SQBI 1303 MICROBIOLOGY

STERILIZATION

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PRINCIPLES OF STERILIZATION

Definitions:

1. Sterility is defined as an absolute term - that means absence of all viable forms of life.

2. Sterilization is the process of achieving sterility.

- In practice sterility is achieved by exposure of the object to be sterilized to chemical or physical agent for a specified time.

PRINCIPLES OF STERILIZATION

The agents used as steriliants are:

1. **Elevated temperature**
2. **Ionizing radiation**
3. **Chemical liquids or gaseous**
4. **Filters**

PRINCIPLES OF STERILIZATION

Definition of death

- In practice, a microbe is defined dead when it cannot be detected in culture media, where it previously grew. Detection requires the development of a single colony on solid medium or turbidity in liquid medium.

PRINCIPLES OF STERILIZATION

Problem detecting cell death :

- As we do not have media to grow all microbes the term absence of viable life is inaccurate, as it cannot be proved.

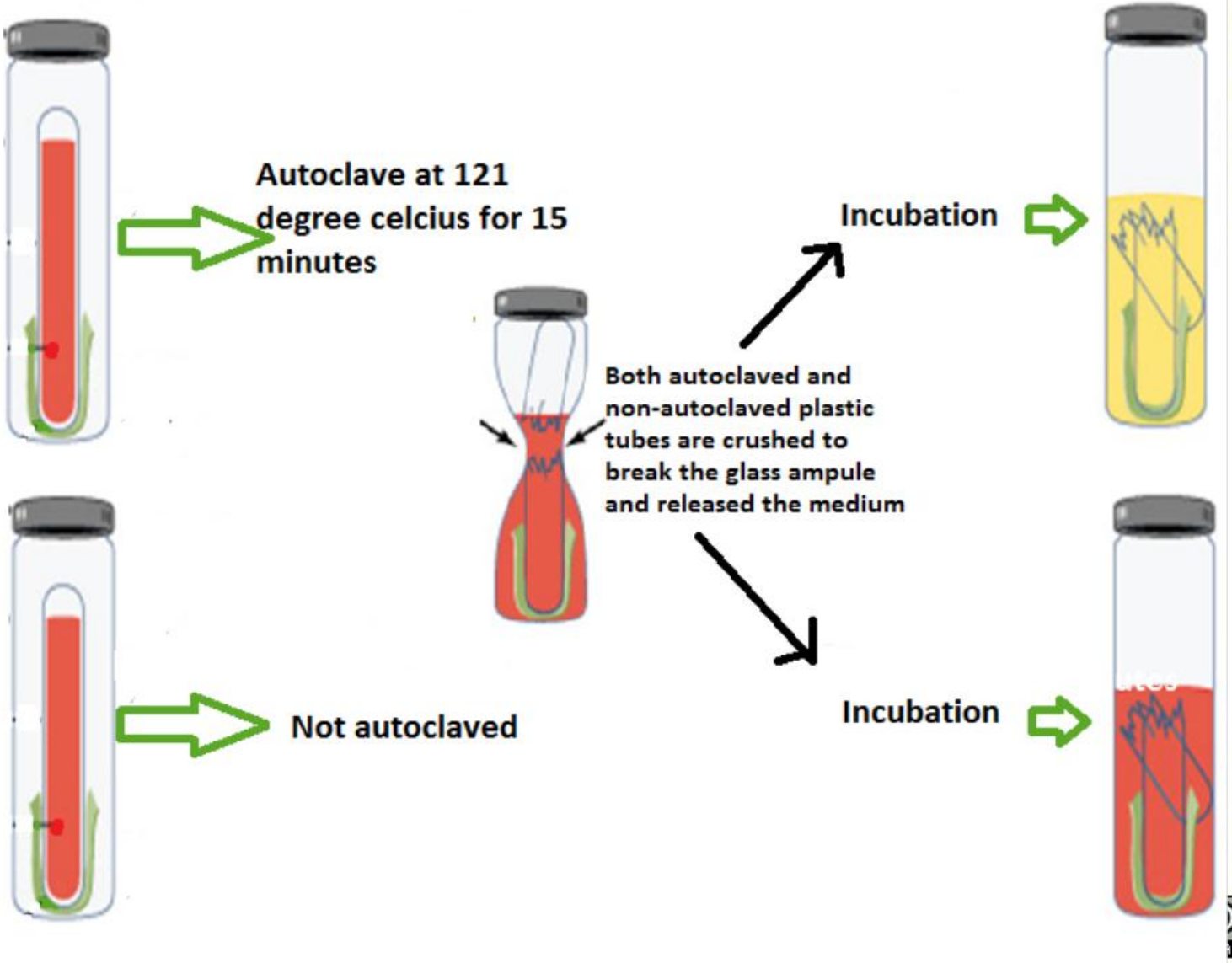
Problem achieving sterility

- is that not all organisms exposed to the same lethal agent die at the same time.

PRINCIPLES OF STERILIZATION

- **Endospores (*Bacillus sp.* & *Clostridium sp.*)**

- standard for sterility



Approximation of number of cell death

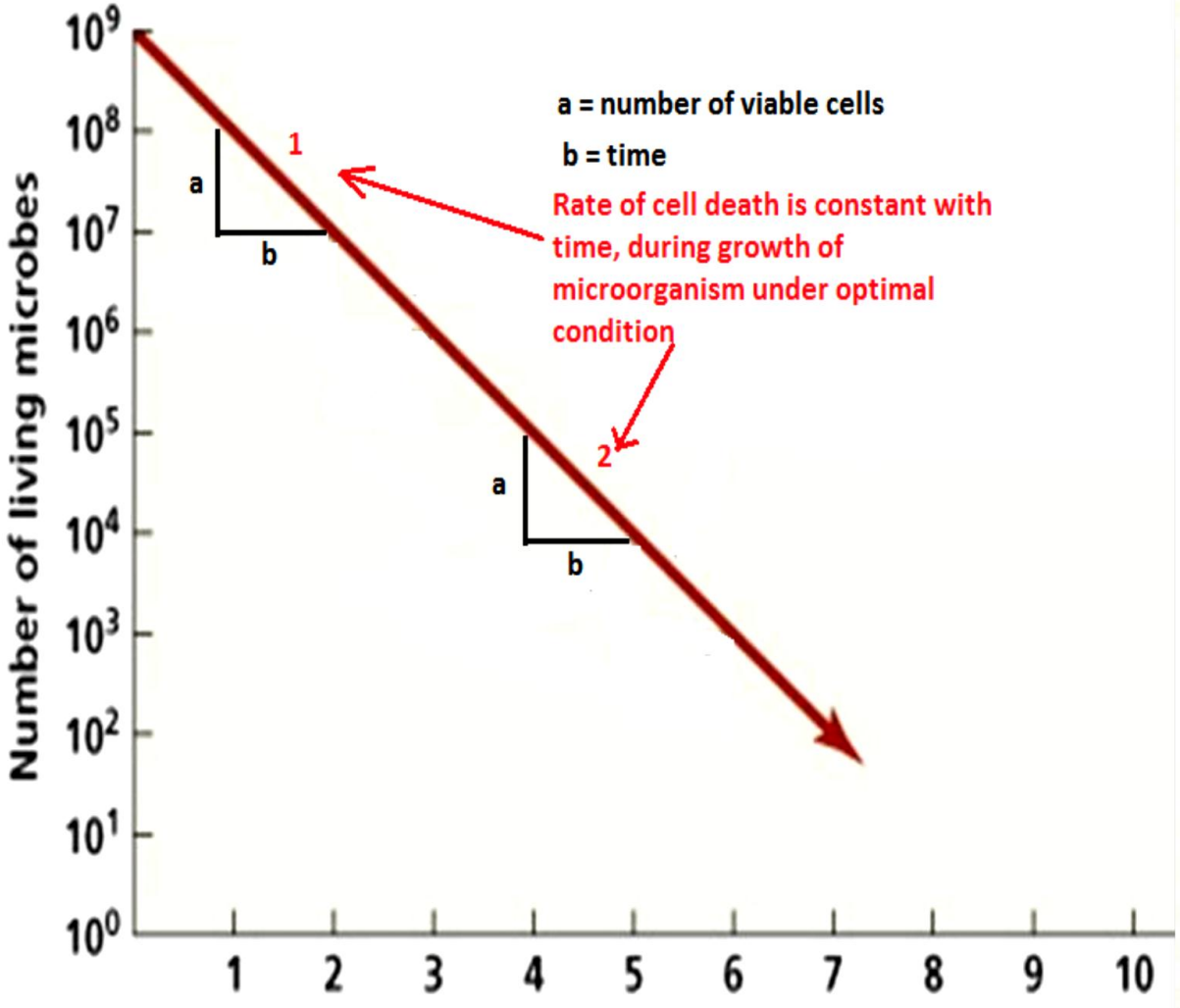
- The number of organisms decreases exponentially with time.

- If you plot the logarithm of the number of survivors (viable cell) as a function of time of exposure, a straight line is obtained.

- The negative slope of the line defines the death rate.

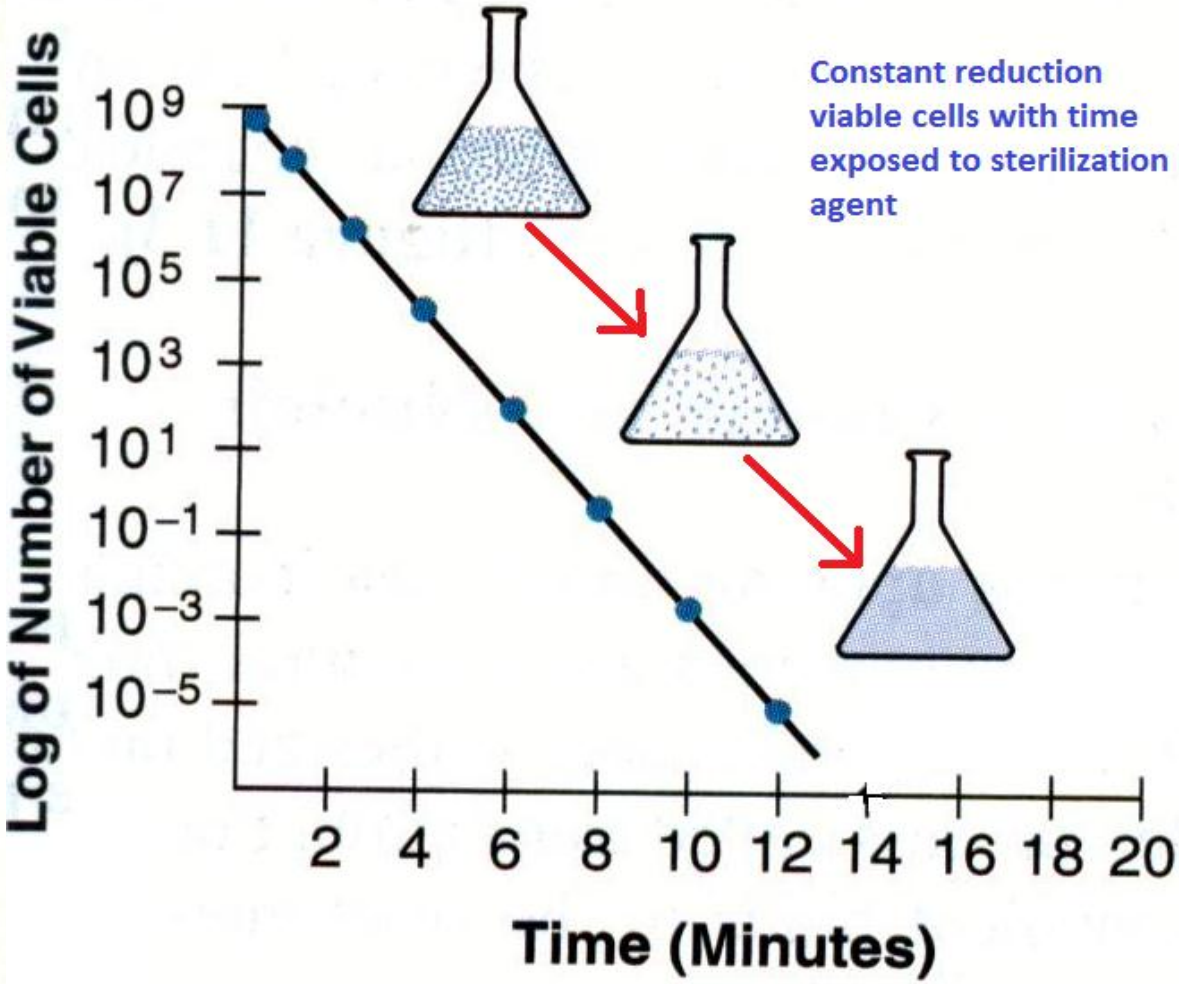
Approximation of number of cell death

- Microbial death : results in the permanent loss of reproductive capability even under optimum growth conditions.



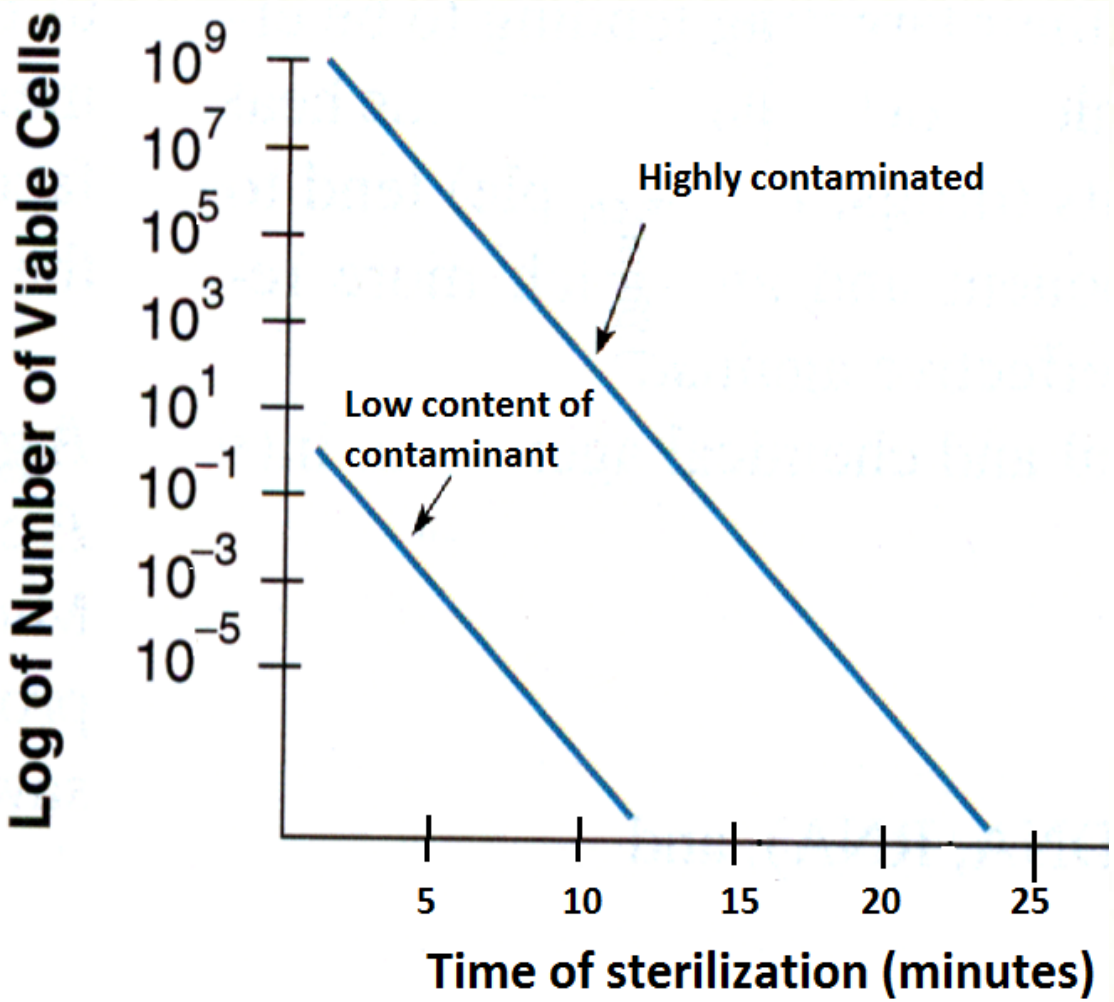
Factors affecting microbial death

- Microbial death is influenced primarily by time exposed to antimicrobial agent.



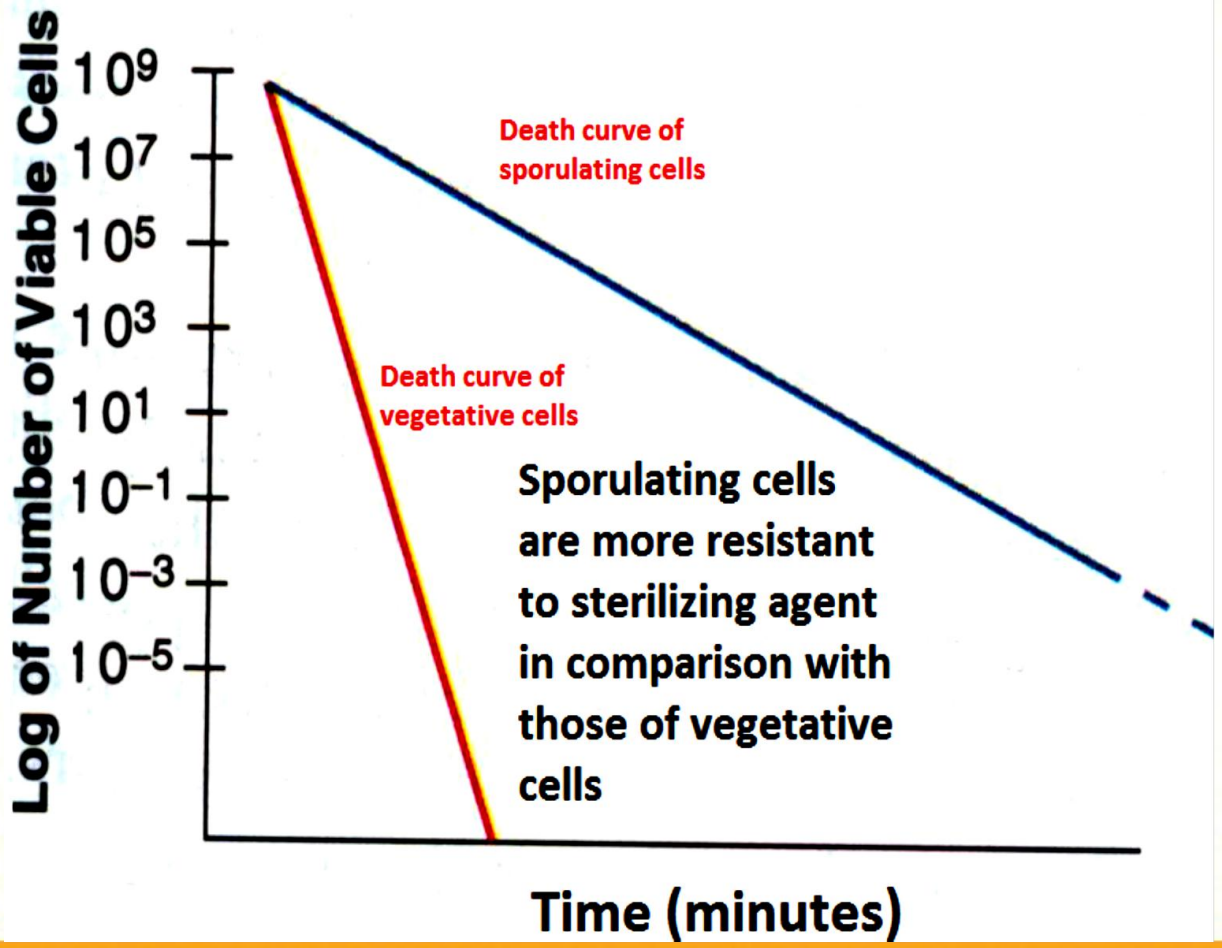
Factors affecting microbial death

- The number of microorganism: a higher load or concentration of contaminants requires more time to be destroyed.



Factors affecting microbial death

- The nature of the microorganisms in the population (microbial resistant to the agent of sterilization used)

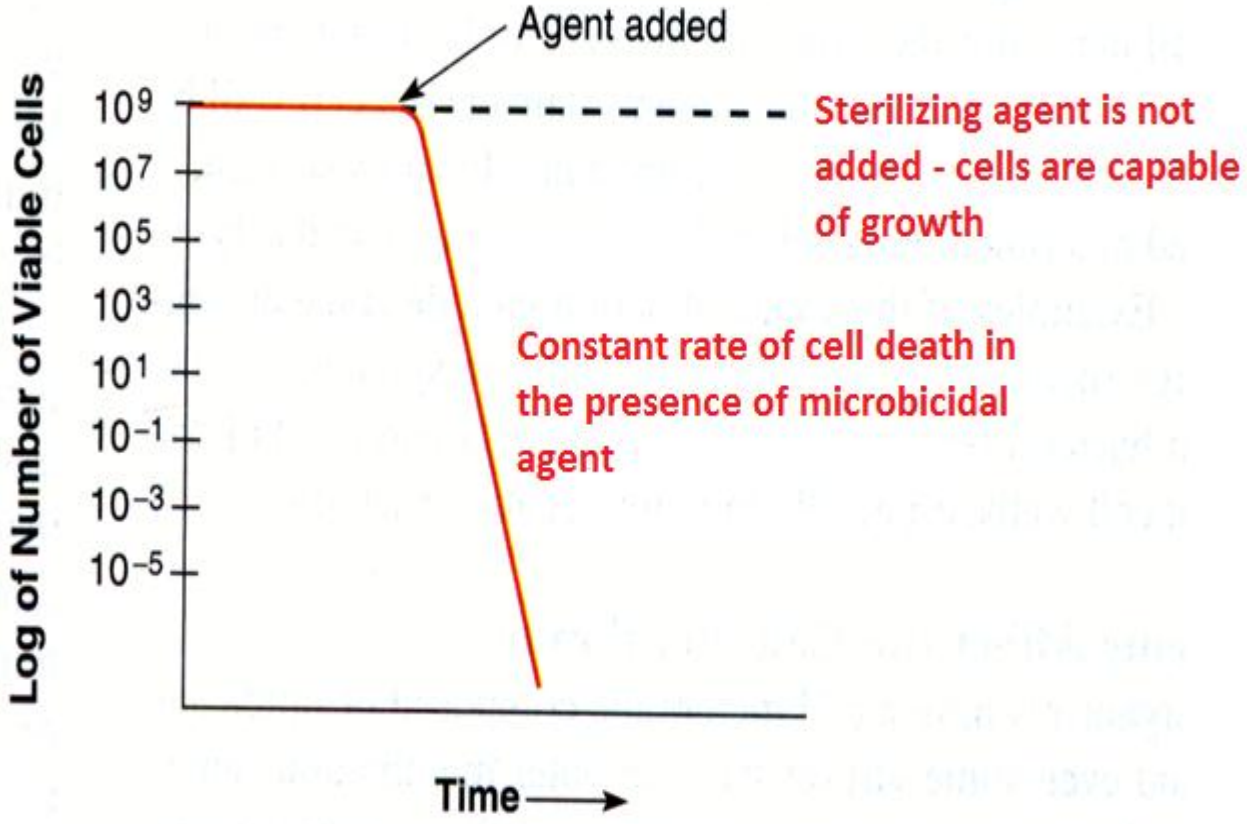


Factors affecting microbial death

- The temperature and pH of the environment.
- The concentration (dosage) of the agent. For example, UV radiation is most microbicidal at 260nm; most disinfectants are more active at higher concentration.
- The presence of solvents, interfering organic matter and inhibitors.

Factors affecting microbial death

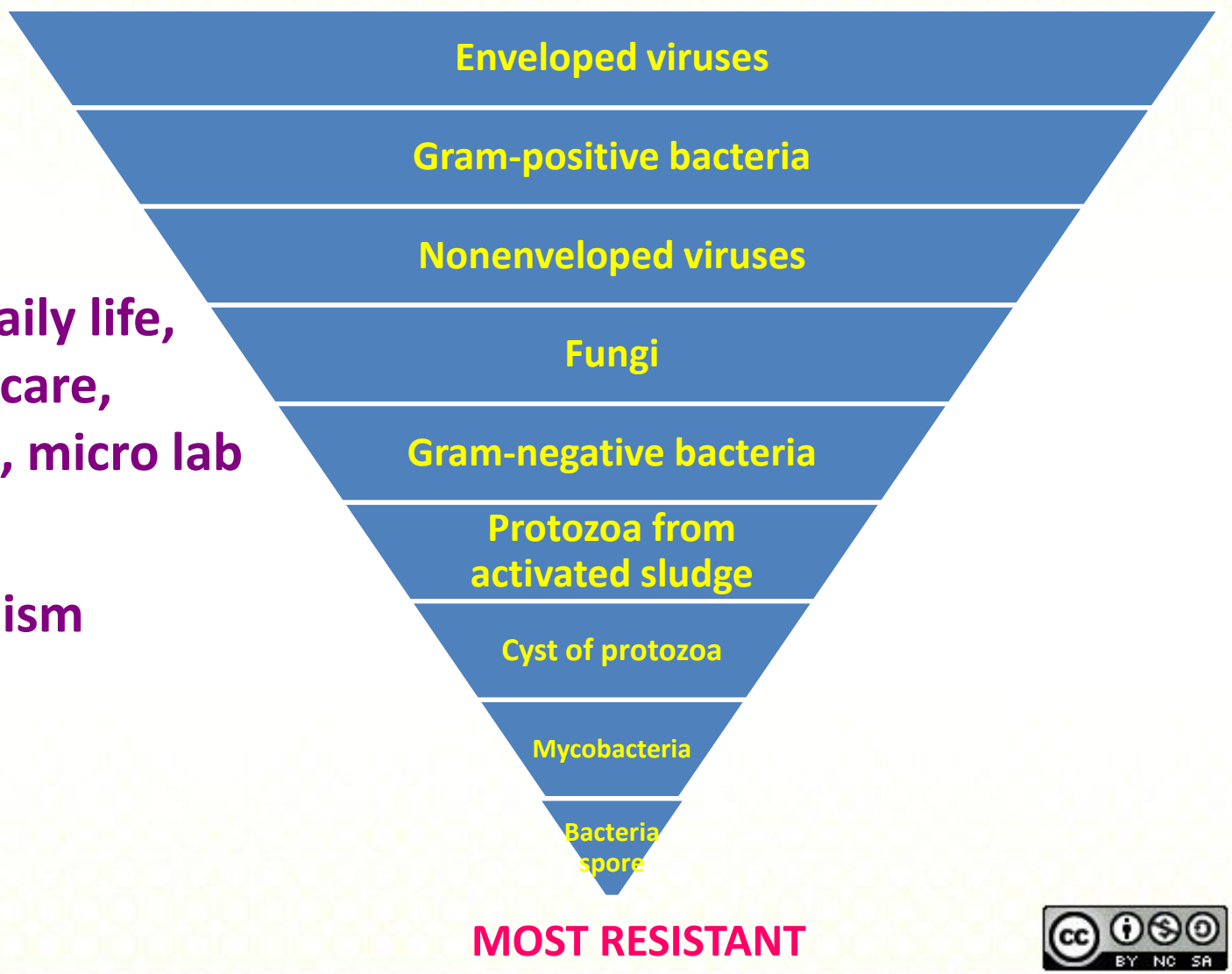
- The mode of action of the agent. How does it kill or inhibit the microorganism?



Factors Affecting Type of Bacterial Control

i. Risk level - daily life, kitchen, day care, hospital, ICU, micro lab

ii. Target Organism



Factors Affecting Type of Bacterial Control

iii. Risk of Infection - pathogen virulence, patient susceptibility

iv. Site to be treated - live tissue, plastic (syringes), metal (surgical equipment), glass

v. Number of Organisms

vi. Environmental Factors - pH, temperature, surface

vii. Toxicity or damage from agent - biodegradability, damage to tissue

viii. Cost and feasibility

Actions of Antimicrobial Agent

1. Attacking cell walls and membranes - destroy integrity

Surfactant inserting in the lipid bilayers, disrupt its integrity and create abnormal channels that alter permeability, thus cause leakage both into and out of the cell.

Actions of Antimicrobial Agent

2. Damaging Proteins and Nucleic Acids -denature and interrupt metabolic processes

(a) the native (functional) state is maintained by bonds that create active sites to fill the substrate. Some agents denature the protein by breaking all or some secondary and tertiary bonds.

Results are:

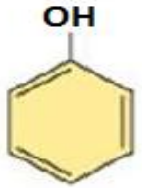
(i) complete unfolding

(ii) random bonding and incorrect folding

(iii) some agents react with functional groups on the active site and interfere with bonding.

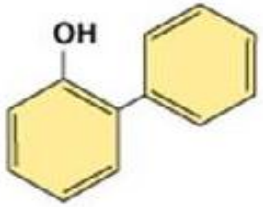
Methods of Evaluating Disinfectants

A. Phenol Coefficient: comparing the activity of a given disinfectant with that of phenol.

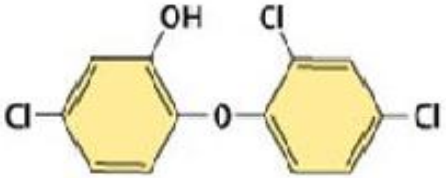


Phenol

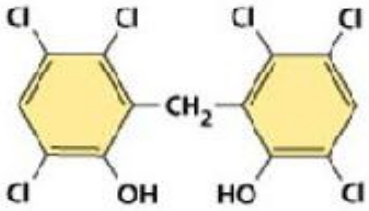
Phenolic compounds commonly available in disinfecting agent



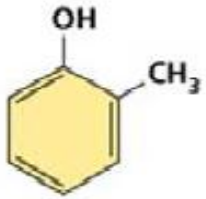
Orthophenylphenol



Triclosan



Hexachlorophene



Orthocresol

Methods of Evaluating Disinfectants

B. Use-Dilution test

- Three bacteria used are *Salmonella choleraesuis, Staphylococcus aureus* and *Pseudomonas aeruginosa*, grown in liquid media.
- Metal carrier rings are dipped into the liquid cultures.
- Cells in the liquid culture are dried at 37^{o}C for a short time.
- Dried cultures are placed into a solution of disinfectant at the concentration recommended by manufacturer and left for 10 minutes at 20^{o}C.
- Carrier rings are transferred into the medium that will permit growth of any surviving bacteria.
- The effectiveness of the disinfectant can be determined by the number of cultures that grow.

Methods of Evaluating Disinfectants

C. Disk Diffusion test

- A disc of filter paper is soaked with a chemical and placed on an agar plate that has been previously inoculated and incubated with the test organism

- If the chemical is effective a clear zone representing inhibition of growth can be seen around the disk.

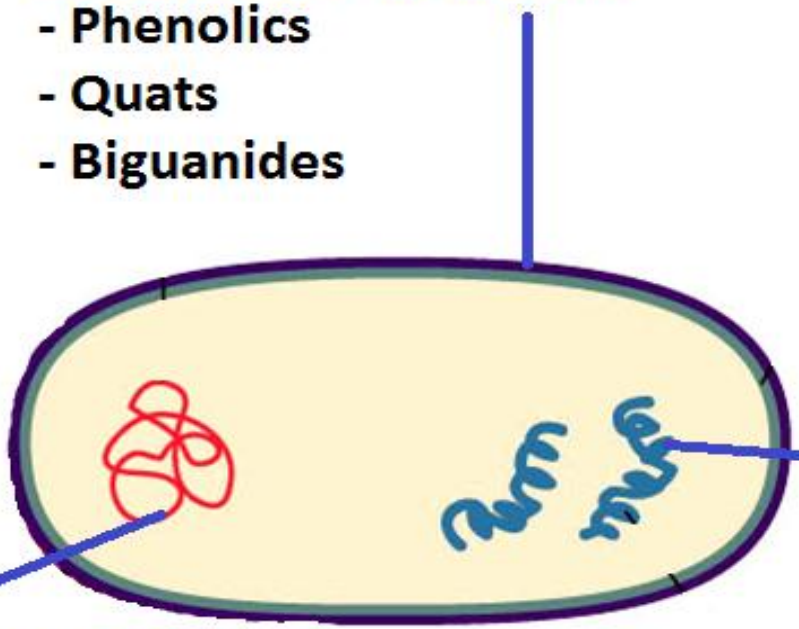
Physical Methods of Disinfection

Method & use	Description	Advantages	Disadvantages
Heat - coagulates - denatures - proteins			
Autoclave (moist heat) lab materials	15psi, 121°C, steam, 15 min	Sterilizes everything including endospores	Destroys heat labile items
Pasteurization (moist heat) milk & wine	30 min @ 63°C & 15 min @ 72°C	Good for liquids, retains flavor and consistency	Does not sterilize, only kills pathogens
Dry Heat food products, glassware, instruments	2hrs@ 170-180°C	Good for dry powders, glassware, instruments, destroys endospores	Destroys heat labile items. Takes a long time
Microwaves food	Molecular movement produces heat	Fast and penetrating	Uneven
Ultraviolet Light air vents, surgical suites	220-300nm light - damages DNA T-T dimers	Disinfects large surfaces	Does not penetrate or go under surfaces potentially harmful if people are exposed
Gamma Radiation Food- Pasta, strawberries, meat	ionizing radiation - produces damaging highly reactive particles called free radicals (O_2^-) (OH•)	Does not alter food flavor, sterilizes indefinitely -	Expensive, potentially harmful if people are exposed
Filtration air, water, viral media	Using a filter to capture organisms	Pore size selects organisms Does not alter fluid composition, good with air	Expensive, can be clogged or contaminated (water, air, fluids)

Chemical Methods of Control

Chemical	Description	Advantages	Disadvantages
Alcohols	denatures proteins dissolves lipids in membranes	Available, cheap, easy to use	inactivated by organic materials contact time
Aldehydes (formaldehyde & gluteraldehyde)	alkylating agent alters protein & nucleic acid	kills all microbial life forms	contact time harmful
Ethylene Oxide gas	alkylating agent alters protein & nucleic acid	kills all microbial life forms, gas penetrates	carcinogenic
Halogens (Cl & I) cleaning, drinking water, wounds	Halogens create free radicals, are strong oxidizing agents destroying essential metabolic compounds	Inexpensive, available, can be used in doses compatible with living tissue	inactivated by organic material, not effect on endospores
Metals ($AgNO_3$)	combine with and inactivate enzymes	oligodynamic - small amounts = lethal to microbes	toxic and expensive
Phenol (hexaclorophene, triclosan)	coagulates proteins & disrupts membranes	can be mixed with detergents, leaves antimicrobial residue	increasing resistance
QUATS Quaternary ammonium compounds benzalkonium chloride	denature cell proteins, disrupts membranes, interfere with metabolic processes	effective in very small quantities, does not destroy instruments	Useable only on inanimate objects
Peroxide (H_2O_2)	creates oxygen and free radicals	inexpensive, physical bubbling action	destroyed by catalase

Summary of Mechanism Using Chemical Method



Cytoplasmic membrane
- Phenolics
- Quats
- Biguanides

DNA
- Ethylene oxide
- Aldehydes

Protein
- Alcohol
- Aldehides
- Halogen
- Metal
- Ozone
- Peroxigen
- Phenolics

Sterilization

MOIST HEAT STERILIZATION

- Moist heat sterilization is the most efficient biocidal agent.
- Moist heat may be used in three forms to achieve microbial inactivation:
  1. Dry saturated steam – Autoclaving
  2. Boiling water/ steam at atmospheric pressure
  3. Hot water below boiling point

I. AUTOCLAVING / STEAM UNDER PRESSURE

Principle

- Most commonly however sterilization by moist heat requires steam under pressure at temperature range of 121-134^{o}C

- The steam used for sterilization must be both Dry and Saturated.

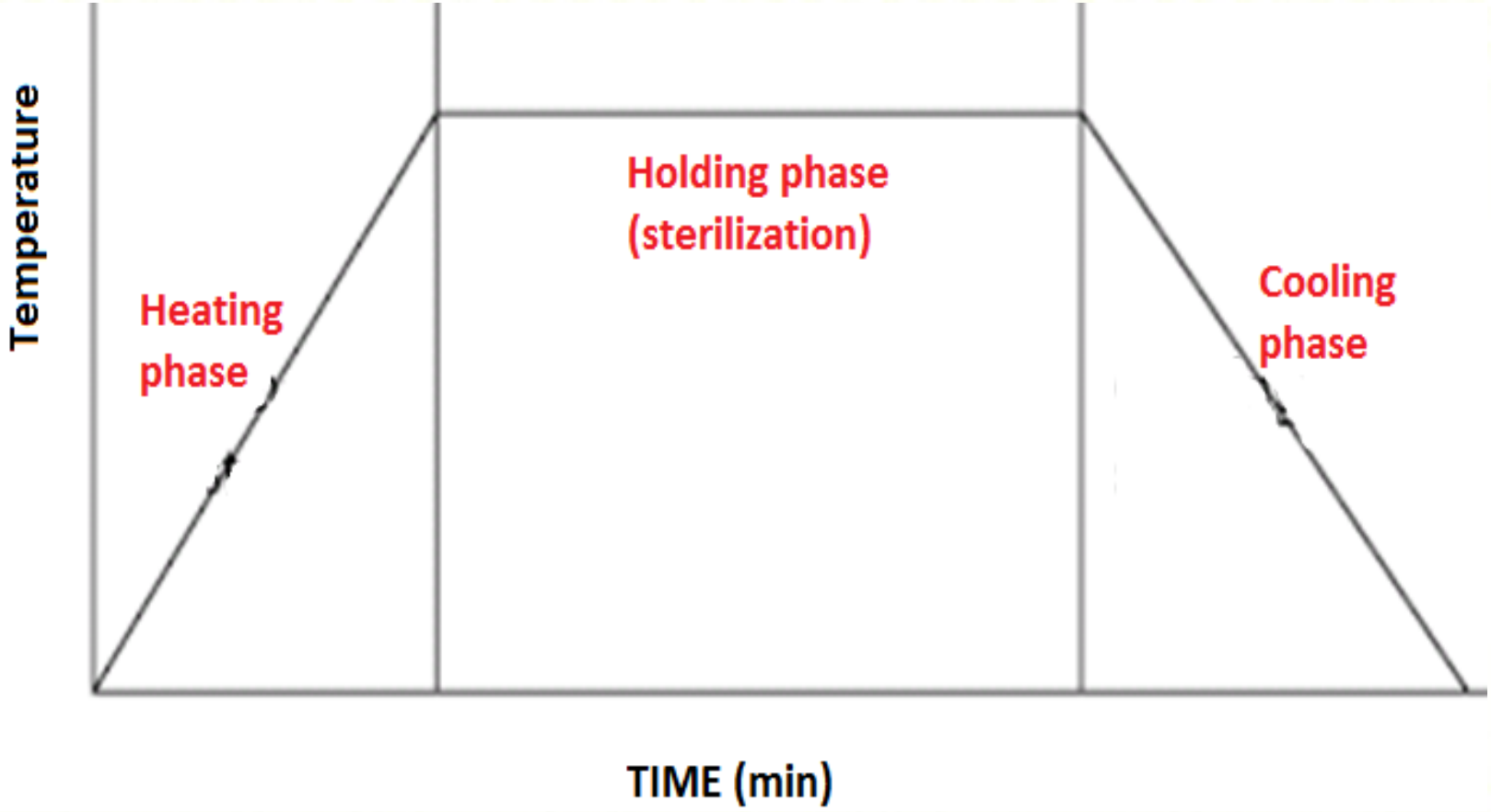
  - Saturated : the steam is at a phase where it is holding all the water that it can, in the form of transparent vapour. It does not contain water droplets and therefore it is described as being dry; if it meets an object cooler than itself it condenses.

I. AUTOCLAVING / STEAM UNDER PRESSURE

Heat sterilization process involves 3 stages :

- **Heating up stage** – the heat raised to sterilization temperature
- **Holding stage** - the objects have to be held at the sterilizing temperature for a sufficient period of time to ensure sterilization.
- **Cooling stage** - the objects are cooled to temperatures that are safe for handling

Stages of heat sterilization



I. AUTOCLAVING / STEAM UNDER PRESSURE

- Moist heat sterilization i.e. Autoclaving is performed in an **autoclave**. The most common conditions for sterilization under pressure include :

PROCESS	TEMPERATURE (°C)	HOLDING TIME (min)	PRESSURE (psi)
MOIST HEAT STERILIZATION	121	15	15
	126	10	20
	134	3	30

Basically operation of an autoclave involves:

i. Ensure there is sufficient water in the autoclave to produce required steam – usually automatic

ii. Load sterilization chamber - Ensure that all products required to be sterilized are accessible

iii. Close chamber door

iv. Air removal –all air must be removed before sterilization can commence

v. Start sterilization cycle (include heating, holding and cooling stages)

vi. Remove objects

vii. All through the process monitor sterilization temp and pressure

There are various types of autoclaves

1. **Simple non-jacketed** - small electrically heated sterilizers with simple air expulsion system – steam sterilization of unwrapped instruments and utensils – used in medical (district hospital) and dental community

Disadvantages:

- Requires personal attention to monitor process
- Method of air discharge inefficient cannot really monitor that all air has been removed
- There is no internal thermometer to check coolest part of load
- There is no mechanism for drying the load

There are various types of autoclaves

2. **Downward displacement autoclaves** - These are usually jacketed horizontal or cylindrical in shape. The air is removed by displacement by steam through an automatic air and condensate discharge valve at the base of the autoclave.

There are 3 main types:

- Laboratory autoclaves
- Hospital - for sterilizing unwrapped surgical instruments used in hospital theaters
- Fluid sterilizers - autoclaves for sterilizing fluid in sealed containers e.g. infusions injections etc.

There are various types of autoclaves

3. **Porous load autoclaves**

– these autoclaves are used for porous materials such as towels surgical gowns, dressings, swabs, gauzes, sheets etc.

- The sterilizer is equipped with a vacuum system to ensure a high degree of air removal and ensure no moisture remains on the sterilized objects.

Sterilization

DRY HEAT STERILIZATION

Examples of Dry heat sterilization are:

- Incineration
- Red heat
- Flaming
- Hot air oven

Dry Heat Sterilization

- bacterial spores are more resistant to dry heat than in the presence of moisture.

- this is due to the fact that sterilization by using hot air made diffusion and penetration of heat into the load is slow, where air is an inefficiency heating medium.

Dry Heat Sterilization

- In general dry heat sterilization requires higher temperatures and extended periods of sterilization

PROCESS	TEMPERATURE (°C)	HOLDING TIME (min)
DRY HEAT STERILIZATION	160	120
	170	60
	180	30

Dry Heat Sterilization

- Due to the high temperatures required for dry heat sterilization can only be used for thermostable, moisture sensitive or moisture impermeable pharmaceutical and medicinal.

These include products like;

- Dry powdered drugs
- Suspensions of drug in non aqueos solvents
- Oils, fats waxes, soft hard paraffin silicone
- Oily injections, implants, ophthalmic ointments, ointment base

Dry Heat Sterilization

Hot air oven

It comprises of :

- an electrically heated, insulated stainless steel chamber fitted with insulated door. The inner surfaces are polished to minimize heat loss.
- Heating arrangements are made around the chamber walls. Heat is delivered to the load primarily by **convection and radiation.**
- A fan is fitted for efficient air circulation and heat distribution within the oven.
- It is also fitted with thermostat to control the temperature.

Dry Heat Sterilization

Used for:

- sterilizing glassware for dry use, for powders, fats, oils and greases that are not penetrable by moist heat.. Powders, oils, fats and grease must be packed in small quantities or in thin layers to enable penetration of heat. Not recommended for plasticware

- The glassware must be dried first before being loaded into a hot oven to avoid cracking.

- The temperature varies between 160^{o}C- 170^{o}C and duration of 1-2 hours.

- Ample spacing must be allowed in the oven to permit free circulation of hot air.

Dry Heat Sterilization

Gaseous sterilization

- Two gases are used for sterilization purposes ethylene oxide and formaldehyde
- Only ethylene dioxide used commonly
- Ethylene dioxide acts as an alkylating agent of proteins, RNA, DNA, as bactericidal

Dry Heat Sterilization

Radiation

i. Ultraviolet light
ii. Ionizing radiation

Dry Heat Sterilization

Ultraviolet light

- Wavelength around 260 (240-280) produced by Mercury vapor lamp is used to kill microbes, but it has little penetrating power for most materials.
- Used to :
  - sterilize air and surface in rooms and biosafety cabinets.
  - to treat water passing in a thin layer under appropriate U.V lamp.
- It is harmful to skin and eyes and workers using it must wear appropriate protective garments.
- U.V light is perhaps the most lethal component in ordinary sunlight used in sanitation of garments or utensils.

Dry Heat Sterilization

Ionizing radiation

- Includes high-speed electrons, X-rays and G-rays. They have good penetrative power.

- G-rays from Cobalt 60 are used to sterilize antibiotic, hormones, sutures, plastics and catheters. It has also been found effective in the pasteurization of meat and other foods such as poultry, fish and fruits.

Dry Heat Sterilization

Filtration

There are two types of filters:

i. Depth filters
ii. Membrane filters

Dry Heat Sterilization

Depth filters

- Consist of fibrous or granular materials so packed as to form twisted channels of minute dimensions.

- They are made of diatomaceous earth (Berkerfield), unglazed porcelain (chamberlain) filter, sintered glass or asbestos.

- ***Setz filter:*** consist of a pad of asbestos secured by clamps at the base of cylindrical structure. The pad assembled in the funnel is heat sterilized and the fluid to be sterilized is passed through the pad by means of suction.

Dry Heat Sterilization

Membrane filters

- These are porous membrane about 0.1mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate, and polyvinylidene fluoride, or some other synthetic material.
- The membranes are supported on a frame and held in special holders.
- Fluids are made to transverse membranes by positive or negative pressure or by centrifugation.

Methods	Mechanism	Merits	Demerits	Applications
Heat sterilization	Destroys bacterial endotoxins	Most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents.	Can be applied only to the thermostable products	Dry heat is applicable for sterilizing glasswares 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.
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.
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 particles in the dry state; including surgical instruments, sutures, prostheses, unit dose ointments, plastics
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 area