# Unit-3

## Pharmaceutical Microbiology

**Unit III** **10 Hours**

- Study of morphology, classification, reproduction/replication and cultivation of Fungi and Viruses.
- Classification and mode of action of disinfectants Factors influencing disinfection, antiseptics and their evaluation.
- For bacteriostatic and bactericidal actions Evaluation of bactericidal & Bacteriostatic.
- Sterility testing of products (solids, liquids, ophthalmic and other sterile products) according to IP, BP and USP.

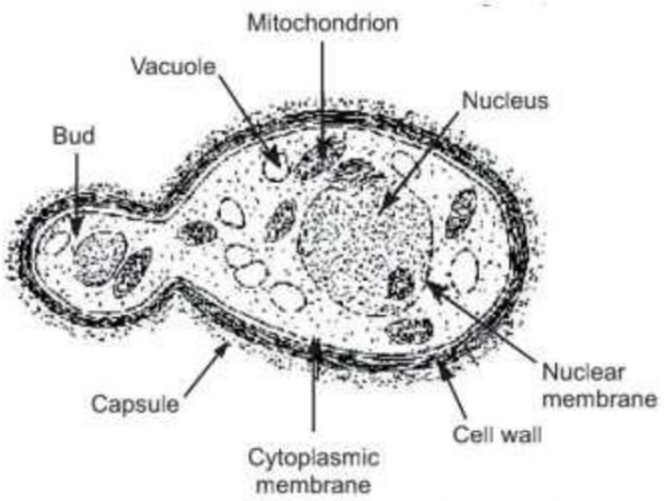
# Fungi:

- Fungi are eukaryotic organisms that play a critical role in the ecosystem and human life.
- They are heterotrophs, meaning they obtain nutrients from other sources, such as dead organic matter or living organisms.
- Fungi are found in a wide range of habitats, including soil, water, air, and on living organisms.
- Fungi reproduce by releasing spores, which are microscopic reproductive units. Spores can be dispersed by the wind, water, or animals.

"The branch of science which deals with the study of fungi is called **Mycology**."

**Depending on cell morphology, fungi can be divided into four classes:**

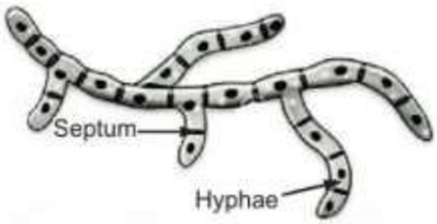
- Moulds
- Yeast
- Yeast like fungi
- Dimorphic fungi




**General structure of Yeast cell**

# Morphology:

- Fungi are eukaryotic organisms that can be unicellular or multicellular.
- Unicellular fungi, such as yeasts, are typically spherical or oval in shape.
- Multicellular fungi consist of threadlike structures called hyphae.
- Hyphae can be septate, meaning that they are divided by cross-walls, or they can be coenocytic, meaning that they are not divided by cross-walls.
- Hyphae can branch and form a network called mycelium.




## Classification of Fungi:

Fungi are classified into several taxonomic groups based on various criteria, including their mode of reproduction, cell structure, and ecological roles. The major groups of fungi include:

1. **Zygomycota:** These fungi reproduce sexually through the formation of zygospores and include molds like Rhizopus.
2. **Ascomycota:** Known as sac fungi, they produce sexual spores in sac-like structures called asci. Common examples are yeasts, truffles, and morels.
3. **Basidiomycota:** Club fungi, such as mushrooms and bracket fungi, reproduce via basidiospores on club-shaped structures called basidia.
4. **Deuteromycota:** Imperfect fungi that lack a known sexual stage, including the common pathogen Aspergillus.
5. **Chytridiomycota and Glomeromycota:** These are primitive fungal groups that exhibit unique characteristics and ecological roles.

## Reproduction

Fungi reproduce sexually and/or asexually. Perfect fungi reproduce both sexually and asexually, while imperfect fungi reproduce only asexually (by mitosis).

**Asexual Reproduction**

- Fungi reproduce asexually by fragmentation, budding, or producing spores. Fragments of hyphae can grow new colonies.
- Mycelial fragmentation occurs when a fungal mycelium separates into pieces with each component growing into a separate mycelium.
- Somatic cells in yeast form buds. During budding (a type of cytokinesis), a bulge forms on the side of the cell, the nucleus divides mitotically, and the bud ultimately detaches itself from the mother cell.

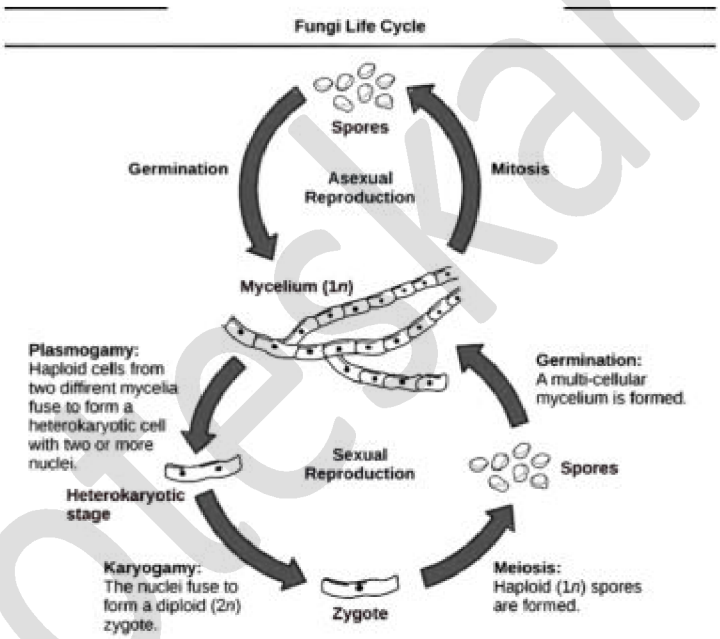
**Sexual Reproduction**

- Sexual reproduction introduces genetic variation into a population of fungi.
- In fungi, sexual reproduction often occurs in response to adverse environmental conditions.

- Two mating types are produced. When both mating types are present in the same mycelium, it is called homothallic, or self-fertile. Heterothallic mycelia require two different, but compatible, mycelia to reproduce sexually.

Although there are many variations in fungal sexual reproduction, all include the following three stages.

- First, during plasmogamy (literally, "marriage or union of cytoplasm"),
- two haploid cells fuse, leading to a dikaryotic stage where two haploid nuclei coexist in a single cell. During karyogamy ("nuclear marriage"), the haploid nuclei fuse to form a diploid zygote nucleus.
- Finally, meiosis takes place in the gametangia (singular, gametangium) organs, in which gametes of different mating types are generated. At this stage, spores are disseminated into the environment.




## Cultivation of Fungi:

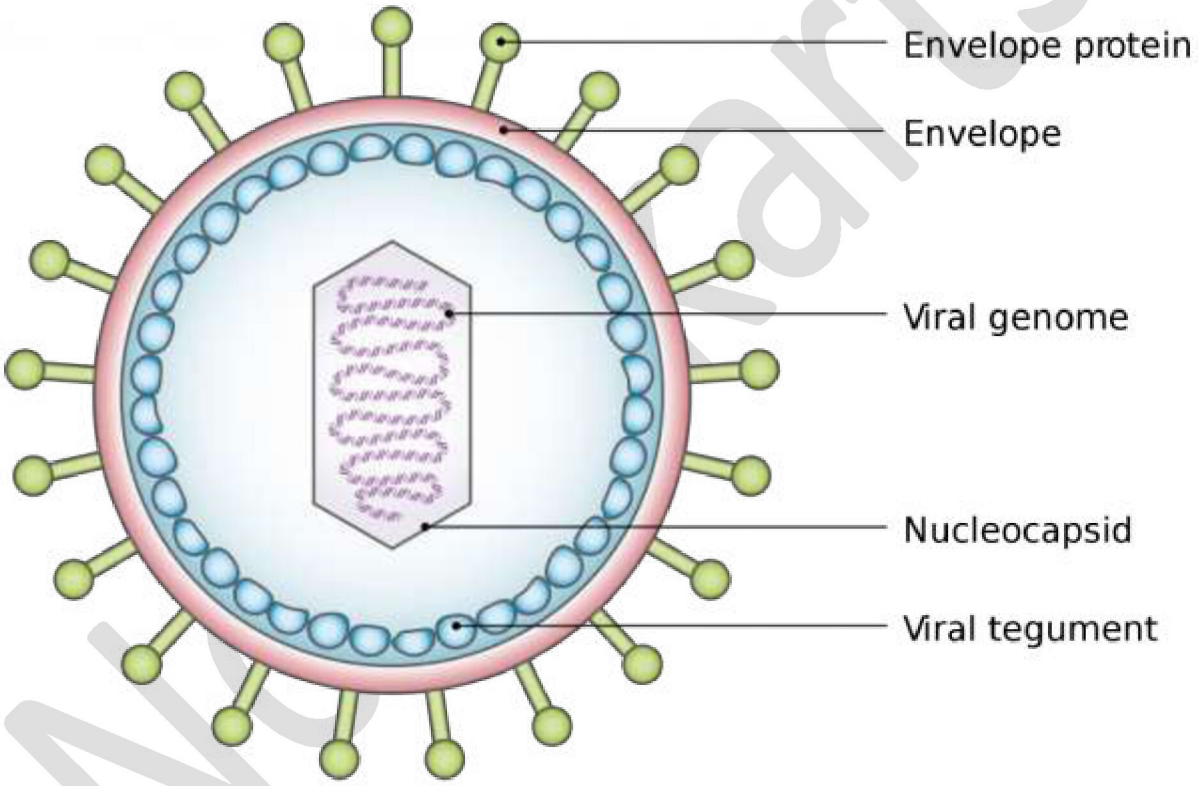
- In cultivation of fungi we have to identify fungi, then separate and grow fungi.
- Sabouraud agar is a type of agar growth medium containing peptones. It is used to cultivate many types of fungi and also grow.
- It has utility for research and clinical care.
- It is developed by Raymond Sabouraud in 1892.
- The standard temperature for incubation of fungi is 30°C in humidified environment for 21 days.
- After 21 days colonies of fungi form on culture medium.

**Media used for growth:**

- Brain heart infusion (BHI) agar
- Czapek's agar (CZA) etc

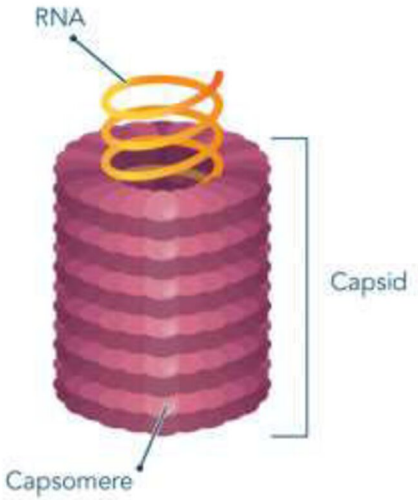
# Viruses:

- Viruses are the smallest of all the microbes.
- They are said to be so small that 500 million rhinoviruses (which cause the common cold) could fit on to the head of a pin.
- They are unique because they are only alive and able to multiply inside the cells of other living things. The cell they multiply in is called the **host cell.**
- A virus is made up of a core of genetic material, either DNA or RNA, surrounded by a protective coat called a capsid which is made up of protein.
- Sometimes the capsid is surrounded by an additional spikey coat called the envelope. Viruses are capable of latching onto host cells and getting inside them.

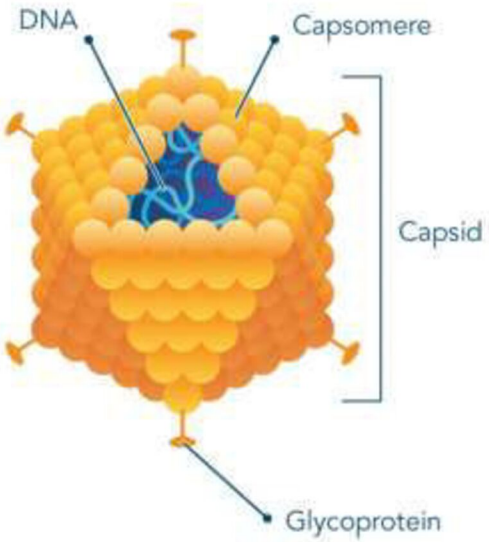



On the basis of their morphology, viruses divided into three types:

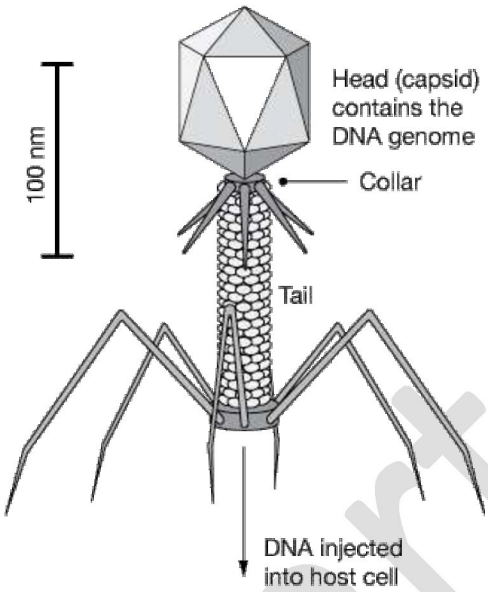
- **Helical virus (Rod Shaped Viruses)**
  - A helical virus is a virus that has a capsid shaped in a filamentous or rod-shaped structure that has a central cavity that encloses its nucleic acid.
  - An icosahedral virus is a virus consisting of identical subunits that make up equilateral triangles that are in turn arranged in a symmetrical fashion.
  - Eg: Rabies Viruses, Tobacco Mosaci Virus




- **Polyhedral (icosahedral) Virus**
  - Polyhedral viruses have nucleic acid encased in a polyhedral (many-sided) shell or capsid, which is commonly shaped like an icosahedron.
  - Eg: Adenoviruses




- **Complex Virus:**
  - It is present a larger variety of components in their capsids than simple viruses.
  - They may contain accessory proteins with specific architectural or functional roles; or incorporate non-proteic elements such as lipids.




### Reproduction/Replication Viruses:

Replication of virus host is necessary host may be bacteria, Plants or an animals .

When virus infect on host cell a host cell is forced to rapidly produce thousands of identical copies of original virus.

Steps for replication:

- Attachment
- Penetration
- Uncoating (Biosynthesis)
- Assembly (Maturation)
- Release

### Attachment:

- In this step virus attached on the surface of host cell, they attached on specific receptor on plasma membrane of host cells.

### Penetration:

- In this step virus enter inside the host cells, (only DNA/RNA of virus enter or complete virus enter) enter of virus.

### Uncoating and Biosynthesis:

- In this step viral capsid is removed by virus enzymes or host enzyme.
- Due to capsid removed viral geromic nucleic acid release in host cells.

**Biosynthesis:**

The replication mechanism depends on the viral genome

- DNA viruses usually use host cell protein and enzymes to make additional DNA that is transcribed to messenger RNA which is then used to direct protein synthesis.
- RNA viruses usually use the RNA core as a template for synthesis of viral genomic RNA.

**Assembly (Maturation):**

- After biosynthesis modified viral protein are packaged with newly replicated viral genome into new virus that are ready for release from the host cell.

**Release:**

**Two method for release:**

- **Lysis:** In this viruses burst the cell membrane of host cell and release (Death of host cell) (Cytolytic)
- **Budding:** In this viruses make envelope from plasma membrane of host cell and release does not harm host cell (Influenza)

## Cultivation of Viruses.

- Viruses are obligate parasites, So they cannot grow on culture media.

**Cultivation of virus are as follow:**

**Laboratory Animals:**

- It is one of the oldest method for cultivation of viruses.
- Animals use for this such as rabbits, guinea pigs, mice, etc.
- The growth of the virus in inoculated animals may be indicated by death disease or visible lesions.
- Animal inoculation is used for the study of pathogenesis, immune response and epidemiology.

**Advantage:**

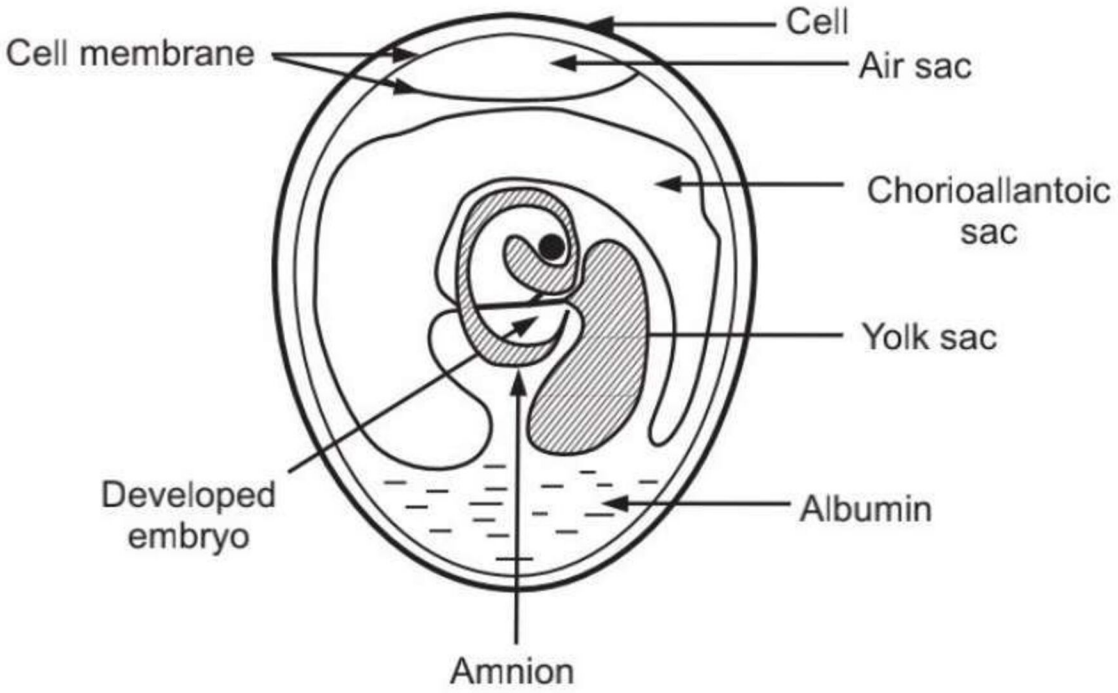
- Antibodies productions are identified.
- Mice are the reliable model for virus replication

**Disadvantage:**

- Animal experiment is costly and difficult to maintain.
- Animal selection for specific viruses is difficult.
- Some human viruses are not grown in animals.
- Vaccine production is not possible with mice model.

**Embryonated Eggs:**

The embryonated Hen's egg offer several sites for the cultivation of viruses.




- Fertile chicken eggs incubated for 5-12 days can be inoculated through the shell aseptically. The opening may be sealed with paraffin wax and the egg incubated at 36°C for the time required for the growth of the viruses.
- Virus may kill the chick embryo and produce specific evidence of viral activity.
- These effect help in the identification of virus.

# Classification and mode of action of disinfectants Factors influencing disinfection, antiseptics and their evaluation

## Classification and mode of action of disinfectants:

**Disinfectants:**

- A disinfectant is a chemical substance or compound used to inactivate or destroy microorganisms on inert surfaces.
- Disinfectants are generally distinguished from other antimicrobial agents such as antibiotics, which destroy microorganisms within the body, and antiseptics, which destroy microorganisms on living tissue.
- They are primarily applied to non-living surface such as for cleaning your countertops or tubes and sterilization of instruments are apparatus.

**Classification of Disinfectants:**

- Acids and alkalies
- Halogens
- Heavy metals
- Phenol and its derivatives
- Alcohols
- Quaternary ammonium compounds
- Dyes
- Detergents and soaps

**Acids and alkalies:** Generally strong acids and alkali kill the bacteria but weak organic acids inhibit their **growth.**

**Halogens:**

Chlorine, fluorine, bromine and iodine in the free state as well as their compound strongly act as germicidal.

**Heavy Metals:**

- The most widely used heavy matels are those of mercury, silver and copper.
- Heavy metals and their compounds act as antimicrobial by combining with the cellular protein.
- High concentration of salts of heavy metals like mercury, copper and silver coagulate cytoplasmic proteins, resulting in the damage or death of cell.

**Phenol and its derivatives:**

- Phenol is the chief products obtained by the distillation of the coal tar.
- Phenol 1% has bacterial action.
- Many derivatives of phenol are more effective and less costly.

**Alcohols:**

- Alcohols have fairly rapid bactericidal action against vegetative bacteria when diluted to the concentration of 60% to 70% v/v with water.
- Ethanol 60 to 70% v/v and isopropanol 50 to 60 % v/v are used as skin disinfectants while methanol vapour has been used as fungicide.
- The higher alcohols (Propyl, Butyl, amyl, etc) are more germicidal than ethyl alcohol.

**Aldehyde:**

- Formaldehyde is the main aldehyde used for disinfection.
- Formaldehyde is solution is usefuls for sterilization of certain instruments.

**Dyes:**

- A number of dyes have been used to inhibit the bacterial growth.
- Basic dyes are more effective bactericides than acidic dyes.
- Acridine and triphenylmethane dyes are commonly used as antimicrobial agents.

**Detergents & Soaps:**

- They are widely used as surface active agents, wetting agents and emulsifiers.
- They are classified into four main group such as anionic, cationic, non-ionic and emulsifiers.
- The most important antibacterial agents are the cationic surface active agents.
- **Eg: Cetrimide, benzalkonium chloride etc.**
- Soaps and Sodium lauryl sulfate are anionic compounds. Soaps prepared from saturated fatty acids are more effective against gram negative bacilli while those prepared form unsaturated acids have greater action against gram positive.
- Nonionic detergents are not ionized. However these substances do not posses significant anti-microbial activity.
- Amphoteric compounds have the detergent properties of anionic surfaces combined with disinfectant properties of cationic surfactants

**Mode of action**:

**Alcohols**

- Coagulation of proteins
- Water-dependent action because water is necessary to make cell membrane absorptive.
- Cytoplasmic elements denature after intracellular congregation.
- Disrupt the cell membrane.

**Aldehydes**

- Activity via alkylation for groups of carboxyl-, amino-, and hydroxyl destroys nucleic acids.
- It eliminates each microorganism and spores.

**Phenol**

- It coagulation the cellular protein and lipids
- Disrupt the membranes

**Halogens**

- Act through oxidation and destroy essential enzyme groups such as sulfhydryl.
- Chlorine and water chemically form hypochlorous acid (microbicidal)

**Hydrogen Peroxide**

- Act through oxidation and react with cellular protein and DNA.
- It functions on microbes through discharging of nascent O2.

## Factors influencing disinfection:

- Concentration
- Temprature
- pH
- Surface Activity:
- Interfering Agents:

**Concentration:**

- The Rate of killing of microorganism warrier directly with the concentration of the disinfectant.

**Temprature:**

- The rate of disinfectant normally increase with the temp the effect of the temp of bacteriosidal activity.

**pH:**

- The bacterial growth it optimum in the pH range 0 to 8 and the growth declines out side this range.

**Surface Activity:**

- The additive of low concentration of surface active compounds may antibacterial agent,
- Eg: Phenols are more active in the presence of soaps.

**Interfering Agents:**

- Interfering agents like organic acids, may directly or indirectly affect the action of disinfectants.
- These interfering agents reduce the activity of disinfectants by adsorbing or inactivating them.

**Antiseptics:**

- Antiseptics are antimicrobial substance that are applied to living tissue skin to reduce the possibility of infection.
- Some antiseptic are germicide capable for destaring microbe.

## Differences b/w Antiseptics and Disinfectants:

| S.No. | Disinfectants | Antiseptics |
|---|---|---|
| 1. | They are manly used to kill the microbes on the non-living objects | They are used to kill the microbes on the skin. |
| 2. | They have power to kill all the microorganisms and bacteria on the surfaces | They do not have power to kill all the microorganisms and bacteria on the surfaces |
| 3. | They are used in laboratory and house hold items cleaning | They are used in surgery and hand washing. |

**For bacteriostatic and bactericidal actions Evaluation of bactericidal & Bacteriostatic.**

## Evaluation of Disinfectants:

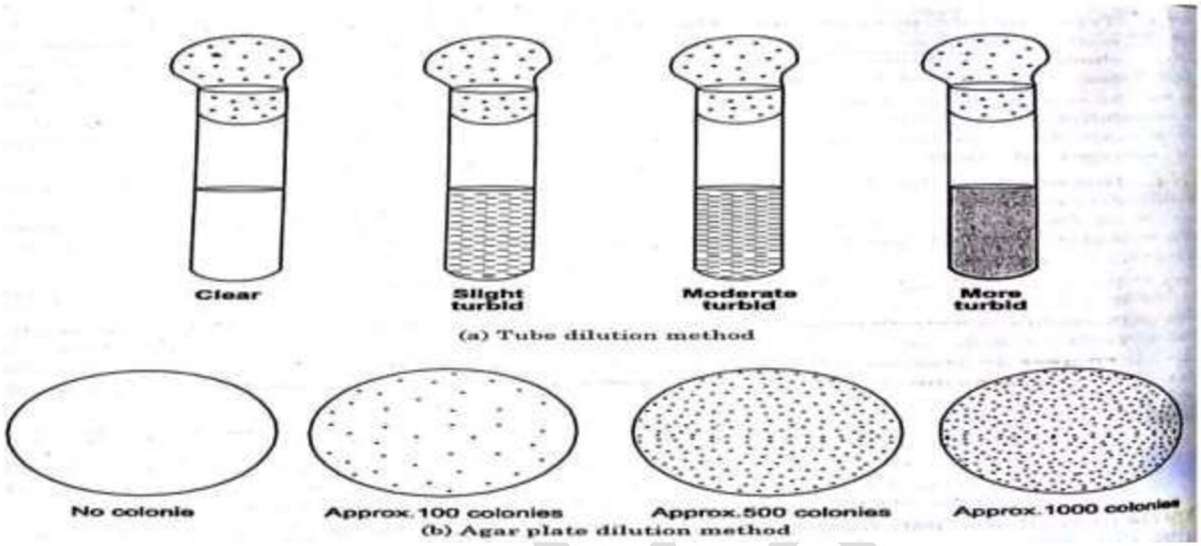
Techniques and methods used for evaluation of Disinfectants:

- **Tube dilution and agar plate method.**
- **Cup plate methods (Cylinder plate method)**
- **Ditch Plate method**
- **Gradient plate technique**
- **Phenol coefficient method**

## Tube dilution and agar plate method.

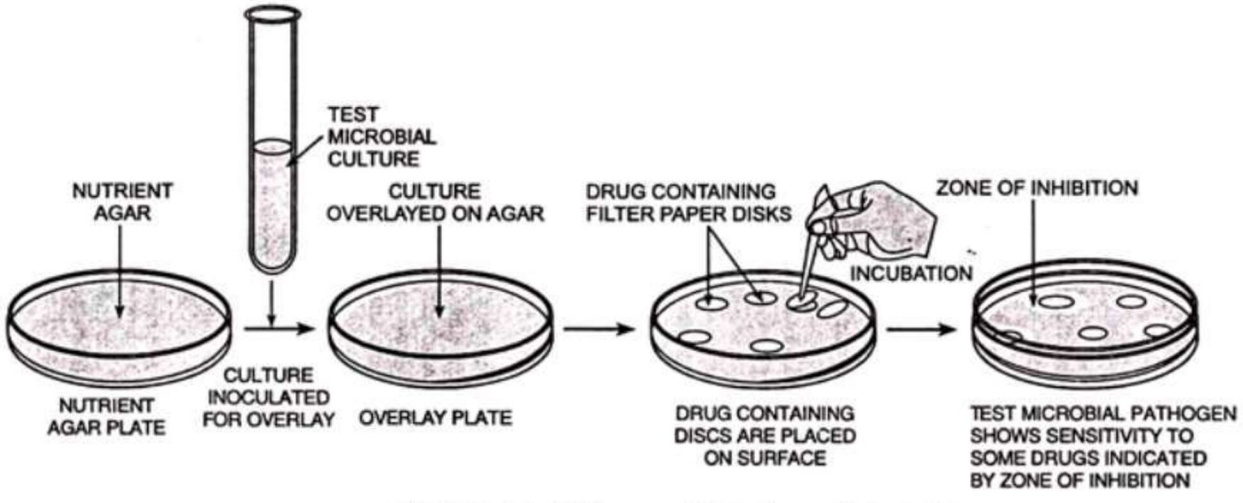
- The chemical agent is incorporated into nutrient broth or agar medium and inoculated with test micro-organisms.
- These tubes are incubated at 30°C to 35°C for 2 to 3 days and then the results in the form of turbidity or colonies are observed.
- The results are recorded and the activity of the given disinfectant is compared.

# 1. Tube Dilution And Agar Plate Method



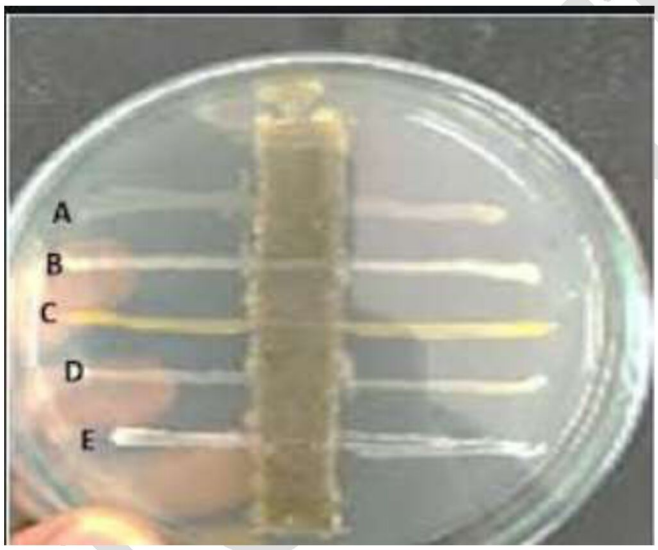

## Cup plate methods (Cylinder plate method)

- The nutrient agar is melted, cooled suitably, poured into petri dish.
- Spread 0.2 ml of known concentration of inoculum on the surface of the solidified agar ( Spread Plate Technique).
- Cups or cavities are made by using a sterile borer.
- Now 0.2 ml of drug is poured into the cups of agar plate and then incubated at 37°C for 24 hr.
- If the drug has any anti-bacterial effect it will show the zone of inhibition.



## Ditch Plate method:

- Agar is melted and then solidified in a petri plate.
- **A ditch is made in the petri plate** by cutting the soliodified agar.
- The **disinfectant solution is made to run through the ditch** carefully.
- The **test organisms are streaked outwards from the ditch**.
- The **petriplate is incubated** at desired temp. And time period.
- The **microorganisms** which are **resistant to the disinfectant grow even near the start at the ditch** itself.
- The **sensitive organisms show a zone of inhibition** near the ditch or at center of the petri plate.
- The **width of the zone of inhibition is an indication of activity** against the test organism.




## Gradient Plate Technique

- This technique is used to isolate the resistant mutants.
- The petri dish is kept in slanting position and; a sufficient amount of melted nutrient agar is poured and solidified in slanting position.
- Another layer of agar is poured over it, which contains antibiotic solution and solidified it.
- After solidification, 0.2ml of bacterial culture was spreaded over the solid surface and incubated it at 370C for 24 to 48 hr.
- The microorganisms will grow, where the concentration of the drug is below the critical level.
- The antibiotics get diluted on the lower layer and the gradient of concentration will be produced.
- Thus the resistant mutant can be islated.

## Phenol Coefficient Method:

- In this method the efficacy of the disinfectant in use is rated by **comparing** it **with** the activity of **Phenol** taking as a standard.

- The test is carried out by adding an increasing amount of phenol and disinfectant in test tubes containing microorganisms.
- In **UK the test organism used is Salmonella typhi** while the **USA uses Salmonella typhi, Staphylococcus aureus and Pseudomonas aeruginosa**.
- The official phenol coefficient tests include,
    - Rideal-Walker Test (RW Test).
    - Chick-Martin Test.
    - United States FDA Test for Phenol Coefficient. (FDA Test)
    - The US Association of Official Agricultural Chemists Test (FDA Test)

A. **Rideal-Walker Tes**t:

- Introduced by **Rideal** and **Walker** the British chemists in **1903** are still in use.
- The test uses Rideal, Walker Broth and **Salmonella typhi** as a test organism.
- **Different dilutions of the phenol and test disinfectants are made** and **5 ml of each is inoculated with the 0.5 ml of the 24 hr broth culture of the test microorganism**.
- All the **test tubes are placed in a water bath at 17.5 °C**.
- **Subcultures from each test tube are taken and transferred to 5 ml sterile broth after 2.5, 5, 7.5 and 10 minutes**.
- The broth tubes are **incubated at 37 °C for 2 - 3 days** and are examined for the presence or absence of the growth and the Phenol coefficient is calculated using the formula,

$$RW Coefficient = \frac{Dilution of Disinfectant Killing In 7.5 But Not In 5 min}{Dilution of Phenol Killing In 7.5 But Not In 5 min}$$

- The Phenol Coefficient for phenol is considered as "1" any value for disinfectant coming below one is considered as less while above it is more.

Sterility testing of products (solids, liquids, ophthalmic and other sterile products) according to IP, BP and USP.

## Sterility testing:

- It is the method required for all articles or substances to be introduced in to raw tissue (injections, ophthalmics etc.).
- The method is reveals the presence or absence of viable microorganisms in any articles.

- All the products labeled sterile indicate that the product must pass through the sterility testing methods as per IP, BP and USP.
- There are two methods are used namely membrane filtration and direct inoculation.

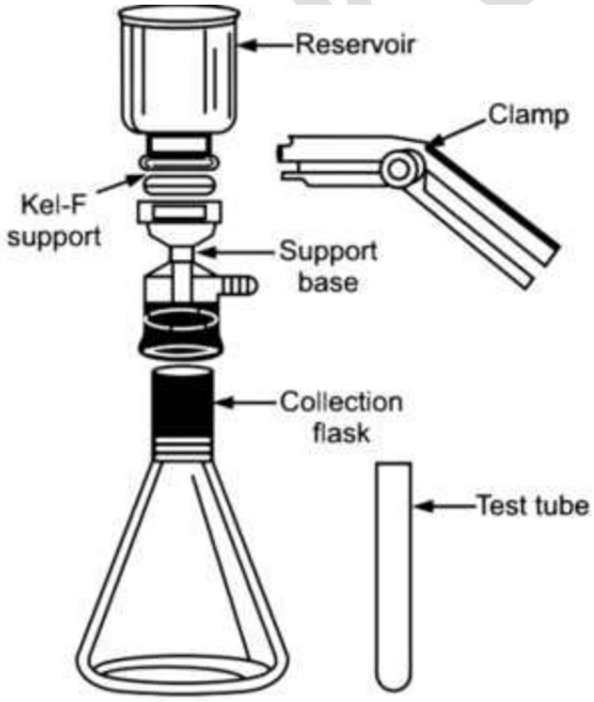
## Methods for Sterility test

### 1. Membrane Filteration:

- This method is used for those substances that are put in to the solution like oil, ointment, non-bacteriostatic solids and a soluble powder or liquids that have themselves bacteriostatic properties etc.
- This method is used for the liquid products which have the volume of the container 100 ml or more

### Apparatus:

- The apparatus contains a reservoir and a container to collect the filtrate.
- In the junction of these two, a membrane is placed which has an proper porosity (0.45 µM) and filter diameter is 47 mm.
- The flow rate of fluid is 55-75 ml/minute at a pressure of 70 mm of mercury. Complete unit should be free from microorganisms.




### 2. Direct inoculation:

- This method is used for small volume samples. The volume of the product is not more than 10% of the volume of the medium.
- It is suitable method for aqueous solutions, oily liquids, ointments and creams. The method involves aseptically opened each sample container from a sterilized batch of product.

- Then using sterile syringe and needle the required volume of sample is withdrawn for both media from the container.
- Half of the volume of the sample is injected in to a test tube containing the required volume of fluid thioglycollate medium and the other half volume of sample into a second test tube containing the required volume of Soybean Casein Digest Medium.

**Method:**