UNIT- III
(CHAPTER-9)
FUNGI AND VIRUS

Points to be covered in this topic

- 1. INTRODUCTION
- 2. FUNGI (CLASSIFY, REPRODUCTION)
- 3. FUNGI- CULTIVATION
- 4. VIRUS- CLASSIFY, REPRODUCTION
- 5. VIRUS - CULTIVATION

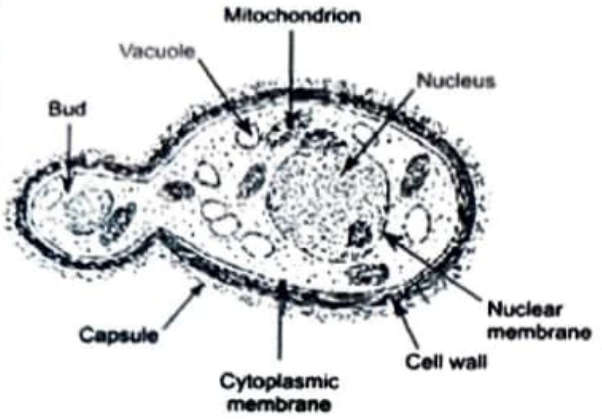
❑ INTRODUCTION

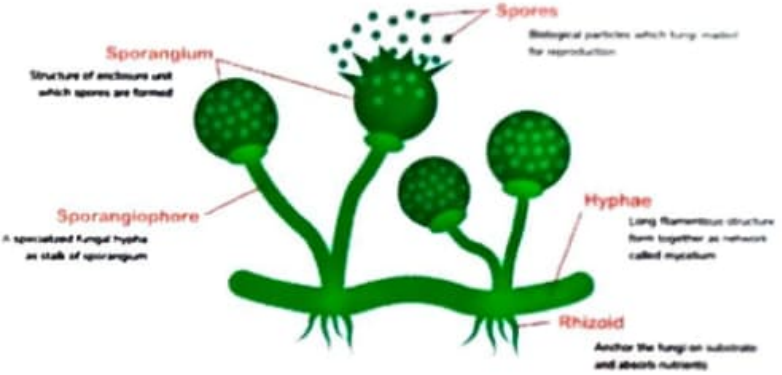
❖ FUNGI

- Fungi are **eukaryotic organisms** that include microorganisms such as **yeasts, molds and mushrooms**. Fungi may be **unicellular** or **multicellular**. Fungi are **heterotrophic** organisms which means they **require organic compound for nutrition or growth.**
- Study of fungi is generally known as **mycology.**

➢ MORPHOLOGY

- Fungi are divided into two morphological forms viz. yeasts and hyphae (mold).





- Yeasts cells are generally **larger than most of the bacteria.**
- Size of yeast ranging from **1 to 5 micrometers** in width and from **5 to 30 micrometers in length.**
- Flagella or other organelles of **locomotion are absent in yeast.**
- Cell wall constituents of fungi are mainly **chitin** and **glucans.**
- Multicellular fungi are composed of networks of long filamentous **branched structure called hyphae.**
- The hyphae often aggregate in a thread like dense network known as **mycelium.**

➢ CLASSIFICATION

BASED ON MODE OF NUTRITION:

1. Saprophytic- The fungi obtain their nutrition by feeding on dead organic substances. Examples: Rhizopus, Penicillium and Aspergillus.

2. Parasitic - The fungi obtain their nutrition by living on other living organisms (plants or animals) and absorb nutrients from their host. Examples: Taphrina and Puccinia.

3. Symbiotic- These fungi live by having an interdependent relationship association with other species in which both are mutually benefited. Examples: Lichens and mycorrhiza.

BASED ON SPORE FORMATION:

Kingdom Fungi are classified into the following based on the formation of spores:

1. **Zygomycetes -** These are formed by the **fusion of two different cells**. The **sexual spores** are known as **zygospores** while the **asexual spores are known as sporangiospores.** The hyphae are without the septa.

2. **Ascomycetes-** They are also called **as sac fungi**. They can be coprophilous, decomposers, parasitic or saprophytic. **The sexual spores are called ascospores.** Asexual reproduction occurs by conidiospores. Example - **Saccharomyces** .

3. **Basidiomycetes - Mushrooms are the most commonly** found basidiomycetes and mostly **live as parasites.** Sexual reproduction occurs by basidiospores. Asexual reproduction occurs by conidia, budding or fragmentation. Example- **Agaricus.**

4. **Deuteromycetes -** They are otherwise **called imperfect fungi** as they do not follow the regular reproduction cycle as the other fungi. **They** do **not reproduce sexually.** Asexual reproduction **occurs by conidia**. Example- **Trichoderma.**

MORPHOLOGICAL CLASSIFICATION :

- Yeast,
- Yeast like fungi
- Molds
- Dimorphic fungi

1. YEAST :

- They are **round, oval, unicellular fungi**.
- It contains only single cell.
- They are **aerobic and some are facultative anaerobic.**
- They forms circular, smooth, creamy white colonies on the surface of media.
- They generally reproduce by **asexual reproduction.**

2. YEAST LIKE FUNGI :

Some yeast like candida albicans, in this the bud remains attached to the mother cell and **elongates followed by repeated budding and forms chains of elongated cells of fungi.**

3. Molds :

- It contain multiple nuclei and grows in the form of mycelium or hyphae of filaments.
- It gives fuzzy appearance on the surface of media and **forms black, green, brown, orange or pink color.**
- They are strictly aerobic.
- They generally **grow under 22-28 degree Celsius.**
- They can reproduce by either asexual or sexual reproduction. **EX- Aspergillus Niger..**

4. DI MORPHIC FUNGI :

- In this dimorphic, di means two and morphic means.
- Some fungi who mainly belongs to pathogenic species exhibits **dimorphism.**
- Such fungi can either grow as a yeast or mold.
- Ex- **Mucor rouxii, Histoplasma capsulatum.**

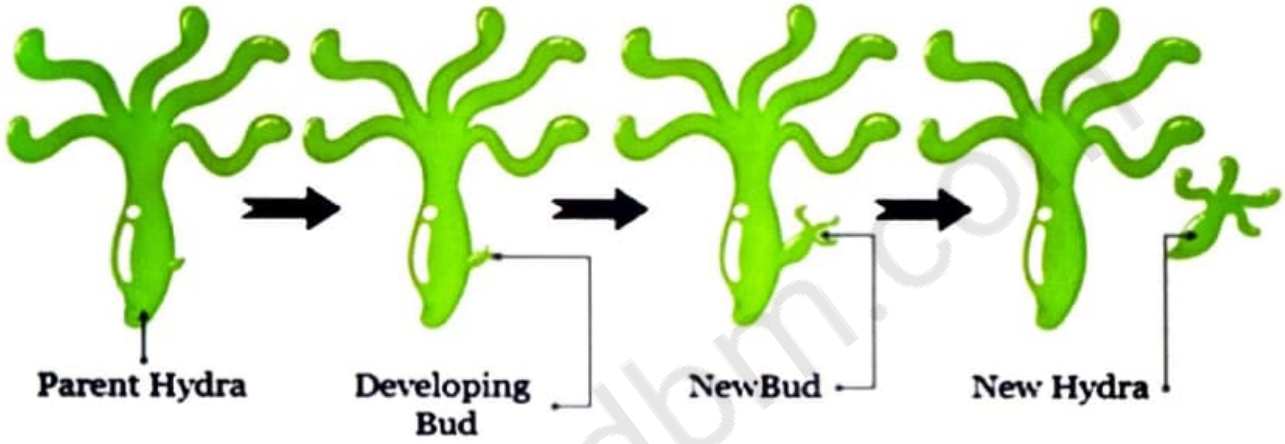
REPRODUCTION

Reproduction in fungi is both by sexual and asexual means. The sexual mode of reproduction is referred to **as teleomorph** and the asexual mode of reproduction is referred to **as anamorph.**

- **Vegetative reproduction** –This takes place by budding, fission and fragmentation.

BUDDING

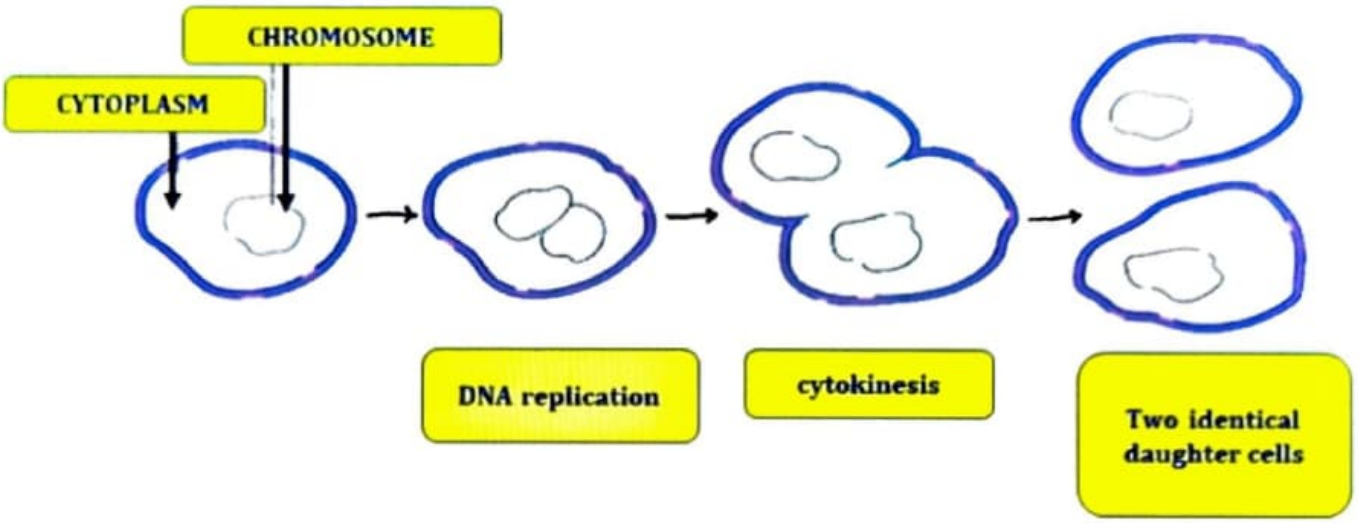
Both hydra and yeast reproduce by the process of Budding.



- Budding in hydra involves a small bud which is developed from its parent hydra through the **repeated mitotic division of its cells.**
- The small bud then receives its nutrition from the **parent hydra and grows** healthy. Growth starts by developing **small tentacles** and the mouth.
- Finally, the small newly produced hydra **gets separated from its parent hydra and becomes an independent organism.**

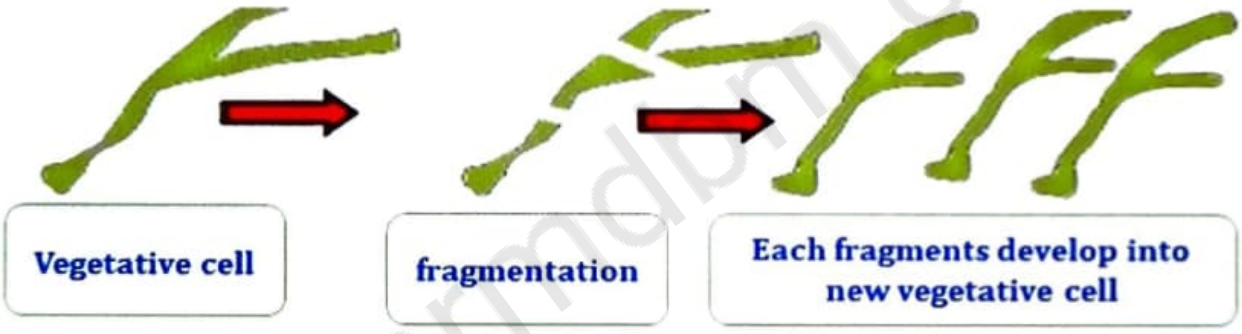
BINARY FISSION

- Separation of the body into two new bodies. In the process of binary fission, an organism **duplicates its genetic material,** or deoxyribonucleic acid DNA and,
- Then divides into two parts cytokines, with each **new organism receiving one copy of DNA.**

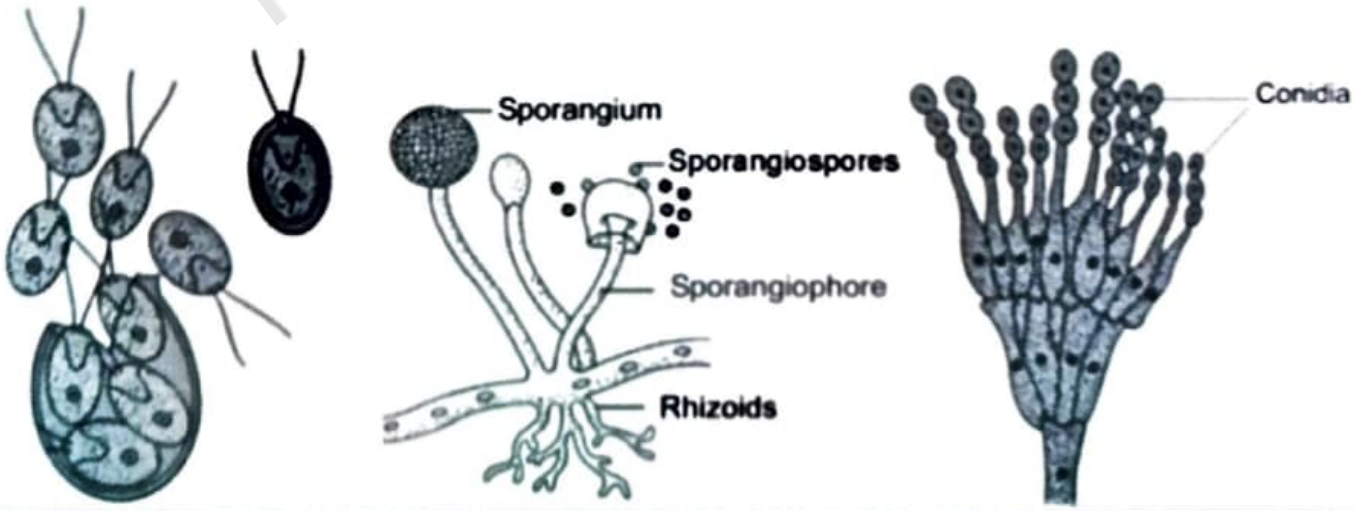


- **FRAGMENTATION**

A parent breaks into multiple pieces, and **each develops into a fully functioning independent individuals.**

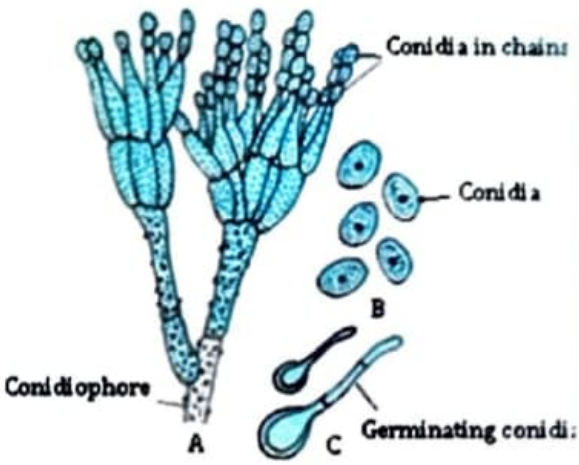


❖ **ASEXUAL REPRODUCTION**



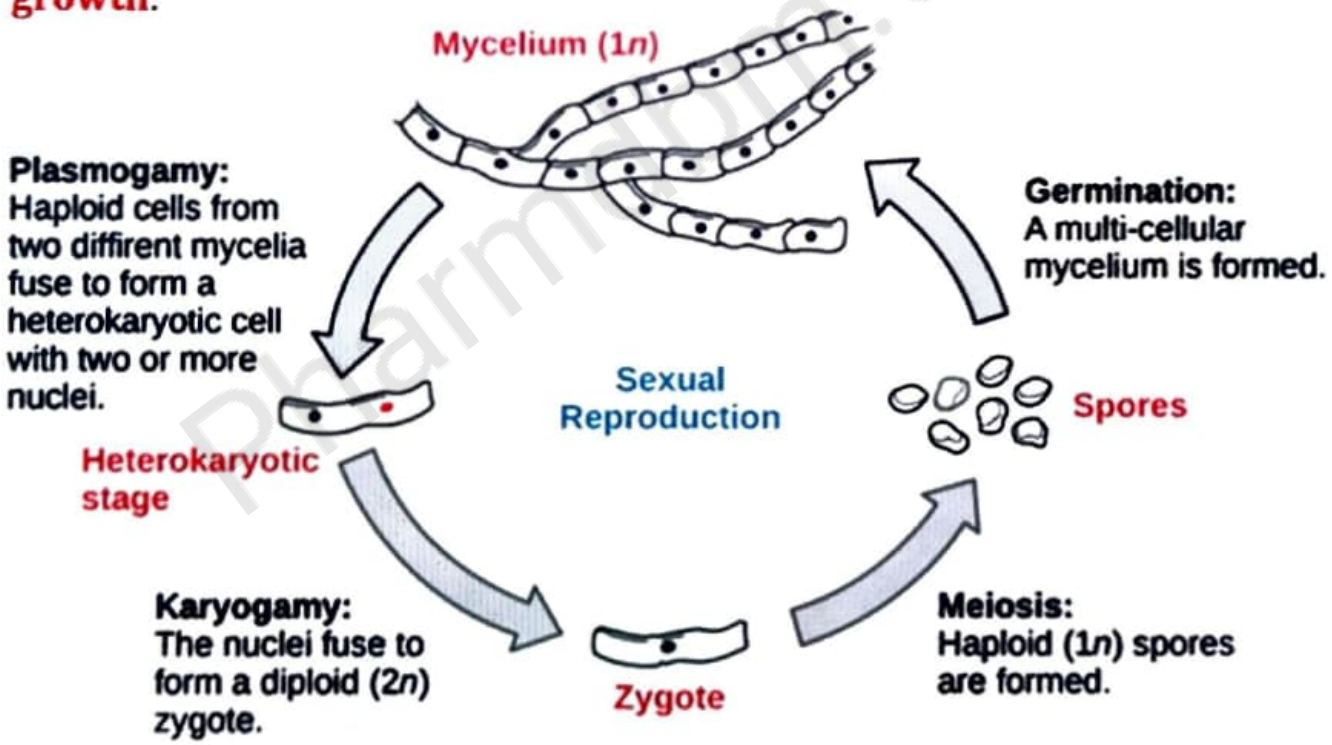
1. ZOOSPORES	2. SPORANGIOSPORES	3. CONIDIA

Conidium, a type of asexual reproductive **spore** of **fungi** (kingdom Fungi) usually produced at the tip or side of hyphae (filaments that make up the body of a typical fungus) or on special spore-producing structures called **conidiophores**. The spores detach when mature.



❖ SEXUAL REPRODUCTION

- During the process of sexual reproduction, **a huge number of sperms are produced from the parents' body.**
- The produced sperms disperse either floating on the wind or hitching a ride on an animal, as they are lighter and smaller than the seeds. **The dispersed sperms land in an environment that will support their growth.**



➢ There are many variations in fungal sexual reproduction, which includes the following three stages.

1. **Plasmogamy:** The fusion of protoplasm.
2. **Karyogamy:** The fusion of nucleus.
3. **Meiosis:** Cell cycle involved with the nuclear division.

CULTIVATION

- Saturated Agar is a type of agar growth medium containing peptones used to cultivate fungi and **can also grow filamentous bacteria**. (Nocardia)
- It was developed by **Raymond sabouraud in year 1892**, it also contains antibacterial agent used to kill the contaminating bacterial species.
- The standard temperature **incubation of fungi is 30 degree Celsius** and cultures should be circulated in a humidified environment for 21 days.
- They should be inspected daily for a work and at least 3 times weakly.
- After the incubation for **21 days the appearance** of fungi is found.

Characteristics	Fungi	Bacteria
Cell type	Eucaryotic	Procaryotic
Optimum pH	4 to 6	6.5 to 7.5
Optimum temperature	25 to 30°C (saprophytes) 32 to 37°C (parasites)	32 to 37°C (mesophilic)
Cell membrane	Sterols present	Sterols absent except mycoplasma
Oxygen requirement	Strictly aerobic (moulds), Facultative anaerobic (some yeasts)	Aerobic to anaerobic
Light requirement	None	Some photosynthetic groups occur.
Carbon source	Organic	Inorganic/organic
Concentration of sugar in laboratory media	4 to 5%	0.5 to 1%
Cell wall components	Chitin, cellulose or hemicellulose	Peptidoglycan
Susceptibility to antibiotics	Sensitive to griseofulvin, Resistant to penicillins, chloramphenicol etc.	Resistant to griseofulvin, sensitive to penicillins, tetracyclines, etc.

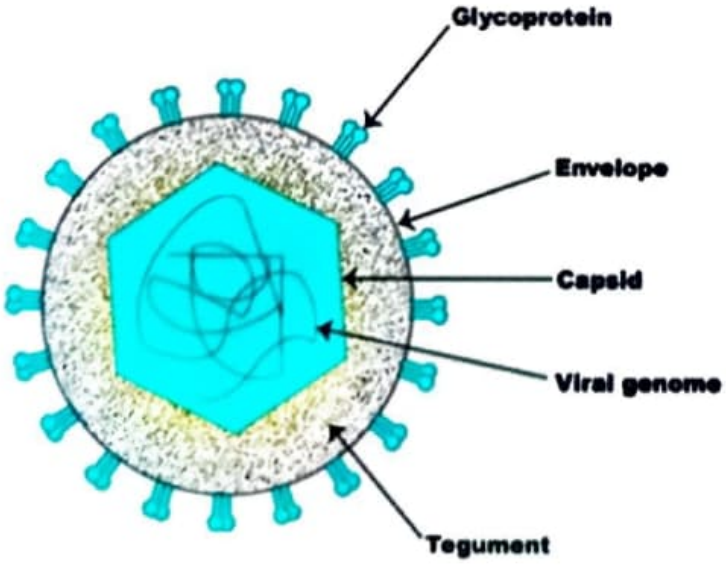
❑ VIRUS

- All viruses are **obligate intracellular parasites**, they inhabit a living or non living both characteristics of both.
- They cannot be observed **using a light microscope.**
- They have no interna cellular structure.

- They are incapable of replication unless **occupying an appropriate living host cell.**
- They are incapable of metabolism.
- Individuals show no **increase in size.**

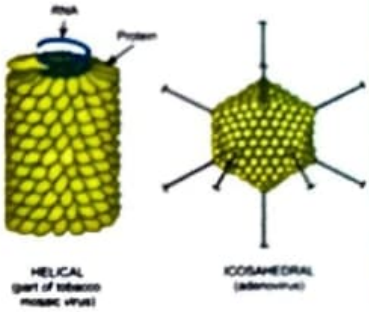
➢ MORPHOLOGY

- A fully assembled infectious virus is **called a virion.**
- The simplest virions consist of two basic components: **nucleic acid** (single or doubled- standard RNA or DNA) and **a protein coat**, the capsid, which functions as a **shell to protect he viral genome from nucleases** and during infection attaches the virion to specific receptors exposed on the host cell.
- The capsid is made up of large number of protein subunits **known as capsomers.**
- The capsid together with nucleic acid is known as nucleocapsid. some virus families have an additional covering, **called the envelope, which is usually acquired during release of progeny virus** by budding process through host cell membrane.
- Viruses containing envelope are **called enveloped viruses and those lacking envelope are generally called naked viruses.**
- N the outer surface of the envelope glycoprotein subunits are exposed in the form of projecting **spikes known as peplomers.**



➢ CLASSIFICATION

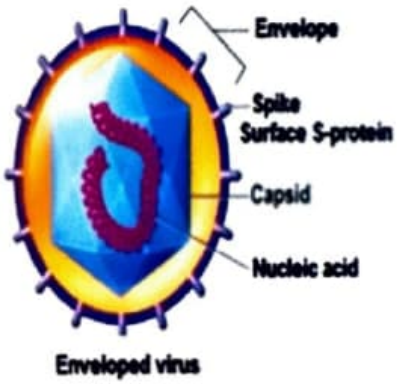
On the basis of their capsid architecture viruses may be classified into several **morphological types:**



1. **Helical viruses:** In helical viruses the viral nucleic acid is found with in a hollow, cylindrical capsid, **which has a helical structure.** The viruses that cause **rabies and EHF (Ebola hemorrhagic fever)** are examples of helical viruses.

2. **Polyhedral viruses:** Many bacterial, plant and animal viruses are many-sided or polyhedral. The capsid of most polyhedral viruses have the shape of an icosahedron, a regular polyhedron with 20 **triangular faces and 12 comers.** The **capsomers of each face from an equilateral triangle. In icosaheclral** capsids the nucleic acid, together with certain proteins, is tightly packed in a central core forming a pool of parallel loops around a cylindrical hole. **eg. Adenovirus, Poliovirus.**

3. **Enveloped viruses:** They are roughly spherical. Helical and polyhedral viruses enclosed by envelopes, are **called as enveloped helical or enveloped polyhedral viruses,** respectively. Influenza virus is an example of **helical virus and herpes simplex virus** is an example of polyhedral (co sahedral) virus.



4. **Complex viruses:** They possess complicated structures, eg: **bacteriophage.** Some bacteriophages have capsids to which additional structures are attached **Poxvirus is another complex virus,** which does not contain clearly identifiable capsids but several coats around the nucleic acid.

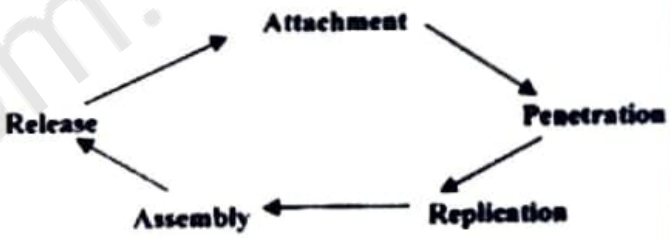
5. **Size and shape:** Most animal viruses are almost spherical. **The pox-viruses are brick shaped with rounded corners**. Influenza virus is usually spherical but sometimes may occur in long filaments.

- classification and taxonomy of viruses is similar to that of bacteria, enzymes and other biological groups.
- In **1948, Holmes** classified viruses on the basis of their host:

(a) **Animal viruses (zoophagineae):** They usually have DNA but may also have RNA and infect man, fowl, pigeon, parrot, dog, cow, etc. and arthropods-insects.

(b) **Plant viruses (phytophagineae):** They have RNA and infect angiosperms like potato, tobacco, sugar cane, cucurbits and any other higher plants.

(c) **Bacterial viruses (phagineae):** They have DNA and are called bacteriophages or simply, phages.



❖ REPLICATION

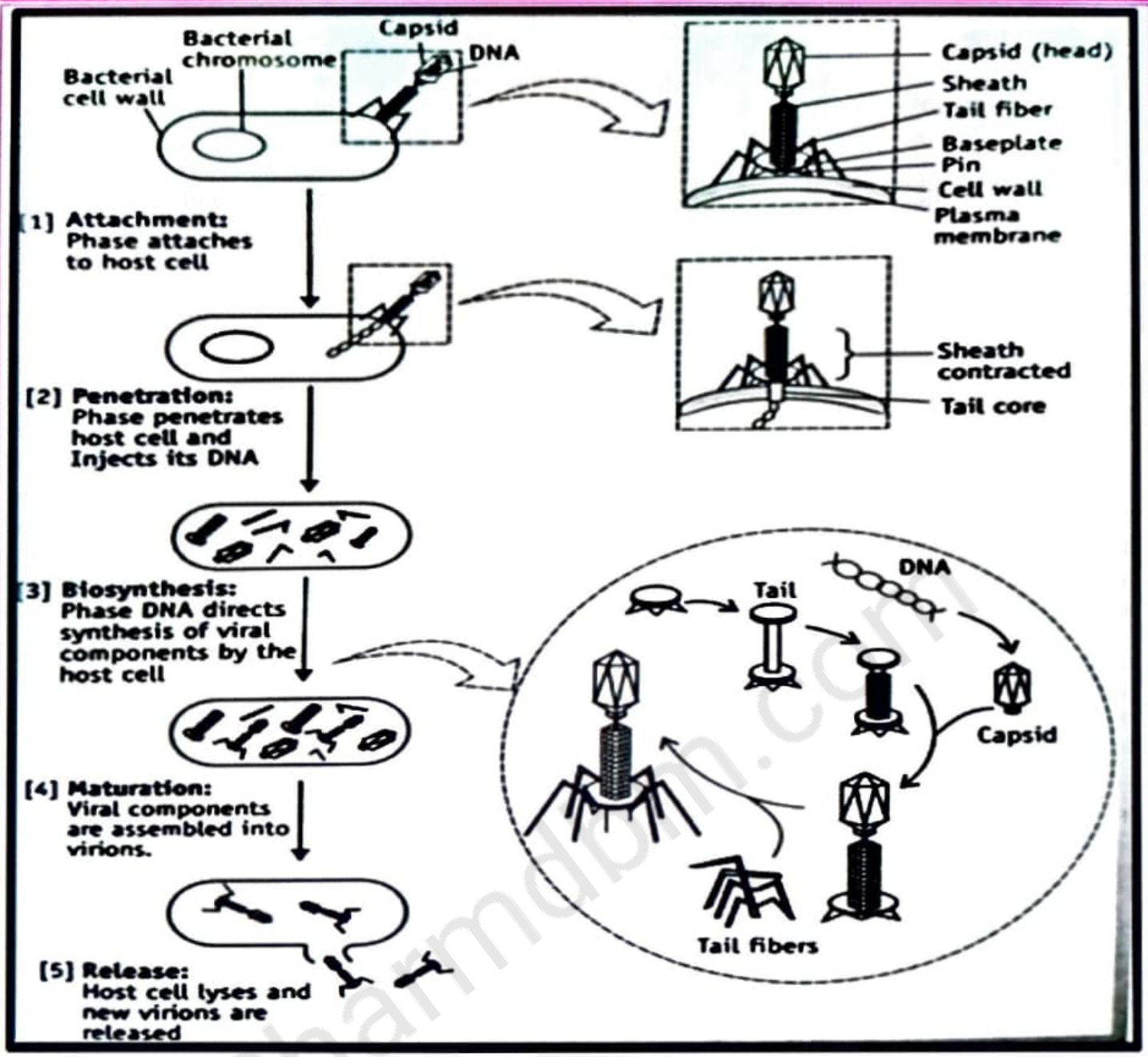
➢ ATTACHMENT

Also called as adsorption.

- It is the first event in any viral infection in which the virus comes in contact with cells by random collision and gets attached to the cells. This attachment or **adsorption is specific and depends on the presence** of specific receptors on the host cell surface (called the host cell receptors).
- Animal viruses have specialized attachment sites disturbed over the surface of the virions **e.g. Orthomyxo viruses and paramyxo viruses** attach through glycoprotein spikes, and adenoviruses attach through the penton fibers.

➢ Penetration

- Penetration of the virus occurs either by engulfment of the whole virus, or by fusion of the viral envelope with the cell membrane allowing only the nucleocapsid of the virus to enter the cell.
- **Thus virus particle is taken inside the cell by one of the following mechanism;**



(a) **Non enveloped viruses** enter the cell by engulfment of virions by invagination of plasma membrane with accumulation of virus particles in cytoplasmic vesicles called phagosomes. This is known as **viropexis, a mechanism resembling phagocytosis.**

(b) **In enveloped viruses,** the envelope may fuse with plasma membrane of the host cell **releasing the nucleocapsid into the cytoplasm.**

❑ UNCOATING

- The process involves the **physical separation of nucleic acid** from its outer structural components. It is assumed that host components and proteolytic enzymes within the lysosomes cause this uncoating process.
- The enveloped viruses get uncoated by action of **lysosomal enzymes in adeno viruses and parvo viruses,** uncoating is accomplished by cellular

➢ BIOSYNTHESIS

- After uncoating the viral genome directs the biosynthetic machinery of the host cell to shut down the normal **cellular metabolism and to produce its own viral components.**

⬇

- In general the nucleic acid genome of most of the **DNA viruses are synthesized in the host cell nucleus** with an exception of poxvirus which synthesizes all their components in cytoplasm.

⬇

- The viral DNA is released into the nucleus of the host cell where it is **translated into early viral proteins**. The early viral proteins are concerned with replication of the viral DNA, so they are transported back into the nucleus where they become involved in **the synthesis of multiple copies of viral DNA.**

⬇

- These copies of the viral genome act as templates for transcription into late **mRNAs which are also transported back** into the cytoplasm for translation into late viral proteins.

⬇

- The late viral proteins are structural proteins (e.g.- coat, envelope proteins) or core proteins (certain enzymes) which are **then transported back into the nucleus for the next stage of the replication cycle.**

- In the case of some RNA virus **(e.g. Orthomyxo, Paramyxo and Retro virus),** the infectious viral RNA enters into the nucleus where it is replicated before transport back to the cytoplasm for translation into viral proteins.

➢ ASSEMBLY (VISION ASSEMBLY)

- Assembly of various viral components into a complete virus particle occurs shortly after replication of viral nucleic acid and may take place in either nucleus (**Adeno and Herpes viruses) or cytoplasm (Pox and Picorna viruses).**

- The capsomere proteins enclose the nucleic acid to form the viral nucleocapsid. **The process is called encapsidation.** In case of enveloped viruses, the envelope is derived from the nucleus (Herpes virus) and from plasma membrane if they assemble in cytoplasm of the host cell **(Paramyxo and Orthomyxo viruses).**

➢ RELEASE

- It is final event in viral replication, and it results in the release of the mature virions from their host cell. Virus maturation and release occurs over a considerable period of time.
- **Non enveloped (naked) viruses** may be released by the lysis of the host cell or released by process which may be **called as reverse phagocytosis** without affecting the host cell, except polio virus which damages the host cell.
- In the case of enveloped viruses, the nucleocapsid acquires its final envelope from the nuclear or **cell membrane by a budding off** process before released out of the host cell.

➢ CULTIVATION

- Cultivation of virus are not possible on artificial medium so, requires live cell or living organism. Because **they contain no metabolic zymes, viruses are unable to use environmental nutrients.**

Method for cultivation :

1. Cultivation in animal
2. Cultivation in embryonated egg.
3. Cultivation in cell culture.

1. Cultivation in animal :

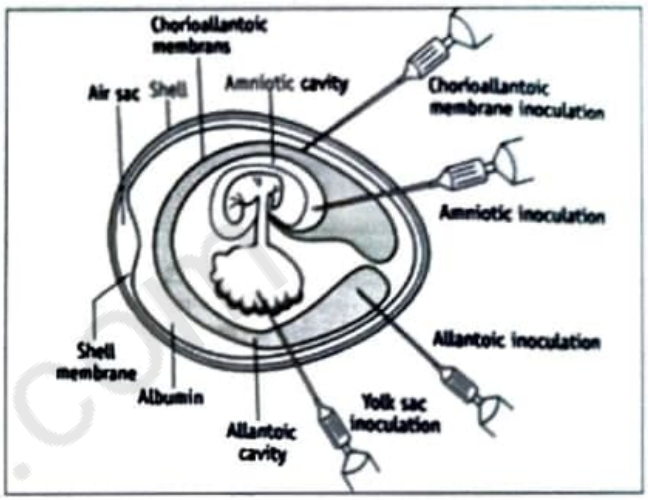
- Virus cultured in living animal such as **mice, rabbit, & guinea pig.**
- Virus particle injected by following route: Intra cerebral, Intra peritoneal and Subcutaneous.
- Example: **Arbovirus, coxsackivirus.**

2. Cultivation in embryonated egg :

The embryonated hen's egg was first used for the cultivation of viruses by good

- pasture and the method was further developed **by Burnet**. Route for cultivation of virus:

- Chick embryos are **live animals** and one procedure for introducing viruses is to inoculate them into the choriallantoic membrane. Viruses can also be **cultivated in embryonated eggs** .



- A hole is drilled in the shell of the embryonated egg, and a viral suspension is injected into the fluid of the egg. Virus is injected in any membrane of the egg, which is most appropriate for its growth. Viral growth is indicated **by the death of the embryo, by embryo cell damage** or

- by the formation of typical pocks or lesions on the egg membranes. Embryonated eggs, one most popular **for isolation and growth of viruses**, are now used only for the production of certain vaccines.

3. Cultivation in cell culture :

1. **Organ:-** Organ cultures are useful for the isolation of highly specialized parasite.

   Example: **Trachea ring culture for corona virus.**
2. **Explant-** Example: Adenoid tissue explant for cultivation of adenovirus
3. **Cell culture:-** In the cell culture required **13 essential amino acid, 9 vitamin salt glucose buffer, Antibiotic and 5% serum.**

- Virus growth in cell cultures can be detected by cytopathic effect, transformation, **metabolic inhibition, interference** and **immunofluorescence.**

UNIT- III
(CHAPTER-10)
DISINFECTANTS

Points to be covered in this topic

1. INTRODUCTION
2. CLASSIFICATION
3. FACTORS INFLUENCING DISINFECTION
4. FACTORS AFFECTING CHOICE OF ANTIMICROBIAL AGENT.

❑ INTRODUCTION

❖ DISINFECTION

- **Disinfection is the process of destruction** or removal of microorganisms and reducing them to a level not harmful to health.
- **Disinfection generally kills the sensitive vegetative cells** but not the **heat-resistant endospores. If the object is inanimate (lifeless)**, such as working areas, dishes, benches etc., the chemical agent is known as a **disinfectant .**

- However, if the object is animate (live) such as a human body tissue, the chemical is known as an antiseptic.
- Disinfectants are usually bactericidal but occasionally they may be bacteriostatic.

IDEAL CHARACTERISTICS OF DISINFECTANTS:

- Broad spectrum: should have a wide antimicrobial spectrum.
- Fast acting: should produce a rapid kill.
- Not affected by environmental factors: should be active in the presence of organic matter (e.g., blood, sputum, feces) and compatible with soaps, detergents, and other chemicals encountered in use.

- Nontoxic: should not be harmful to the user or patient.
- Surface compatibility: should not corrode instruments and metallic surfaces and should not cause the deterioration of cloth, rubber, plastics, and other materials.
- Residual effect on treated surfaces: should leave an antimicrobial film on the treated surface.
- Easy to use with clear label directions.
- Odorless: should have a pleasant odor or no odor to facilitate its routine use.
- Economical: should not be prohibitively high in cost.
- Solubility: should be soluble in water.

CLASSIFICATION OF DISINFECTANTS:

❖ **According to the Drugs and Cosmetics Rules (Rule 126, Schedule O), disinfectants are classified as :**

(a) Black fluids

(b) White fluids

(a) **Black fluids :** These are homogeneous **dark brown solution of coal tar acids or similar acids** derived **from petroleum** with or **without hydrocarbons, and/or other phenolic compounds**, and their **derivatives; and a suitable emulsifier.**

(b) **White fluids :** These are finely dispersed **homogeneous white to off-white** emulsion consisting **of coal tar acids** or similar acids derived from **petroleum, with or without hydrocarbons**, **and/or other phenolic compounds, and their derivatives.**

❖ Chemical classification :

1. Phenols and related compounds
2. Halogens
3. Aldehydes
4. Alcohols
5. Dyes
6. Heavy metals
7. detergents

❖ 1. PHENOLS RELATED COMPOUNDS

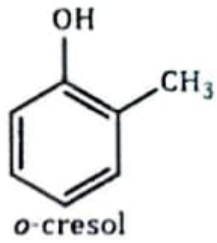
Phenols contain many chemicals **like- phenol, cresol, chlorohexidine, chloroxylenol.**

➢ Phenols

- Phenol also called **carbolic acid** is the first chemical **agent used as an antiseptic.**
- These are **obtained by distillation of coal tar** and have a powerful microbicidal action
- Introduced by **Lord Joseph Lister (1854).**
- **Mechanism of action: disruption of cell membrane** and precipitation of protein. **Inactivation of enzyme and leakage** of amino acid from the cells.

- **Phenol (1%)** has **bactericidal action** but it is readily **absorbed by skin & mucous membrane and causes toxicity.**

Cresols



o-cresol

- cresol is used as **a solution of cresol in soaps** (lysol). It is **used for disinfection of infected glass wares.** In laboratory disinfection of **excreta, cleaning floors of** wards **& operation theatres.**

Chlorohexidine

- **It is bactericidal** at high dilution. It is an **active ingredient of savlon** which is **widely used in burns, wounds, pre- operative disinfection** of skin etc.

Chloroxylenol

- It is an active ingredient in Dettol.

4-chloro-3,5-dimethylphenol

2. HALOGENS

- **Chorine, iodine, bromine and fluorine** in the free state as well as their **compounds strongly act germicidal. Bromine and fluorine** are irritants and are difficult to handle.
- **Chlorine & iodine are used** as disinfectants **being strong oxidizing agents.**

Iodine

- Mechanism of action : **protein inactivation.**
- It is used **as a skin disinfectant** and for **cold sterilization** of surgical sutures **e.g. weak iodine solution** B.P, aqueous iodine solution BP' etc.
- It **is good disinfectant** in **aqueous or alcoholic solutions.**
- It is **sporicidal, fungicidal** and active **against many viruses.**

Chlorine

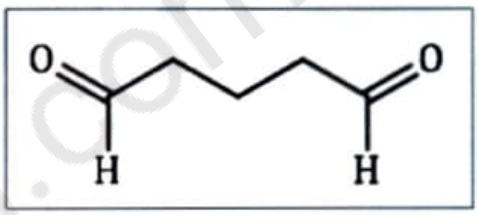

- Mechanism of action : **oxidizes proteins** and enzymes.
- **Chorine is available** in **organic, inorganic** and gaseous forms.
- It **keep bacterial** population at **low levels in** municipal water.

- The germicidal action of chorine and its compounds is due to the formation of hypochlorous acid when free chlorine reacts with water.

$$Cl_2 + H_2O \longrightarrow HCl + HClO$$

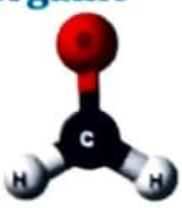
- Products containing calcium hypochlorite are used for sanitizing utensils in restaurants.
- Sodium hypochlorite is used as disinfectant for laboratory gloves, linen, syringes, and reagent bottles.
- Chlorine compounds have been used to disinfect open wounds to treat athlete's foot and other infections.

3. ALDEHYDES



➢ Glutaraldehyde

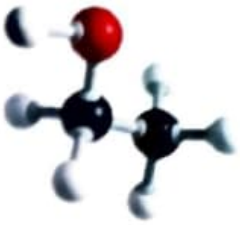
- Is active against bacteria (including Mycobacterium tuberculosis and Pseudomonas aeruginosa), spores, viruses and fungi. It is one of the most effective chemical liquids for sterilization purposes.
- useful for disinfecting surgical instruments, anesthetic equipment and cystoscopes.
- Vegetative bacteria (including S. aureus and M. tuberculosis) and viruses are killed in 10 minutes and spores in 3 hours.
- The activity of glutaraldehyde is neither greatly reduced by organic matter nor does it damage delicate objects.



➢ Formaldehyde

- Mechanism of action : Formaldehyde kills microorganisms through its action as alkylating agent, on amino and hydroxyl groups of nucleic acids and proteins, and with carboxyl and sulfhydryl groups in proteins.
- Agent for disinfecting clothing, blankets, pillows, mattresses, toys and similar articles but the process is not reliable.

- It is **bactericidal, sporicidal, virucidal.**
- A **10% of formalin solution** is used for **better results.**



4. ALCOHOL

✓ Ethyl alcohol / ethanol

- EA in concentration **between 50- 70%** is effective against viruses.

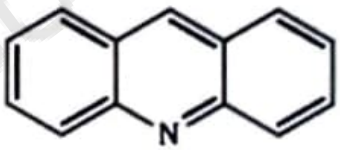
✓ Methyl alcohol / Methanol

- **Effective against fungal spores** but it is toxic to eyes.

✓ Isopropyl alcohol (50- 70%)

- Better **than ethyl alcohol** in bactericidal property used for **disinfection of clinical thermometers.**

5. DYES



acridine

- Two groups of dyes (**aniline dyes & acridines**) have been extensively used **as skin de wound antiseptics.** Both these groups are **bacteriostatic in high dilution but have low bactericidal activity.**
- **Aniline dyes includes** malachite green, brilliant green & crystal violet. They are used on **skin & on mucous membranes** as antiseptics & have been **also used for some fungal infections like oral thrush.**
- **Acridine dyes** Includes **acriflavine, proflavine, aminacrine le euflavine.** They are **bacteriostatic & used for treating wounds.** for irrigation **of bladder & vagina.**

6. HEAVY METALS

- The **most widely** used compounds **of heavy metals** are those of **mercury, silver and copper.** Heavy metals and their compounds **act antimicrobial by combining with cellular proteins.**

- High concentrations of salts of heavy metals like mercury, copper, and silver coagulate cytoplasmic proteins, resulting in damage or death of the cell.
- Mercurochrome destroys vegetative bacteria and also fungi excluding their spores.
- Silver nitrate 1% has been used in past for treatment of ophthalmia neonatorum and is instilled into eyes of newborn after delivery.

7. DETERGENTS AND SOAP

- They are widely used as surface active agents, wetting agents and emulsifiers. They are classified into four main groups such as anionic, cationic nonionic and amphoteric. The most important antibacterial agents are the cationic surface active agents, eg. cetrimide, benzalkonium chloride etc.
- Non-ionic detergents are not ionized. However, these substances do not posses significant antimicrobial activity.

➢ Chemical disinfectant and their uases

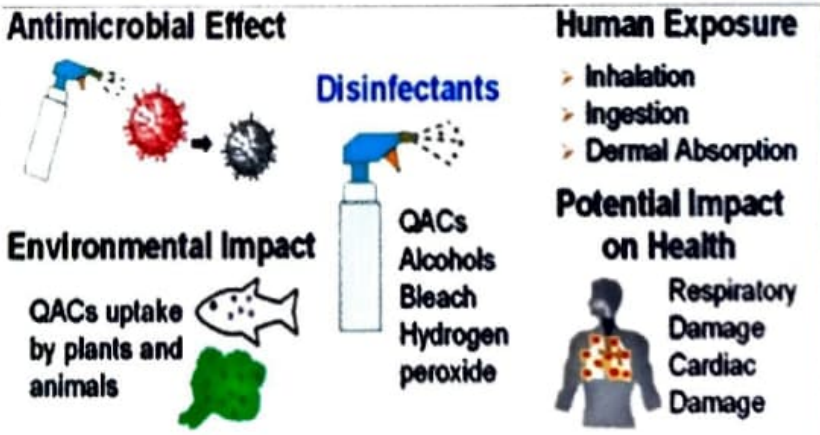
CHEMICAL DISINFECTANT	MODE OF ACTION	USES
Alcohol	Protein denaturation and damage lipid complexes.	Surface disinfectants, preservatives and antiseptics.
Phenol	Disruption of cell membrane and pptn. Of cell protein.	Disinfectant, antiseptics
Mercury compounds	Coagulation of cytoplasmic proteins.	Surface disinfectants and antiseptics.
Silver nitrate	Protein denaturation.	antiseptics. And bactericidal
Iodine	Protein inactivation.	Skin disinfectant and water or air.
Chlorine	Inhibit cellular oxidation process.	Disinfectant in water treatment, antiseptic.
Glutaraldehyde	Denaturation of proteins.	Sterilization of heat sensitive instrument

Difference between disinfection & sterilization

DISINFECTION	STERLIZATION
In this the number of harmful microbes is minimized to a negligible level.	In this medium is made completely free from all microbes.
It kills only vegetative cells and not the spores.	It kills both vegetative cells and spores.
Wounds are disinfected- with agents such as hydrogen peroxide or rubbing alcohol.	Wounds cannot be sterilized as it may kill surrounding healthy cells.
Disinfection only reduces the effect of microbes.	Sterilization completely rids microbes from the surface.
Chemical methods are used for disinfection.	Combination of heat, irradiation, high pressure, chemical and physical methods are used for sterilization.
Phenol, alcohol, chlorine, iodine, are some of the disinfecting agents.	High temperature, steam, radiation, filtration are some of the sterilization techniques.
Only adequate cleanliness.	Extreme cleanliness
Used in daily life	Used primarily for medical and research purposes.
For e.g., pasteurization, disinfecting urinals, etc.	For e.g., sterilization of instruments used during surgery by autoclaving.

❖ FACTORS INFLUENCING DISINFECTION

The rate and extent of antimicrobial action of chemical disinfectants are influenced by a number o factors:



1. Concentration of disinfectants
2. Temperature
3. Time of contact
4. Ph of the environment
5. Surface tension
6. Formulation of disinfectant
7. Chemical Structure Of Disinfectant
8. Type and number of microorganisms present
9. Interfering substances in the environment
10. Potentiation, synergism and antagonism of disinfectants

1. CONCENTRATION OF DISINFECTANTS

- The rate of killing of **microorganisms varies directly** with the **concentration of the disinfectant.** However, the **effectiveness is generally related to the concentration exponentially**, not linearly. There is an **optimum concentration of phenol at about 1%.** Beyond this **concentration, the disinfecting effectiveness becomes less.**
- The dilution coefficient can be calculated from the following equation:

$$n = \frac{\log t_2 - \log t_1}{\log C_1 - \log C_2}$$

- Where **n is the concentration** exponent or **dilution coefficient** for the disinfectant,
- t_1 **is the death time** with disinfectant **concentration** C_1 **and** t_1 **is the** death time with **disinfectant concentration** C.
- Concentration **exponent or dilution coefficient** is an important characteristic of each disinfectant and **is useful in determining** the **effect of dilution on the disinfectant.** $^{(T+10)^\circ}$

2. TEMPERATURE

- The rate of **disinfection normally increases** with the temperature.
- The **effect of temperature on bactericidal** activity may **be expressed quantitatively by means of a temperature coefficient.**
- The **temperature coefficient per degree** rise in **temperature is denoted by 'B' whereas per 10°C rise in temperature is expressed by** θ^{10} **or** Q_{10} **values.**

$$\text{Thus, } \theta^{10} \text{ or } Q_{10} = \frac{\text{Time required to kill at } T^\circ}{\text{Time required to kill at } (T+10)^\circ}$$

$$\theta^{(T_2 - T_1)} = \frac{t_1}{t_2}$$

- The **value for** Q_{10} for phenol **is 4, which means that over the** 100 C **range** used to determine the Q_{10} the activity will be **increased by factor 4.**
- Thus θ_{10} **or** Q_{10} **=** ***time required to kill.***

3. TIME OF CONTACT

- **Sufficient time of contact** must be allowed for the **disinfectant to exert its action.**
- It is shown that the **principles of first order kinetics** may be **applied to the disinfection process** and the rate or velocity constant, **K is a measure of the efficiency of the disinfectant**

$$K = \frac{1}{t} \log {N_o}/{N_t}$$

where t = time for the viable count to fall from No to N,

N_o = **initial number of microorganisms**

N_t = **final number of microorganisms**

4. PH OF THE ENVIRONMENT

- A **change of pH during** the disinfection **process can affect the rate of growth inoculum.**
- A **pH of 6-8 is optimal for the growth** of many bacteria and **the rate of growth declines** on either side of the range.
- **Phenolic and acidic antimicrobial agents** usually have **greatest activity in acidic conditions.**
- Acridine dyes and **quaternary ammonium compounds** are usually **more active in alkaline** then in acidic solutions.

5. SURFACE TENSION

- **Contact between** aqueous **solutions of disinfectants Is** facilitated if they have surfactant properties. **This helps in adsorption of surface active disinfectants on the surfaces** of cells as well as in **wetting and spreading properties of the solutions.**
- A combination of soap **with crude phenol (carbolic acid)** has excellent disinfecting properties. **Soaps can be used to lower the** surface **tension and the extinction time, until the soap** concentration Is equivalent to the **critical concentration for micelle formation.**

6. FORMULATION OF DISINFECTANT

- **Formulation may be** important for the **effective use of disinfectants**. Effectiveness **of chlorhexidine and quintenary** ammonium compounds **may be greater** in **70% alcohol than in aqueous solution.**
- **Iodine is virtually insoluble** in water and **is dissolved in alcohol, potassium iodide solution or solutions** of surface active agents.
- The presence of a **suitable surfactant can moderate the staining** and **corrosive properties of Iodine** and **increase the stability of the preparation.**
- For **convenience and economy it is essential** to formulate a disinfectant solution **to be as concentrated as possible** which is suitable for **dilution with water immediately** before use.

7. CHEMICAL STRUCTURE OF DISINFECTANT

- **Chemical structures of compounds** affects the disinfectant activity.
- Substitution of **an alkyl chain up to 6 carbons** in length in para position to phenolic - **OH group increases activity but greater than 6- cartons in length decreases water solubility and disinfectant activity.**
- Generally, halogenation **increases the antibacterial activity of phenol** but nitration increases antibacterial activity and systematic toxicity also.

8. TYPE AND NUMBER OF MICROORGANISMS PRESENT

- The **efficiency of disinfection greatly** depends on the nature and the number of **contaminating microorganisms** and especially on the **presence and absence of bacterial spores.**
- It can be seen that **most vegetative bacteria** are rapidly **killed by most chemical disinfectants.**
- Bacterial spores are **difficult to destroy but some disinfectants e.g aldehyde are sporicidal.**

9. INTERFERING SUBSTANCES IN THE ENVIRONMENT

- Material such as **blood, body fluids, pus, milk**, food residues **or colloidal proteins may reduce the** effectiveness of disinfectant **if present in small amounts.**
- The presence of oil **and fat markedly reduces the** disinfecting **ability of phenolics.**

10. POTENTIATION, SYNERGISM AND ANTAGONISM OF DISINFECTANTS

- **Potentiation of a disinfectant** leads to enhanced antimicrobial activity.
- Synergistics **effects are often shown by two antimicrobial agents which is giving an increased activity.**
- Antagonism effects are often shown by **two antimicrobial agents which is giving an decreased activity.**

❖ FACTORS AFFECTING CHOICE OF ANTIMICROBIAL AGENT

Selection of the **most appropriate antimicrobial compound** for **specific practical applications depend on:**

1. Properties of chemical agents
2. Environment
3. Types of microorganisms
4. Intended application
5. Toxicity
6. Cultural state

1. PROPERTIES OF CHEMICAL AGENTS

- **Properties of chemical agents:** The process of killing or inhibiting the growth Microorganisms using antimicrobial agent is basically a chemical reaction. The rate and extent of a chemical reaction is generally influenced by concentration of the chemical, temperature, pH and formulation.

2. ENVIRONMENT

- Organic matter, blood, body fluids, pus , milk, colloidal proteins and food residues, mainly reduce the effectiveness of antimicrobial agents. Organic matter can have a drastic effect on antimicrobial activity by adsorption or chemical inactivation. Dried organic deposits may inhibit penetration of the chemical agent.

3. TYPES OF MICROORGANISMS

- The types of microorganisms present and the levels of microbial contamination (bioburden) both have a significant effect on the outcome of chemical treatments.
- Long exposure times or higher concentration of antimicrobials may be required for higher bioburden. Chemical agents are not equally effective against bacteria, fungi, viruses and other microorganisms.
- Vegetative bacteria and fungi are sensitive to antimicrobial agent. Mycobacterium tuberculosis and other rnycobacteria are resistant to many bactericides. Spores are more resistant than vegetative cells.

4. INTENDED APPLICATION

- The intended application of an antimicrobial agent whether for preservation, antisepsis or disinfection will influence its selection and also affect its performance. Many chemicals adversely affect the instruments at the time of disinfection. Chemicals may cause corrosion of metals and also affect clarity of lenses or change the texture of synthetic polymers.

5. TOXICITY

- Control of **Substances Hazardous to Health (COSHH)** Regulations **which specify the precautions** required in handling to agents.
- **Phenolics, formaldehyde and glutaraldehyde** are mainly to antimicrobial agents. **Toxic volatile substances are kept in covered containers to reduce the level of exposure to Irritant vapour** and they are used with an or facility.
- **Alcohols, chlorine compounds and phenolics** mainly affect the eyes and skin.
- **Aldehydes mainly affect the respiratory system** and also **produce contact dermatitis.**

6. CULTURAL STATE

- When the bacterial cells **are actively dividing** in **the log phase of growth, they** are more sensitive to antimicrobial age.
- The **sensitivity may be due to a minor interference** with the **replication of nucleic acids and with protein** synthesis **having a profound effect on the continuation of high metabolic aces.**
- All **microorganisms are more susceptible** to chemicals at the **point of division.**

UNIT- III
(CHAPTER- 11)
Sterility testing and Evaluation

Points to be covered in this topic

1. INTRODUCTION
2. EVALUATION
3. STERLITY TESTING
4. CULTURE MEDIA
5. METHOD FOR STERLITY TESTING

❑ INTRODUCTION

❖ BACTERIOSTATIC AND BACTERIOCIDAL

- **Bactericidal** antibiotics kill the bacteria.
- **Bacteriostatic** antibiotics suppress the growth of bacteria.

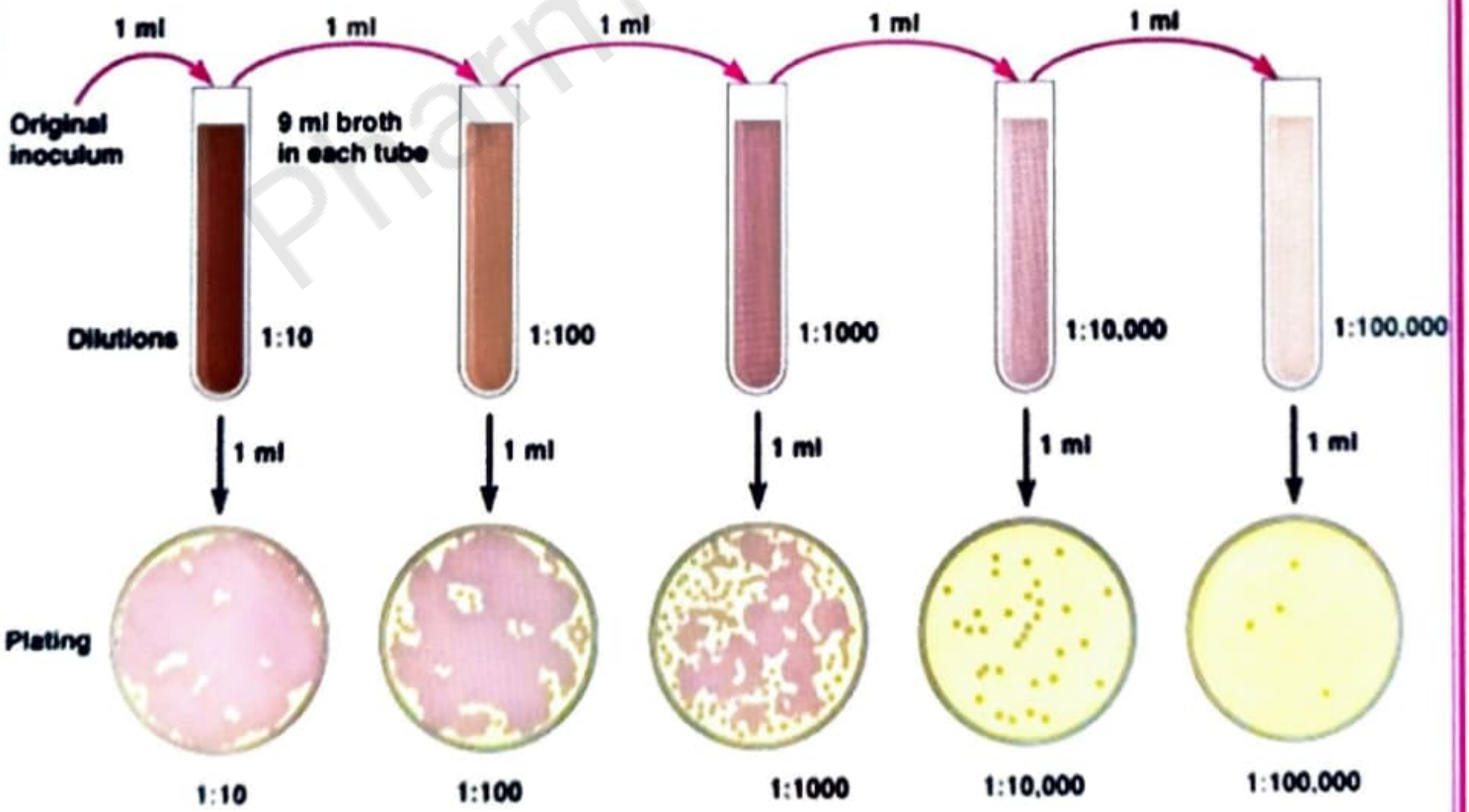
❖ EVALUATION

Techniques or methods used for the evaluation of disinfectants are:

1. Tube dilution and agar plate method.
2. Filter paper and cup plate method.
3. Ditch-plate method.
4. Phenol coefficient method.
5. Kelsey Sykes method.

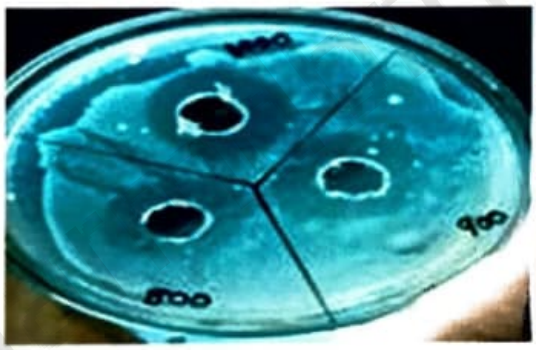
1. TUBE DILUTION AND PLATE METHOD

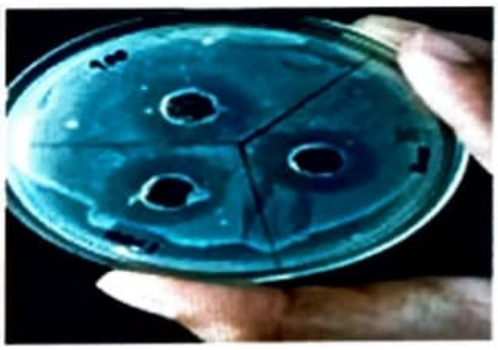
- The chemical agent is incorporated into **nutrient broth or agar medium and inoculated** with the **test microorganisms and** are incubated **at 30 to 33 degree Celsius for 2 to 3 days** and then the results **in the form of turbidity or colonies are observed.**
- The results are recorded and the **activity of the given disinfectant is compared.**



2. FILTER PAPER AND CUP PLATE METHOD

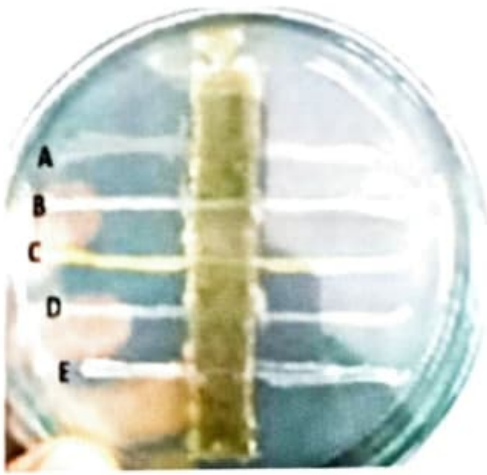
- In this methods, the **agar is melted, cooled at 45°C,** inoculated with **the to microorganisms and poured** into a sterile Petri plate.
- In the cup plate method, **when the Inoculated agar** has solidified, holes **about 9 mm in diameter** are cut in the medium with **a sterile cork borer.**
- The antimicrobial agent is **directly placed in the holes.** In the filter paper and **cylinder plate method,** the **antimicrobial agent is applied to the surface of the solidified,** inoculated agar by using a **filter paper disc and cylinder respectively.**
- The **zone of inhibition** is **observed** atter incubation at **30 to 35°C for 2 to 3 days.**
- The diameter of the **zone of inhibition** gives an **indication of the relative activities** of different **antimicrobial substances** against the test microorganisms.



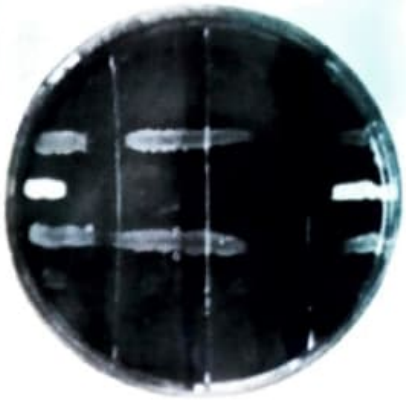


3. DITCH PLATE METHOD

- A solution of the **antimicrobial substance** is **carefully run into the ditch which** is prepared in **an agar plate.**
- **A loopful of each** test microorganism is then **streaked outwards** from **the ditch on the agar surface. Microorganisms** resistant to the **antimicrobial grow right** up to the **ditch whereas** susceptible microorganisms show a zone of inhibition adjacent **to the ditch** or **center of plate.**



- The **width of the inhibition zone** gives an indication of the **relative activity** of **the antimicrobial substance** against the **various test microorganisms.**



4. PHENOL – COEFFICIENT METHOD

- The **similar quantities of** microorganisms **are added to** rising **dilutions of phenol and of the disinfectant** to be tested. In the UK the organism used is **Salmonella typhi and the USA, Salmonella typhi Staphylococcus aureus and Pseudomonas aeruginosa are used.**
- The phenol coefficient test includes:

1. **Rideal-Walker test** (RW test)
2. **Chick-Martin test** (CM test)
3. United States Food and Drug Administration test (**FDA test**).
4. The US Association of Official Agricultural Chemists test (**AOAC test**).

Phenol coefficient test is suitable **for testing disinfectants miscible with water and which test exert their** antimicrobial action in a manner similar **to that of phenol.**

➢ Rideal-Walker test (RW test)

- If a phenol coefficient or **Rideal-Walkar coefficient** of the **given test disinfectant is 1,** it means **that the disinfectant** has the same **effectiveness as phenol.**
- **Rideal-Walker (RW) test:** The Rideal-Walker test is a **measure of the total** possible. **germicidal work a disinfectant** can do in **terms of an arbitrary**. standard whose **absolute value is unknown.**

➢ Chick martin test

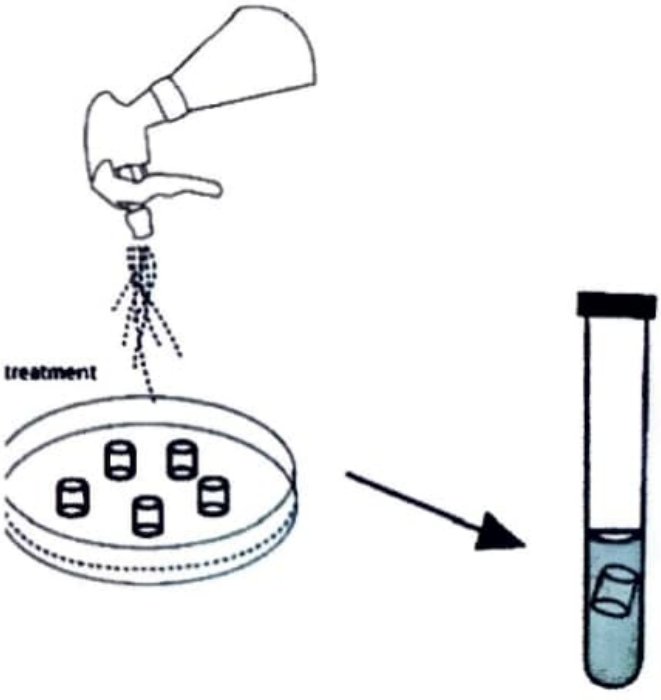
- It is an obsolete **method for determining** the in **vitro efficacy of a disinfectant.**
- In this, a **standardized quantity** of Salmonella typhi is **added to sterilized feces,** and various **dilutions of the disinfectant** are compared with the efficacy of phenol as a **disinfectant**, yielding the **phenol coefficient**.

➢ AOAC test

- The **AOAC Use Dilution method** is a **method of testing the efficacy** of **disinfectants,** originally developed **in 1955.**
- Throughout its **numerous revisions**, it has become the **standard for evaluating liquid** and dilutable liquid **disinfectants** for hard surfaces.

5. KELSEY- SYKES METHOD

- In this **method several test bacteria** such **as Staphylococcus aureus, Proteus vulgaris,** Escherichia coli and **Pseudomonas aeruginosa** are used. This test can be carried out in **clear or dirty conditions**.
- In both cases the **final concentration** of bacterial **cells should** be about **10/ml.**
- Clean **conditions** are simulated by using broth as the **suspending fluid and dirty conditions** by the use of a yeast **suspension or inactivated** horse serum as the **suspending fluid.**
- The dilutions of the **disinfectant** are made in **hard water.**



Disinfectants test :-

CHARACTERISTICS	RIDEAL WALKER	FDA TEST	CHICK MARTIN	AOAC
Medium PH	7.3 to 7.5	6.8	7.3 to 7.5	6.8
Vol. medium(ml)	5.0 ml	10.0 ml	10.0 ml	10.0 ml
Dilute for test disinfectant	Water	water	yeast	Water
Test microorganisms	Salmonella typhi	Salmonella typhi	Salmonella typhi	Salmonella typhi, staphylococcus aureus
Calculation of phenol coefficient	Dilution test killing in 7.5 but not 5 min divided by same for phenol.	Dilution test killing in 7.5 but not 5 min divided by same for phenol.	Highest phenol concentration inhibiting and lowest permitting growth divided same for phenol.	Greatest dilution of test killing in 10 min divided by same for phenol.

Disinfectants test :-

DISINFECTANT TO BE USED	BACTERICIDAL TESTS	BACTERIOSTATIC TEST
Solid disinfectants and disinfectant powders.	Modified end- point or extinction time method.	Inhibition on seeded agar
Semi- solid formulations :	Modified end point or extinction time method.	Cup plate methods
Liquid disinfectants	In vivo tests (e.g. skin tests)	Ditch- plate technique
	End point or extinction time method	Serial dilution in fluid media.
	Counting methods	Serial dilution in solid media.
	Turbidimetric methods	Cup plate, fish- spine bead and filter paper methods
	In use and other tests	Gradient- plate methods. Ditch plate method.
	In vivo tests	All methods may be used quantitatively but final method id used qualitatively.
Aerial disinfectants	Use of slit- sampler in test chamber.	

➢ STERILITY TESTING

- Process of removing all viable **forms of microorganisms**. **Sterility test** – A **test that critically assesses** whether a sterilized **pharmaceutical product is free** from contaminating **microorganisms.**
- **(or) Acc to IP –** The **sterility tests are intended** for testing the absence of viable **forms of microorganisms in or** on the **pharmacopeial preparations.**
- Products which **are necessary to** be sterilized : • **Injections** • **Implants** • **Syringes** • **Ophthalmic preparations** • **Ointments & creams** • **Bandages** • **Surgical dressings & devices** • **needles.**
- **No. of containers** in the batch Minimum **no. of containers** recommended to be tested.

Sterility testing table -

S. No	Preparations	Plumber of containers in the batch	Minimum number of containers are recommended to be tested
1.	Parenteral preparations	Not more than 100 containers	10% or 4 containers (whichever is greater)
		More than 100 but not more than 500 containers	10 containers
		More than 500 containers	2% or 20 containers (whichever is less)
		For large-volume parenterals	2% or 10 containers (whichever is less or specified)
2.	Ophthalmic and other non parenteral preparations	Not more than 200 containers	5% or 2 containers (whichever is greater)
		More than 200 containers	10 containers

3.	Surgical dressings and devices	Catgut, surgical sutures and other sterile medical devices for use	2 % or 5 packages (whichever is greater, maximum 20 packages)
		Not more than 100 packages	10% or 4 packages (whichever is greater)
		More than 100 but not more than 500 packages	10 packages
		More than 500 packages	2% or 20 packages (whichever is less)
4.	Bulk solids	Upto 4 containers	Each container
		More than 4 containers but not more than 50 containers	20% or 4 containers (whichever is greater)
		More than 50 containers	2% or 10 containers (whichever is greater)

❑ CULTURE MEDIA

➢ Fluid thioglycolate medium (FTM):

- It is used with ear fluid products. FTM is primarily intended for the culture of anaerobic bacteria; however it will also detect aerobic bacteria.
- If more than the upper one-third of the medium has acquired a pink color, the medium may be restored once by reheating in a water bath or in free flowing steam until the pink color disappears and cooling rapidly, taking care to prevent the introduction of non-sterile air into the container. When ready for use, not more than the upper one-tenth of the medium should have a pink color.

➢ Alternative thioglycolate medium (ATM):

It is used with turbid or viscid products and for devices having tubes with small lumina. ATM is incubated in such way that, to assure anaerobic conditions.

➢ Soyabean casein digest medium (SCDM):

- **SCDM is suitable** for the culture **of both fungi and aerobic bacteria**.
- Medium 1 and 2 are **adjusted to pH 7.1 ± 0.2**, medium 3 is adjusted to pH **7.3 ± 0.2 and sterilized** by autoclaving at **121°C for 20 minutes**.
- **Anaerobic conditions are** required for growth of **some microbial species**.

❖ Test for Bacteriostatic and Fungi stasis (test Sample to be tested as per IP 1996):

- Prepare **cultures of bacteria** and fungi from the **strain of** microorganisms.

↓

- **Incubate the sterility test media** with about **100 viable** microorganisms using volumes of media.

↓

- Add the **specific portion** of the preparation being **examined to half of a suitable** number of the **containers already** containing the inoculum and **culture medium.**

↓

- **Incubate** the containers at the **appropriate temperatures** and under the condition for **not less than 7 days.**

↓

- If **growth of the test microorganisms** in the **preparation-medium mixture** is visually comparable to that in the **control vessels**, use some **amount of the preparation** and medium regularly.

↓

- If the test preparation is **bacteriostatic and/or fungistatic** when tested as described above, use a suitable **sterile neutralizing agent.**

➢ ANTIMICROBIAL AGENT METHOD OF INACTIVATION

ANTIMICROBIAL AGENT	METHOD OF INACTIVATION/INACTIVATING AGENT
Penicillin	Penicillinase

Cephalosporins	Cephalosporinase
Streptomycin	Streptomycin adenyl transferase/ Streptomycin phosphotransferase
Aminoglycosides	Acetyl-coenzyme 'A'
Barbiturates	Dilute to 0.2% in culture medium with a pH 7.0
Sulfonamides	p-aminobenzoic acid
Chloramphenicol	Acetyl transferase
Phenolic disinfectants	Dilution
Halogens	Sodium thiosulphate
Quaternary ammonium compounds	Lecithin+ Lubrol or Tween 80
Dyes	Membrane filtration
Heavy metals	Thioglycolic acid
Ethyl alcohol	Dilute to less than 1%
Other antibiotics	Membrane filtration

➢ METHOD FOR STERLITY TESTING

Sterility tests can be carried out by using the following methods:

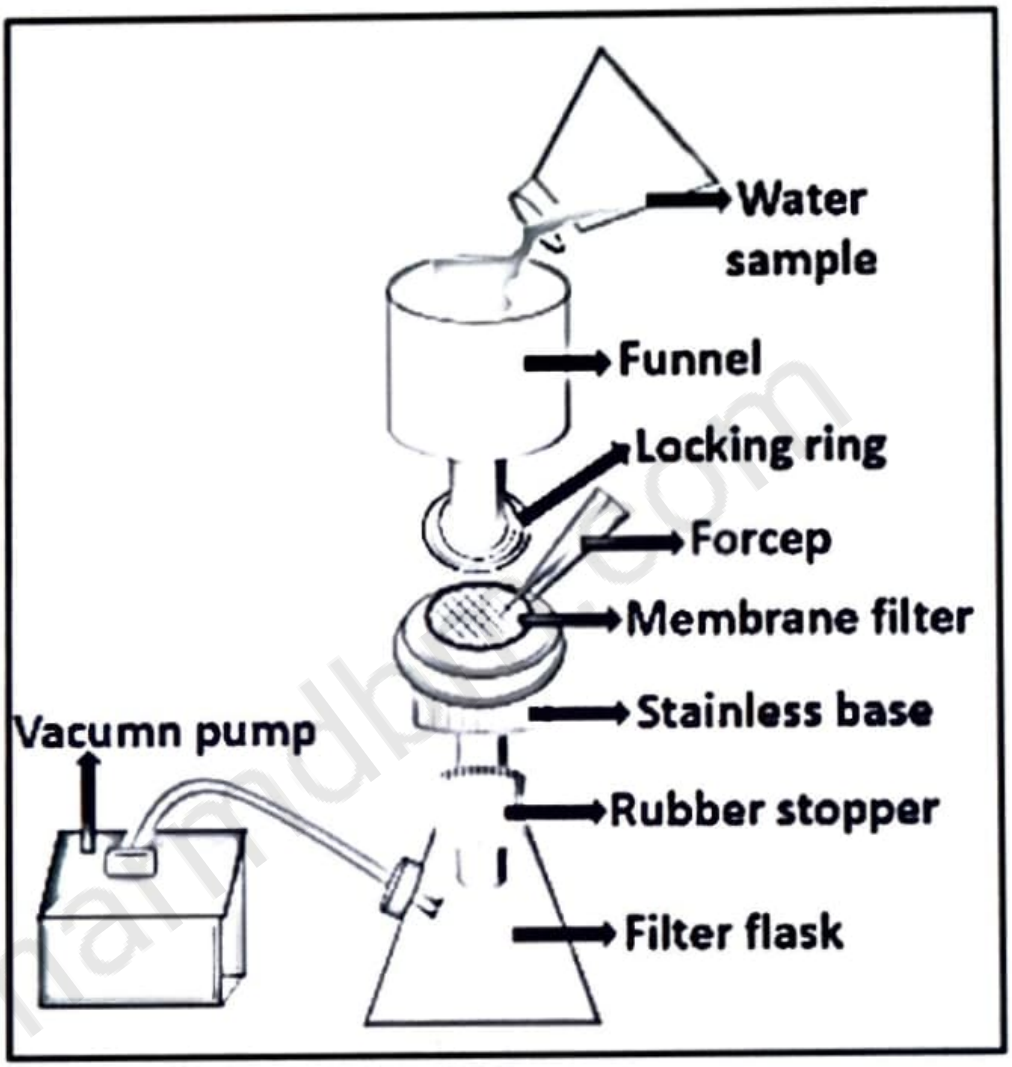
1. Method A: Membrane Filtration
2. Method B: Direct Inoculation.

✓ **Method A: Membrane Filtration**

- This method is to be preferred **where the substances** being examined is (a) **an oil**, (b) **an ointment** that can be put into a solution, (c) **a non-bacteriostatic solid** not readily soluble in the **culture medium**, and (d). **soluble powder or a liquid** that possesses inherent **bacteriostatic and fungistatic properties.**
- For **liquid products where the** volume in the **container is 100 ml** or more, only **Method A should** be employed.

- This **method needs good skill** and **special knowledge** and it also calls for the routine use of **positive and negative controls**.
- A positive **control small number** (not more than 100 CFU) of microorganisms specified in **separate portion of each medium**.

➢ **APPARATUS:**



- **Sterility test apparatus** consists of a closed reservoir and a **container to collect the filtrate,** between which a properly supported **membrane of appropriate porosity** is placed. Membrane generally suitable for **sterility testing** has nominal **porosity of 0.45 um** diameter **about 50 mm flow rate 55-75 ml of water**/minute at a pressure of **70 mm of mercury.**
- Cellulose **nitrate filters** are used for **aqueous, oily and weekly alcoholic solutions** and **cellulose acetate titers**, for strongly alcoholic solutions. Complete unit should be **free** from microorganisms **including** the membrane,

and operation should be carried out aseptically. Preferably assemble and sterilize the entire unit with the membrane in place prior to use.

➢ DILUTION FLUIDS:

Generally two types of dilution fluids are used:

1. Fluid A: Dissolve 1 gm of peptic digest of animal tissue (such as bacteriological peptone) in one litre of water. Filter and adjust the pH to 7.1 ± 0.2. Disperse fluid into the flasks (100 ml) and sterilize at 121 degree Celsius for 20 minutes.
2. Fluid B: The test sample contains lecithin or oil use fluid A, to each litre of which has been added 1 ml of polysorbate 80. Adjust pH to 7.1 ± 0.2. and sterilize at 121°C for 20 minutes using an autoclave.

➢ METHOD OF TEST:

1. For aqueous solutions:

- Transfer aseptically the quantities of the preparation being examined prescribed in the two media onto one membrane.
- Draw the liquid rapidly through the filter with the aid of vacuum.
- The membrane is removed aseptically, cut into two, one part is then immersed in 100 ml of soyabean casein digest medium and incubated to 20 to 25°C for not less than 14 days.
- Similarly, the other part is immersed in 100 ml of liquid thioglycolate medium and incubated at 30 to 35°C for not less than 14 days.

2. For liquids immiscible with aqueous vehicles and suspensions:

- Carry out the test described under, for aqueous solutions but add a sufficient quantity of fluid 'A' to the pooled sample to achieve rapid filtration.

3. For oils and oil solutions:

- Filter oils or oily solutions of sufficiently low viscosity without dilution through a dry membrane. Dilute viscous oils are necessary with a suitable diluent.

4. For ointments and creams:

- Dilute ointments in a fatty base and emulsions of the water-in-oil type to give a fluid concentration of 1% w/v, by heating, if necessary, to not more than 40°C with a suitable sterile diluent such as isopropyl myristate. If ointments and oils are insoluble in isopropyl myristate then use Method B.

5. For soluble solids:

- Dissolve not less than the quantity of the substance being examined as prescribed in a suitable sterile solvent such as fluid 'A'.

6. For solids for injection other than antibiotics:

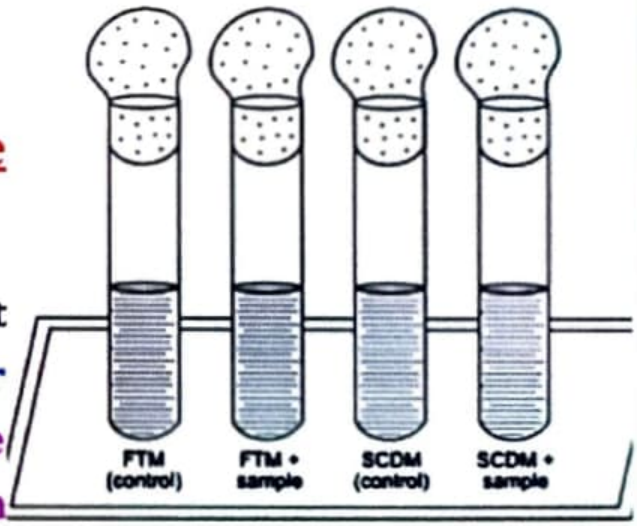
- Constitute the test articles as directed on the label and carry out the test as described under for aqueous solutions or for oils and oily solutions.

7. For antibiotic solids, bulks, and blends:

- Aseptically remove a sufficient quantity of solids from the appropriate amount of containers prescribed, mix to obtain a composite sample, equivalent to about 6 g of solids, and transfer to a sterile flask. Dissolve in about 200 ml of fluid A, and mix.

✓ Method B: direct inoculation

The quantity of the substance or preparation being examined which is to be used for inoculation in the culture media varies according to the quantity in each container.



➢ **METHOD OF TEST:**

1. For aqueous solutions are suspensions:

- Remove the liquid from the test containers with a sterile pipette or syringe. Transfer the quantity of the preparation under examination directly into the culture medium.

- **Examination** is not more **than 10% of** the volume of the **medium, unless otherwise prescribed.** When **the quantity** in a **single container is insufficient to carry out the tests,** the combined contents of two or more containers are to be used to **inoculate the media.**
- The inoculated medium is **incubated for 14 days at 30 to 35°C** in the case of **fluid thioglycolate medium** and at **20 to 25°C** in the **case of soyabean-casein digest medium.**
- If **the preparation** under examination has **antimicrobial activity**, carry out the test after **neutralizing this with a** suitable **neutralizing substance or by dilution** in a sufficient **quantity of culture medium.**

2. For oils and oily solutions:

- Use media to which have been **added 0.1%, w/v** of (4-tert-octylphenoxy) **polyethoxyethanol** or **1% w/v of polysorbate 80** or other **suitable emulsifying agents,** in an appropriate **concentration and proceed with the test.**

3. For ointments and creams:

- Prepare by **diluting to ten-fold by emulsifying** with the chosen **emulsifying agent** in a suitable **sterile diluent such as fluid A.** Transfer the **diluted product to a medium not containing** an emulsifying agent.

4. For solids:

- Transfer the required quantity of the **material to the medium.**

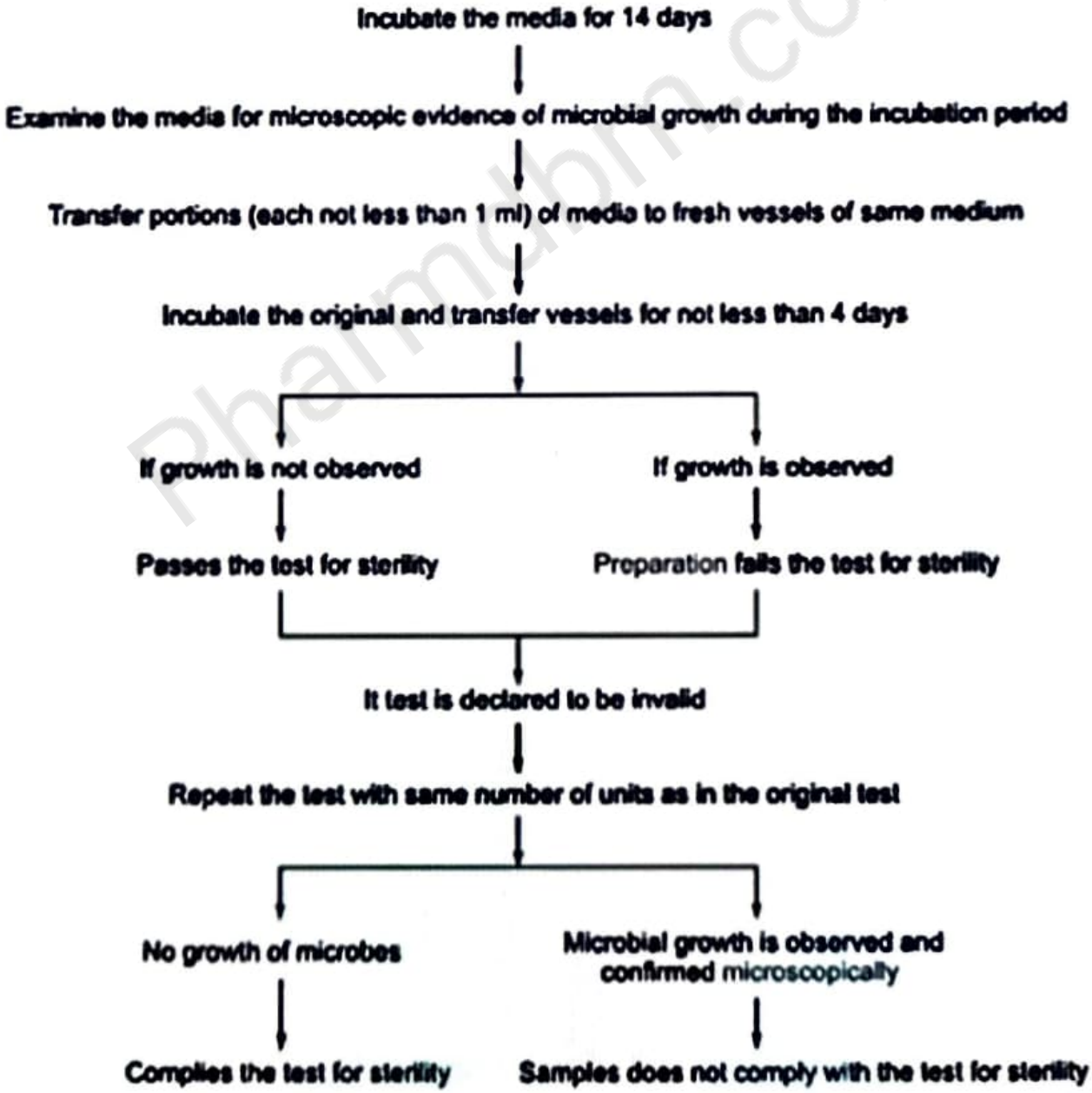
5. For surgical dressings and related articles:

- Aseptically **remove two or more** portions of **100 to 500 mg** each from the innermost **part of the sample** or **package under examination.** From individually packaged, **single use materials, aseptically remove the entire article.** Immerse the **portions or article** in each medium and **proceed with the test.**

6. For sterile devices:

- Immerse the device in 1000 ml of culture medium. If immersion Is impracticable, flush the lumen of 20 units with the fluid thioglycolate medium and soyabean-casein digest medium separately and recover 15 ml of each medium.
- Incubate with not less than 100 ml of each of the medium. For catheters where the inside lumen and outside surface are required to be sterile, either cut them into pieces such that the medium is in contact with the entire lumen or full the lumen with medium and then immerse the intact unit.

➢ INTERPRETATION OF RESULTS



The test may be considered invalid only when one or more of the following conditions are fulfilled:

1. **Microbial growth** is found in the **negative controls**.
2. **Data on microbial monitoring of the sterility testing** facility show a fault.
3. A review of the **testing procedure** used for the test in **question reveals** a fault.
4. After **identifying the microorganisms isolated** from the containers showing microbial growth, the **growth may be ascribed without** any doubt to faults **with respect to the materials or technique** used in conducting the test procedure.