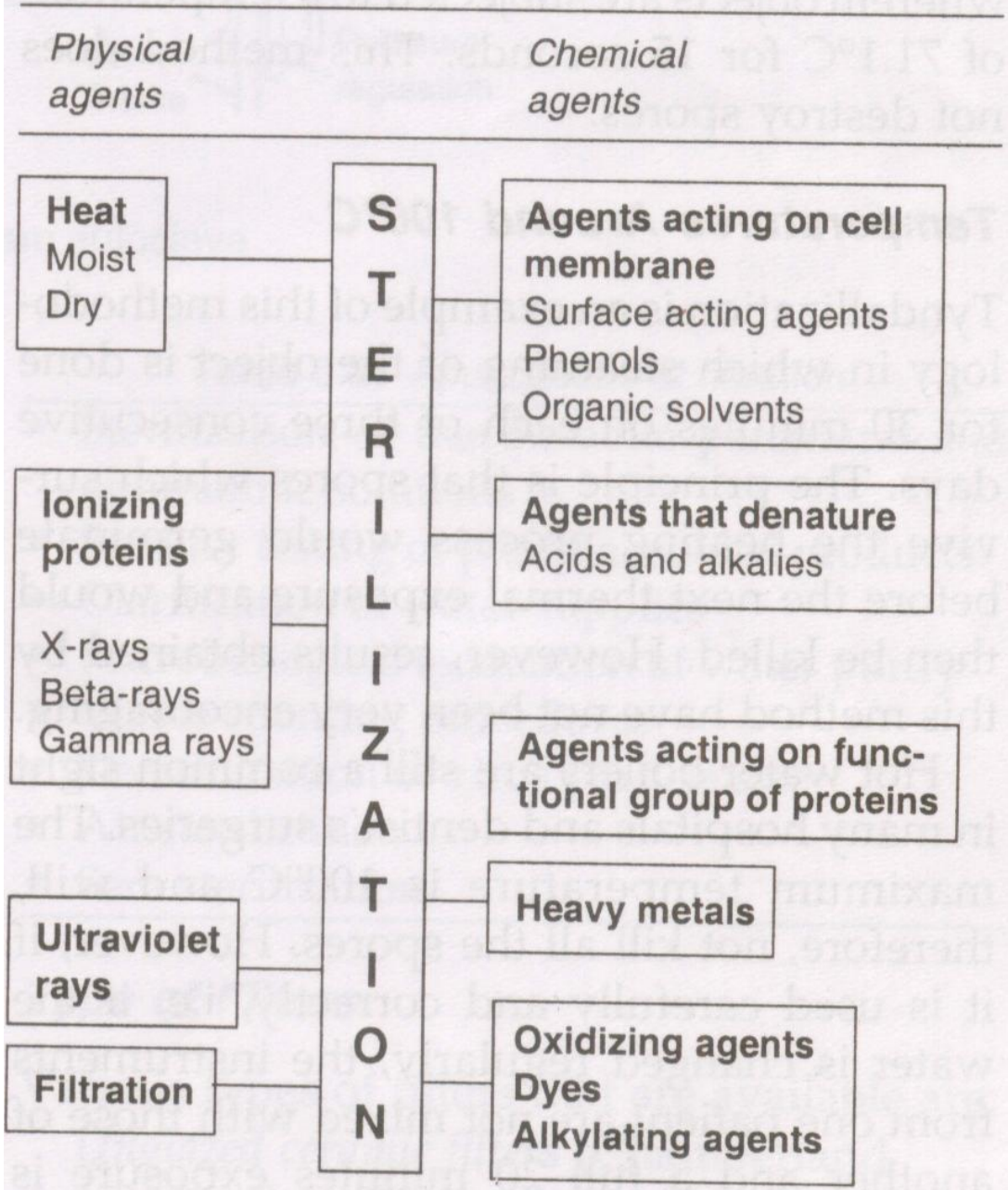


Fig. 3.1: Methods of sterilization

Physical agents

- Usually preferred over chemical agents. The most important physical agent is heat, both moist as well as dry.

➢ **Principle and application of heat killing**

✓ Heat destroys microorganisms by:

1. Denaturing protein
2. Melting lipids
3. Incineration

It includes:

1. Moist heat
2. Dry heat

A. Moist heat sterilization

- Is heating in the presence of water, and can be employed in the following ways:

1. Temperature below 100°C
2. Temperature around 100°C
3. Temperature above 100°C

Temperature below 100°C

- It includes holder method of pasteurization.
- Pasteurization kill most pathogens in milk, other dairy products and beer but does not sterilize.
- A temperature of 60°C for 30 minutes is employed for sterilization.
- In flash modification: objects are subjected to a temperature of 71.1°C for 15 sec.
- This method does not destroy spores.

Temperature around 100°C (boiling)

- **Tyndallization** is an example of this methodology, in which the steaming of the object is done for 30 minutes on each of three consecutive days.
- The principle is that spores which survive the heating process would germinate before the next thermal exposure and then will be killed.
- The results obtained by this method are not very encouraging.

- Hot water boilers are still common in many hospitals and dentists surgeries.
- Maximum temperature is 100°C this will not kill all spores.
- But if this method is used carefully and correctly (changing water regularly, do not mixing instruments from patients, and ensuring exposure for 20 minutes).
- all vegetative bacteria and viruses can be destroyed, provided that the instruments were cleaned before putting them in boilers.
- Killing is by denaturing enzymes and some proteins and disrupting cell membrane.

Temperature above 100°C (autocalving)

- Dry saturated steam is used
- It cause coagulation of proteins and nucleic acids and kill all living microorganisms.
- Autoclaves have been designed on basis of moist heat under pressure = 15 PSI, temp = 121°C for 20 minutes.

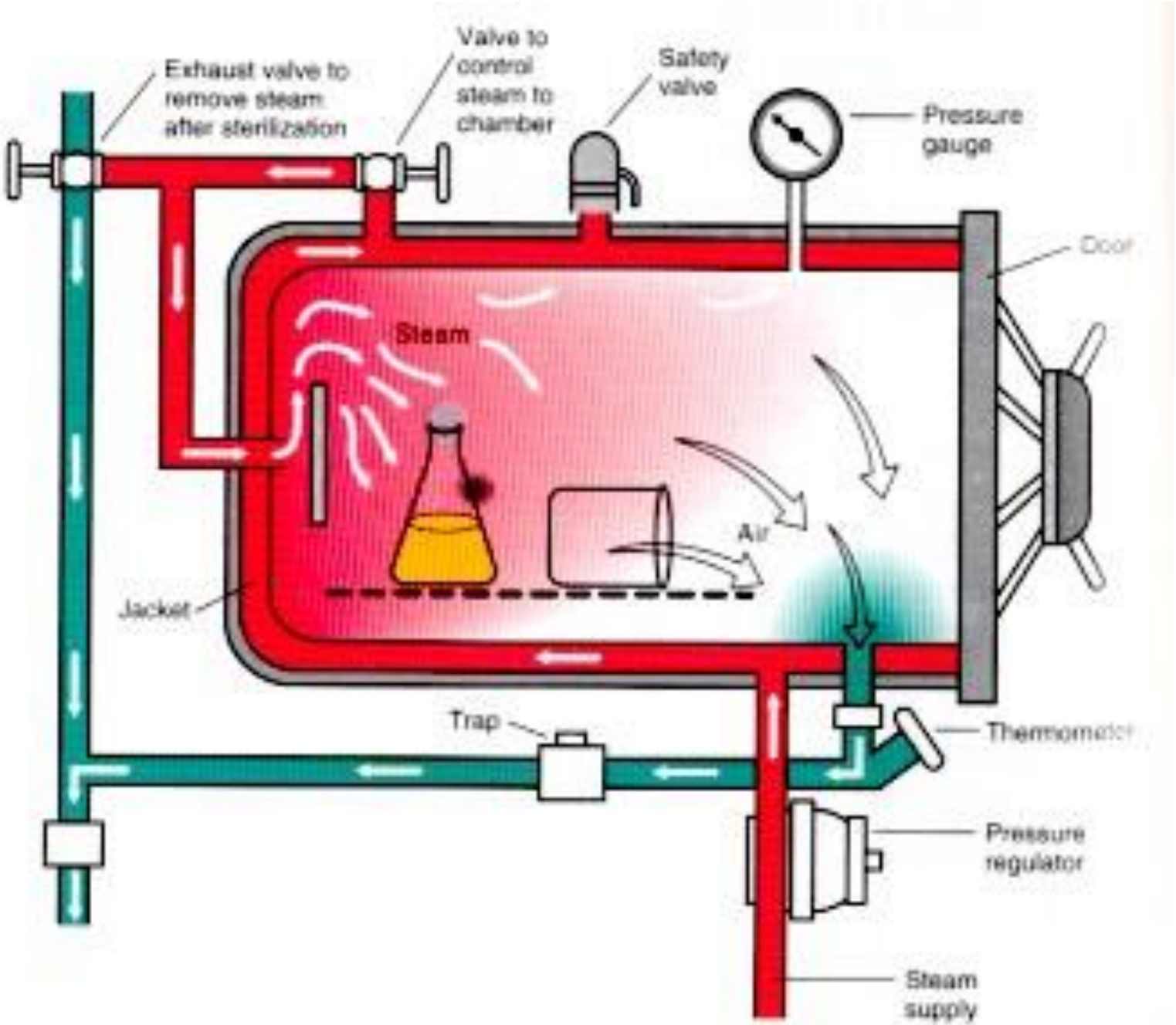

Exhaust valve to remove steam after sterilization
Valve to control steam to chamber
Safety valve
Pressure gauge
Door
Steam
Air
Jacket
Trap
Thermometer
Pressure regulator
Steam supply

Table 3.1 Time-temperature relationship in heat sterilization

Process	Temp	Holding period
Moist heat (autoclaving)	121^{o}C 126^{o}C 134^{o}C	15mins 10 mints 3 mints
Dry heat	>160^{o}C >170^{o}C >180^{o}C	>120 mints >60 mints >30 mints

Temperature above 100°C (autocalving)

This method is used for sterilization of:

- Surgical instruments
- Surgical dressings
- Rubber cloves
- Bacteriological media
- Decontaminating laboratory and hospital reusable goods

B. Dry heat sterilization

- Less efficient as compared to moist heat.
- Used for thermostable substances (non-aqueous liquids and powders).
- Bacterial spore resist dry heat (they require 140°C for 3 hrs. to be killed).
- Is carried out by:

1. Flaming
2. Hot air oven

1. Flaming

- Example: sterilizing inoculating loops and contaminated surgical dressings or contaminated material from laboratories.

2. Hot air oven:

- High temperature for long time (Fig 3.1) can be used.
- Is essential that hot air must circulate in between the objects.
- Microbial inactivation in this method is an oxidation process.

Dry heat sterilization

Used for sterilization of :

- Glassware
- Glass syringes
- Oil and oily injections
- Metal instruments

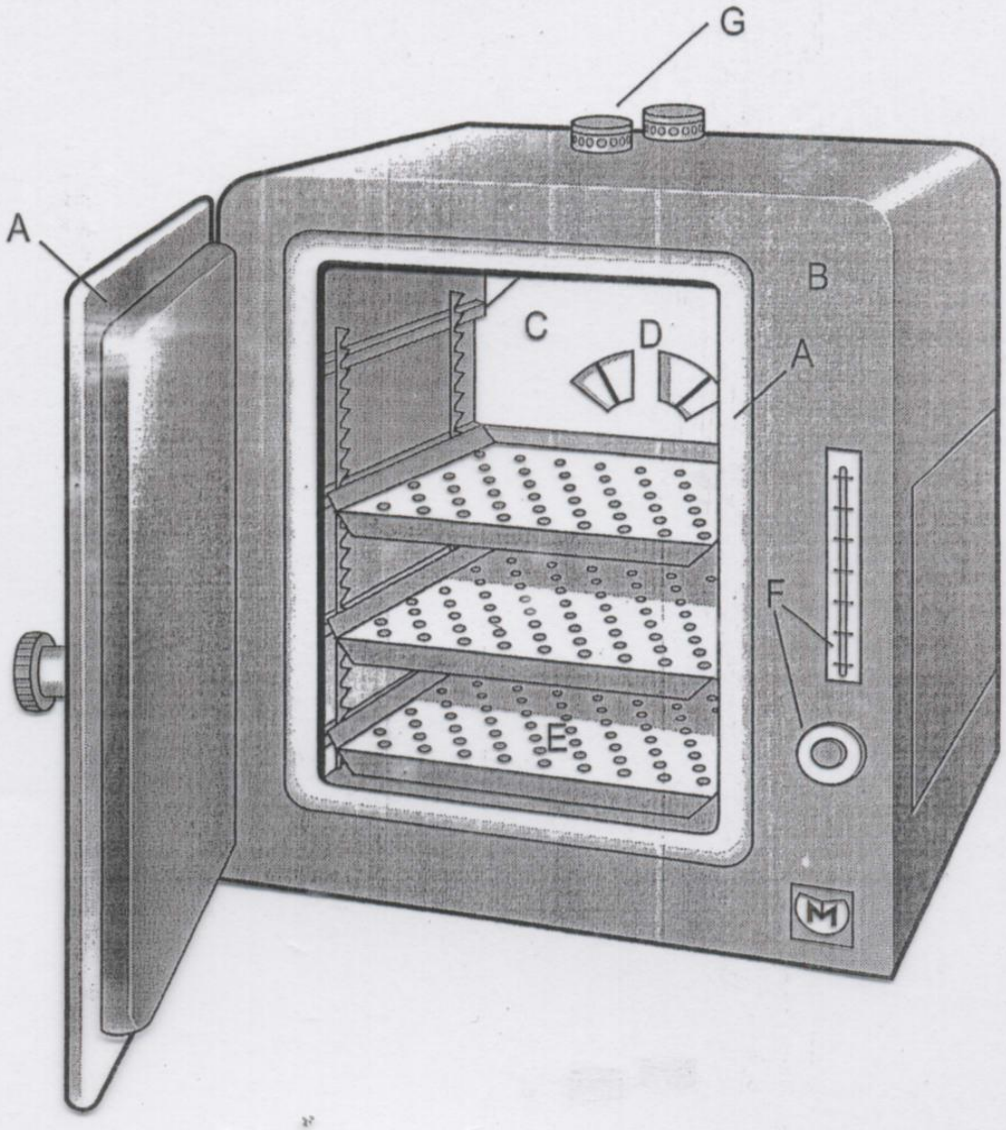


Fig. 12.6 Hot-air oven. A, Asbestos gasket; B, outer case containing glass-fibre insulation, and heaters in chamber wall; C, false wall; D, fan; E, perforated shelf; F, regulator; G, vents.

Refrigeration, freezing, drying and freeze-drying

- Can be used to retard the growth of microorganisms.
- **Lyophilization**, drying in the frozen state can be used for long term preservation of live microorganisms.

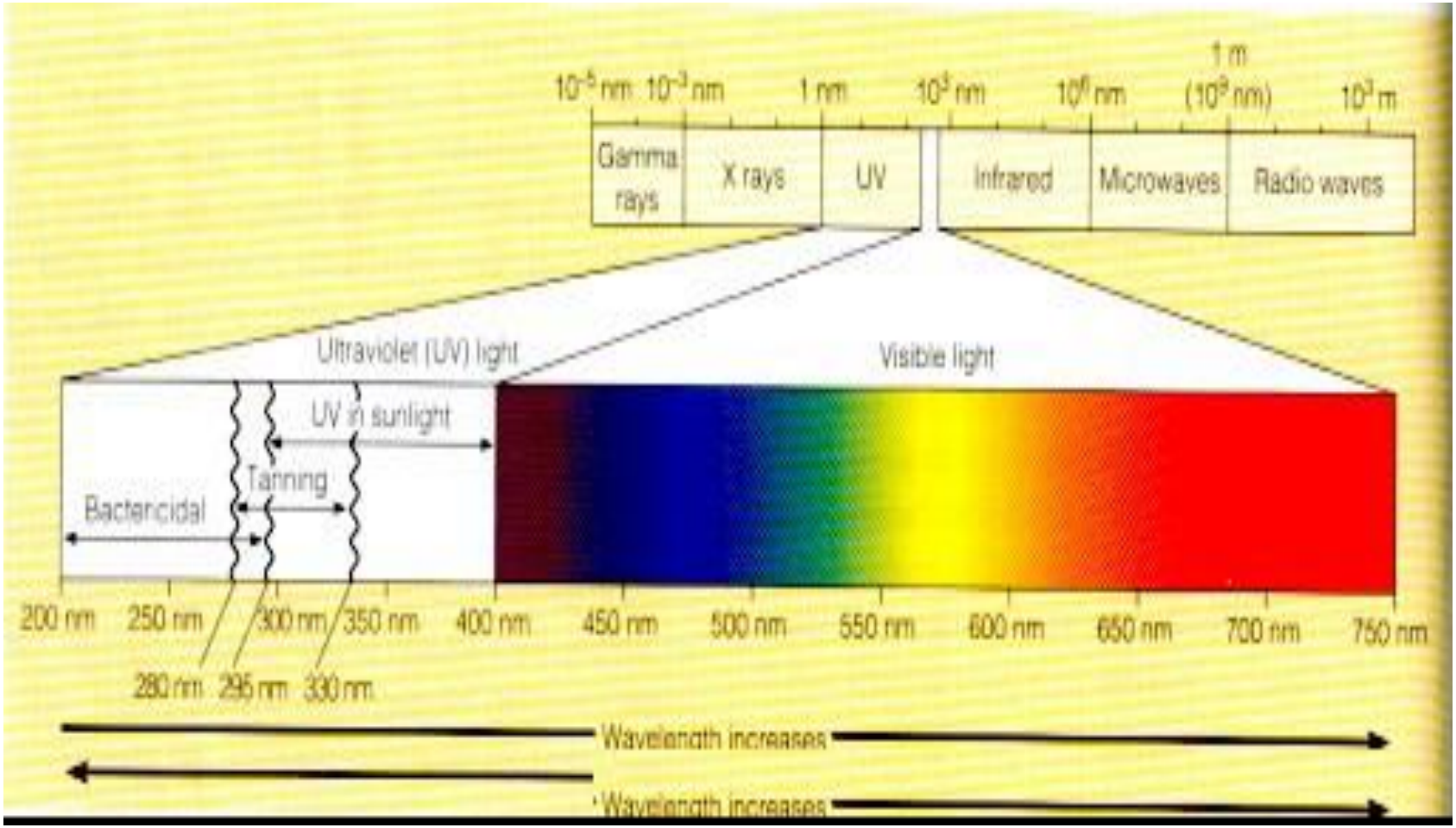
Radiation

- There are 2 kinds of radiation
- **Non-ionizing radiation (UV-radiation)**
  - Ultraviolet radiation of wave length 240-280nm.
  - ✓ It damages DNA, resulting into non coding lesions in DNA and causing bacterial death.
  - The bactericidal activity of sunlight is due to the presence of UV rays.

Radiation

- It should be used for clean surfaces only, because it does not penetrate glass, dirt films, water, etc.
- Is effective for surface sterilization, used in hospitals for sterilization of small and closed chambers, such as inoculating chambers in laboratories.

Radiation


10^{-5} nm
10^{-3} nm
1 nm
10^{3} nm
10^{6} nm
1 m (10^{9} nm)
10^{3} m
Gamma rays
X rays
UV
Infrared
Microwaves
Radio waves
Ultraviolet (UV) light
Visible light
UV in sunlight
Tanning
Bactericidal
200 nm
250 nm
300 nm
350 nm
400 nm
450 nm
500 nm
550 nm
600 nm
650 nm
700 nm
750 nm
280 nm
295 nm
330 nm
Wavelength increases
Wavelength increases

Ionizing radiation

- It includes X rays, gamma rays and beta rays.
- It kills microorganisms by damaging DNA and proteins.
- It gives good penetration and no residual radioactivity.
- Is known as cold sterilization, because there is no increase in temperature
- Used for sterilization of single use disposable medical items such as syringes, swabs, plastic catheters, and cultures.

Microwave radiation

- Absorbs water molecules, then release microwave energy to surroundings as heat.
- Cannot be used reliable to destroy microbes except in special media sterilizing equipment.

Strong visible light

- Oxidation of light-sensitive materials.
- Can be used with dyes to destroy bacteria and viruses, may help to sanitize clothing

Sonic and ultrasonic waves

- Cause cavitation
- Is not a practical means of killing microorganisms, but useful in fractionating and studying cell components.

Filtration

- It removes microbes mechanically.
- Used to sterilize media, pharmaceutical products, and vitamins, in manufacturing vaccines, and in sampling microbes in air and water.
- Filtration used for heat-sensitive substances

Filtration

There are 3 types of filters:

1. **Depth filters:** remove microorganisms by physical screening relying on mechanism of adsorption and entrapment.
2. **High-efficiency particulate air filters:** Used for filtration and sterilization of gases, to supply sterile air to hospital isolation units and operation theaters.

Filtration

3. Membrane filters: are widely used

- Made up from cellulose ester
- Suitable for preparing sterile solutions
- Require pore size 0.2-0.22 mm.

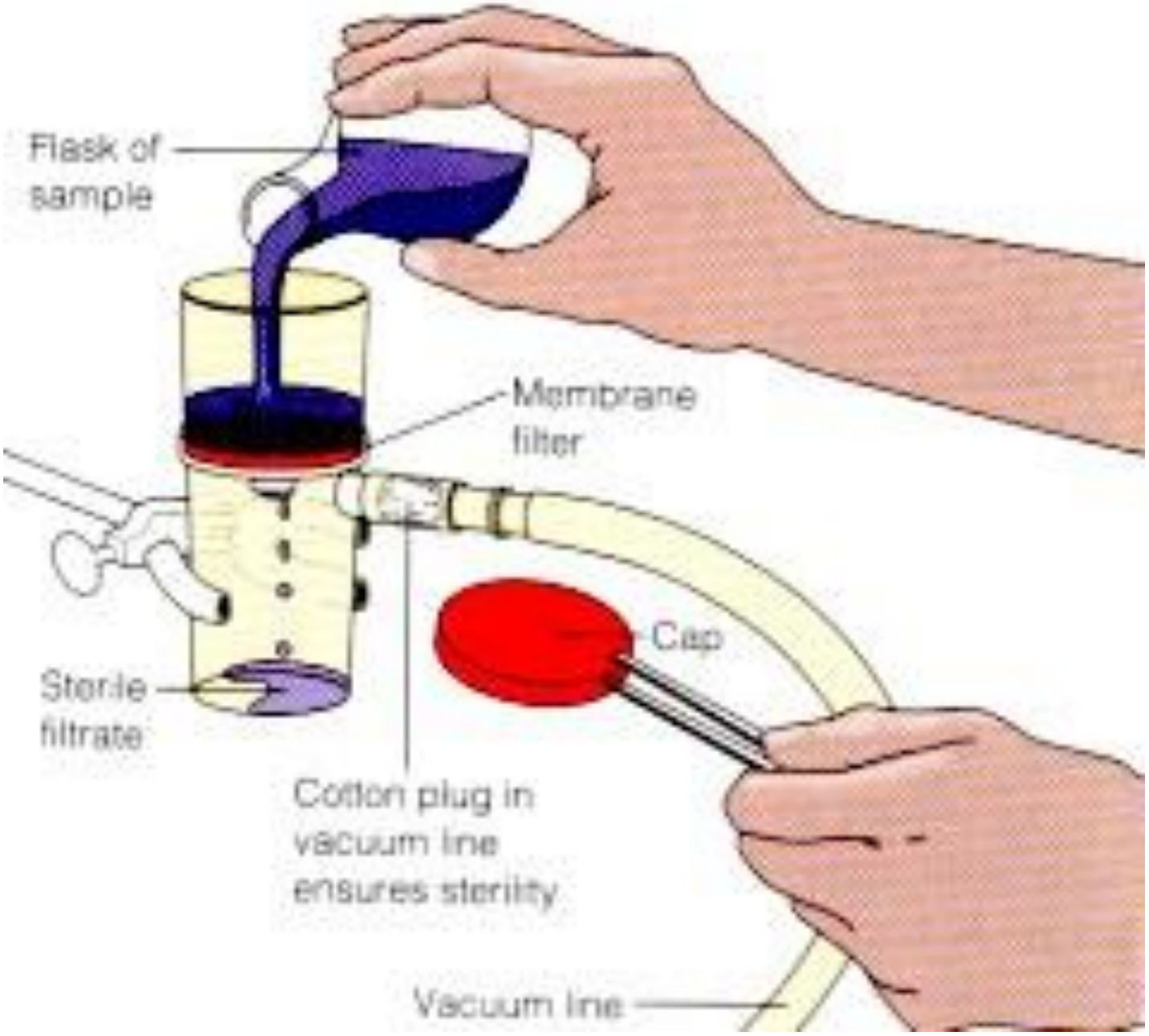
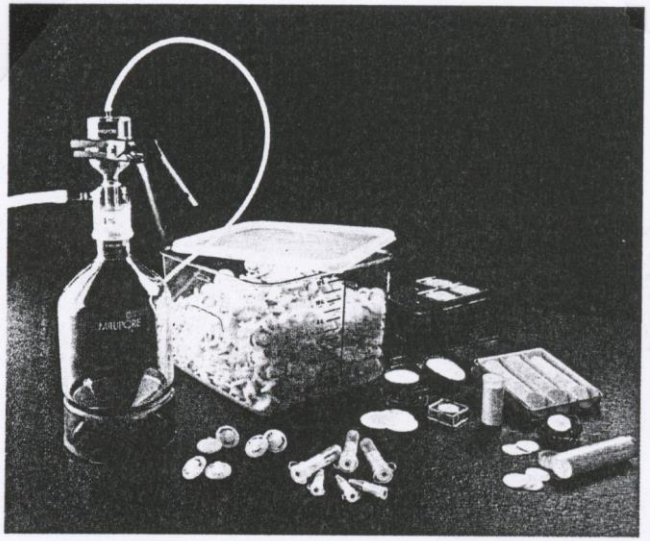

Flask of sample
Membrane filter
Cap
Sterile filtrate
Cotton plug in vacuum line ensures sterility
Vacuum line

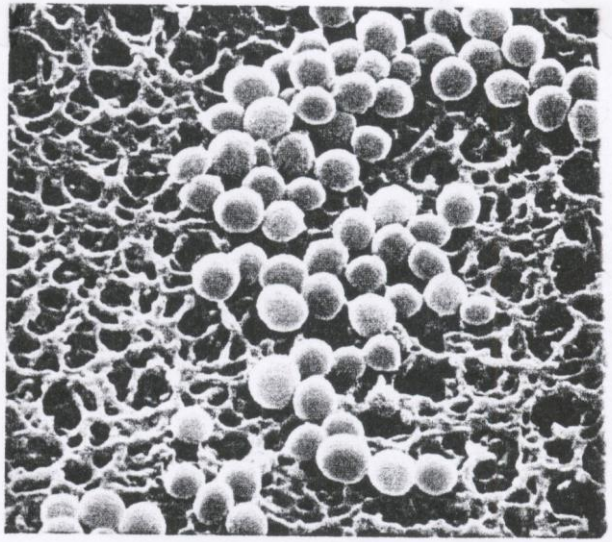
TABLE 12.4

Pore Sizes of Membrane Filters and Particles That Pass Through Them

Pore Size (in μm)	Particles That Pass Through The
10	Erythrocytes, yeast cells, bacteria, viru molecules
5	Yeast cells, bacteria, viruses, molecules
3	Some yeast cells, bacteria, viruses, mol
1.2	Most bacteria, viruses, molecules
0.45	A few bacteria, viruses, molecules
0.22	Viruses, molecules
0.10	Medium-sized to small viruses, molecu
0.05	Small viruses, molecules
0.025	Only the very smallest viruses, molecul
Ultrafilter	Small molecules



(a)



(b)

FIGURE 12.17 Sterilization by filtration. (a) Various types of membrane filters are available to sterilize large or small quantities of l uids. Some can be vacuum-filtered, ensuring that what is forced into the bottle or flask will be sterile. (b) Scanning electron micrograph of *Staphylococcus epidermidis* cells trapped on the surface of a 0.22 μm Millipore membrane filter. Membrane pore size can be selected to allow viruses, but not bacteria, to pass through or to prevent both from passing.

Chemical agents

- Are highly toxic for all types of cells.
- Can be classified according to:
  - Their site of action OR (Table 6)
  - Mechanism of inactivating organisms (Table 3-3)

Table 3.3: Classification of chemical sterilizing agents

Chemical disinfectant	*Examples*
• *Interfere with membrane functions*	
Surface acting agents	Quaternary ammonium compounds, Tween 80, Soaps and fatty acids
Phenols	Phenol, cresol, hexylresorcinol
Organic solvent	Chloroform, alcohol
• Denature proteins	
Acids and alkalies	Organic acids, Hydrochloric acid, sulphuric acid
• *Destroy functional groups of proteins*	
Heavy metals	Copper, silver, mercury
• Oxidizing agents	Iodine, chlorine, hydrogen peroxide
Dyes	Acridine orange, acriflavine
Alkylating agents	Formaldehyde, ethylene oxide

Table 3.4: Applications and in-use dilution of chemical disinfectants

Agent	*Common uses*	*Use dilution(%)*
Alcohols	Skin antiseptic Surface disinfectant	70
Mercurials	Skin antiseptics Surface disinfectant	0.1
Silver nitrate	Antiseptic (eyes and burns)	1
Phenolic compound	Antiseptic skin washes	0.5-5
Iodine	• Disinfects inanimate object • Skin antiseptic	2
Chlorine compounds	Water treatment Disinfect inanimate object	5
Quaternary ammonium compounds	Skin antiseptic Disinfects inanimate object	< 1
Glutaraldehyde	Heat sensitive instruments	1-2

Table 3.5: Preferred methods of sterilization for common use articles

Autoclaving	*Hot air oven*	*Ethylene oxide*	*Autoclaving*	*Ethylene oxide*
Animal cages	Glassware	Fabric	Test tubes	
Sugar tubes	Beakers	Bedding	Enamel metal trays	Plastics
Lab. coats	Flasks	Blanket	Wire baskets	Flasks
Cotton	Petri dish	Clothing	Wood	Petri dish
Filters	Pipette	Matteresses	Tongue depressor	Tubes
Instruments	Slides	Pillows	Applicator	Tubing
Culture media	Syringes	Disposable instruments	Endodontic instruments	Rubber
Rubber	Test tubes	Instruments	Orthodontic pliers	Catheters
Gloves		Blades	Orthodontic kits	Drains
Stopper	Glycerine	Knives	Saliva ejector	
Tubing	Needles	Scalpels	Handpieces	
	Oils	Scissors	Cavitron heads	Special items
Slides		Talcum powder	Steel burs	Bronchoscope
Syringe and	Paper		Steel tumbler	
Wax needles	Matrix band	Books	Hand instruments	Gloves
	Saliva ejector	Cups, plates		Heart lung machine

* Suitable method in some cases but not recommended routinely Cystoscope

Properties of Chemical Antimicrobial Agents

Agent	Actions	Uses
Soaps and detergents	Lower surface tension, make microbes accessible to other agents	Hand washing, laundering, sanitizing kitchen and dairy equipment
Surfactants	Dissolve lipids, disrupt membranes, denature proteins, and inactivate enzymes in high concentrations; act as wetting agents in low concentrations	Cationic detergents are used to sanitize utensils; anionic detergents to launder clothes and clean household objects; quaternary ammonium compounds are sometimes used as antiseptics on skin.
Acids	Lower pH and denature proteins	Food preservation
Alkalis	Raise pH and denature proteins	Found in soaps
Heavy metals	Denature proteins	Silver nitrate is used to prevent gonococcal infections, mercury compounds to disinfect skin and inanimate objects, copper to inhibit algal growth, and selenium to inhibit fungal growth.
Halogens	Oxidize cell components in absence of organic matter	Chlorine is used to kill pathogens in water and to disinfect utensils; iodine compounds are used as skin antiseptics.
Alcohols	Denature proteins when mixed with water	Isopropyl alcohol is used to disinfect skin; ethylene glycol and propylene glycol can be used in aerosols.
Phenols	Disrupt membranes, denature proteins, and inactivate enzymes; not impaired by organic matter	Phenol is used to disinfect surfaces and destroy discarded cultures; amylphenol destroys vegetative organisms and inactivates viruses on skin and inanimate objects; chlorhexidine gluconate is especially effective as a surgical scrub.
Oxidizing agents	Disrupt disulfide bonds	Hydrogen peroxide is used to clean puncture wounds, potassium permanganate to disinfect instruments.
Alkylating agents	Disrupt structure of proteins and nucleic acids	Formaldehyde is used to inactivate viruses without destroying antigenic properties, glutaraldehyde to sterilize equipment, betapropiolactone to destroy hepatitis viruses, and ethylene oxide to sterilize inanimate objects that would be harmed by high temperatures.
Dyes	May interfere with replication or block cell wall synthesis	Acridine is used to clean wounds, crystal violet to treat some protozoan and fungal infections.

Chlorine and Iodine

- These are most useful disinfectants
- Iodine is a skin disinfectant and chlorine is a water disinfectant both gives good results
- Their activity is exclusively bactericidal; they are effective against sporulating organisms also.
- Mixture of various surface acting Iodine (Iodophores)
- Iodophores are used for sterilization of dairy products
- Hypochlorite (inorganic chloramines) and organic chloramines are all good disinfectants.

Hydrogen peroxide

Hydrogen peroxide in a 3% solution is harmless, but very weak disinfectant

- Used for cleaning wound

Potassium permanganate: is oxidizing agent and used in the treatment of urethritis.

Formaldehyde

- Is the least selective agent, is a gas usually used as 37% solution, (formalin)
- In high concentration; it destroys bacteria and their spores.
- Used for equipment that cannot be sterilized by heat, and which are not common source of spreading infection (cold sterilization).

- Cold sterilization is achieved by dipping, precleaned instrument in 2% solution of glutaradehyde for 15-20 mints by this method, bacteria and spores are killed.
- Instruments sterilized by this method are cystoscopes and endoscopes.

Ethylene oxide

- Is alkylating agent, extensively used in gaseous sterilization.
- Active against all kinds of bacteria and their spores
- It sterilizes any object that can be damaged by heat. e.g. heart lung machine.
- Also used to sterilize fragile, heat sensitive equipment, powders and components of space crafts.