Course/Subject Name	Pharmaceutical Microbiology
Course/Subject Code	BP303T
Module no.	3
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Learning Outcome of Unit-3

LO	Learning Outcome(LO)	Course Outcome Code
LO1	Students will learn about the morphology, classification, reproduction/replication and cultivation of Fungi and Viruses.	BP303.1
LO2	Students will learn about the classification and mode of action of disinfectants.	BP303.3
LO3	Students will learn about theEvaluation of bactericidal and Bacteriostatic and sterility testing of products	BP303.3

Content of Module:

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

FUNGI

Introduction:

The **fungi** (singular, **fungus**) include several thousand species of eukaryotic, sporebearing organisms that obtain simple organic compounds by absorption. The organisms have no chlorophyll and reproduce by both sexual and asexual means. The fungi are usually filamentous, and their cell walls have **chitin.** The study of fungi is called **mycology**, and fungal diseases are called **mycoses.**

Together with bacteria, fungi are the major decomposers of organic materials in the soil. They degrade complex organic matter into simple organic and inorganic compounds. In doing so, they help recycle carbon, nitrogen, phosphorous, and other elements for reuse by other organisms. Fungi also cause many plant diseases and several human diseases.

Two major groups of organisms make up the fungi. The filamentous fungi are called molds, while the unicellular fungi are called yeasts. The fungi are classified in the kingdom Fungi in the Whittaker five-kingdom system of classification.

Morphology:

There are wide varieties that range from the smallest unicellular fungi such as yeast to larger multicellular capable of forming hyphal threads or false roots. For this reason, fungi are also classified according to their morphologies.

The following are classifications of fungi based on morphology:

Yeast

Yeast is single celled fungi that can be found in a variety of environment from soil and plants to animal and aquatic environments. Unlike bacteria, yeasts are also eukaryotic, which means that they have different types of organelles that are common in the cells of higher animals.

The Saccharomyces cerevisiae is a good example of yeast that ranges between 1 and 7 micrometers in size. When viewed under the microscope, these organisms may be pigmented on their surface.

As with the cells or higher organisms, the S. cerevisiae contains such organelles as a membrane bound nucleus, a vacuole, mitochondria and the Golgi apparatus as well as the E.R (endoplasmic reticulum). The cell wall of these yeast is composed of glucan (a polysaccharide compound) and mannoproteins.

As for the genome, research has shown these yeast to carry a single, linear double stranded DNA that consists of several repeated sequences. For yeast, the primary mode of reproduction is through budding.

Following the copying of the genetic material, a bud is formed on the surface of the cell that ultimately breaks off with its genetic material and grows to form a new cell.

Yeast-like Fungi

Yeast-like fungi are yeast that partly grows like normal yeast. However, they also attach to each other to form what is known as pseudo hyphae (not a true hyphae).

Candida albicans is one of the most common. When viewed under the microscope, these organisms have been shown to consist of several layers that make up the cell wall.

As with yeast, the wall of C. albicans contains layers of mannoproteins, lipids and a beta glucon, a chitin inner layer that strengthen the cell wall. Like yeast, C. albicans also appear spherical or ovoid in shape and measure between 4 to 8 micrometers.

Since they also reproduce through budding, like yeast, C. albicans may end up creating an elongated chain of cells as they continue dividing to form the pseudohyphae. However, some studies suggest that some of the yeast-like fungi tend to form true hyphae in the process.

Yeast-like fungi such as C. albicans are also described as being polymorphic fungus. This is because they present four types of morphology including the yeast cell, pseudohyphae, hyphae as well as chlamydospores. As such, they are likely to be seen having varying appearance when viewed under the microscope depending on such conditions as the availability of nutrition, pH and temperature among others.

The type of morphological appearance of these cells has also been associated with the pathogenicity of the organism. Given that they are not bacteria, some of these organisms (yeast-like fungi) have characteristics associated with eukaryotic cells in that some have been shown to contain a nucleus and other essential organelles.

Molds

Mold (Mould) are a type of fungi that often grow well in favorable environments with warmth and moisture. They can be found growing on various surfaces such as food surfaces from which they obtain their nutrients.

Compared to yeast, molds are multicellular organisms. As such, they can be seen with the naked eye without using a microscope. However, when viewed under the microscope, it is possible to observe numerous filaments (Hyphae) that are collectively referred to as Mycelium.

While these organisms are microscopic, it is their numerous hyphae (that form the mycelium) that make it possible to see mold as it grows on food surface (bread, oranges etc). There are two main types of mycelium depending on their functions.

These include:

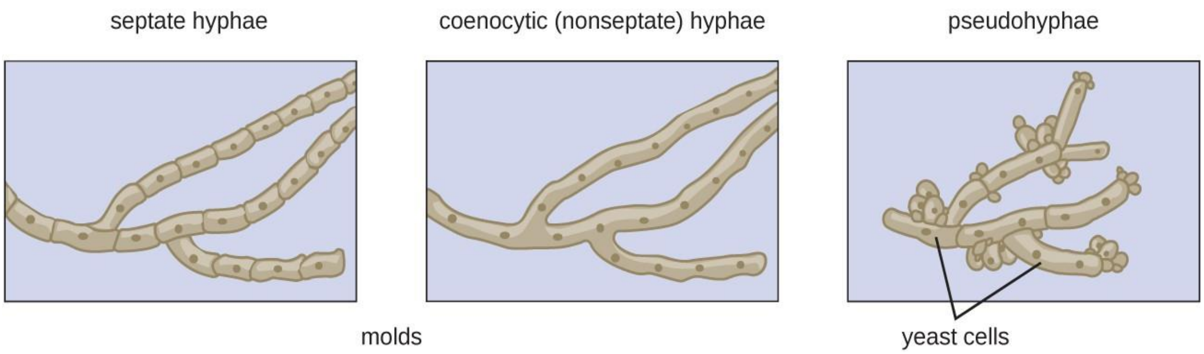
Vegetative mycelium - Vegetative mycelium include the hyphae that penetrates into the substrate and absorbs nutrients for the continued growth and development of the organisms. These hyphae therefore act like plant roots.

Aerial mycelium - Aerial mycelium are the hyphae that are located above the food substance. When viewed closely under the microscope, aerial mycelium contain a spherical structure at the top of the hyphae. These are known as the conidia and sere as the reproductive part of the mold. This part produces spores (asexual reproduction) that can grow in favorable conditions.

Dimorphic Fungi

Dimorphic fungi exist in the form of mold and yeast. Examples of these fungi include Penicilliummarneffei and Mucorcircinelloides.

Most of these organisms are capable of switching from between the two forms in a process commonly referred to as dimorphic switching. As such, they are viewed as having hyphal threads or as single celled organisms under the microscope (yeasts).



Classification of Fungi

The kingdom Fungi contains five major phyla that were established according to their mode of sexual reproduction or using molecular data. Polyphyletic, unrelated fungi that reproduce without a sexual cycle, are placed for convenience in a sixth group called a “form phylum.” Not all mycologists agree with this scheme. Rapid advances in molecular biology and the sequencing of 18S rRNA (a part of RNA) continue to show new and different relationships between the various categories of fungi.

The five true phyla of fungi are the Chytridiomycota (Chytrids), the Zygomycota (conjugated fungi), the Ascomycota (sac fungi), the Basidiomycota (club fungi) and the recently described Phylum Glomeromycota. The Deuteromycota is an informal group of unrelated fungi that all share a common character – they use strictly asexual reproduction.

Chytridiomycota: The Chytrids

The only class in the Phylum Chytridiomycota is the **Chytridiomycetes**.Like all fungi, chytrids have chitin in their cell walls, but one group of chytrids has both cellulose and chitin in the cell wall. Most chytrids are unicellular; a few form multicellular organisms and hyphae, which have no septa between cells (coenocytic). They produce gametes and diploid zoospores that swim with the help of a single flagellum.The ecological habitat and cell structure of chytrids have much in common with protists. Chytrids usually live in aquatic environments, although some species live on land. Some species thrive as parasites on plants, insects, or amphibians (Figure 1), while others are saprobes.

Zygomycota: The Conjugated Fungi

The zygomycetes are a relatively small group of fungi belonging to the Phylum **Zygomycota**. They include the familiar bread mold, *Rhizopusstolonifer*, which rapidly propagates on the surfaces of breads, fruits, and vegetables. Most species are saprobes, living off decaying organic material; a few are parasites, particularly of insects. Zygomycetes play a considerable commercial role. The metabolic products of other species of *Rhizopus* are intermediates in the synthesis of semi-synthetic steroid hormones.

Zygomycetes have a thallus of coenocytic hyphae in which the nuclei are haploid when the organism is in the vegetative stage. The fungi usually reproduce asexually by producing sporangiospores (Figure 2). The black tips of bread mold are the swollen sporangia packed with black spores (Figure 3). When spores land on a suitable substrate, they germinate and produce a new mycelium. Sexual reproduction starts when conditions become unfavorable. Two opposing mating strains (type + and type –) must be in close proximity for gametangia from the hyphae to be produced and fuse, leading to karyogamy. The developing diploid **zygospores** have thick coats that protect them from desiccation and other hazards. They may remain dormant until environmental conditions are favorable. When the zygospore germinates, it undergoes meiosis and produces haploid spores, which will, in turn, grow into a new organism. This form of sexual reproduction in fungi is called conjugation (although it differs markedly from conjugation in bacteria and protists), giving rise to the name "conjugated fungi."

Ascomycota: The Sac Fungi

The majority of known fungi belong to the Phylum **Ascomycota**, which is characterized by the formation of an **ascus** (plural, asci), a sac-like structure that contains haploid ascospores. Many ascomycetes are of commercial importance. Some play a beneficial role, such as the yeasts used in baking, brewing, and wine fermentation, plus truffles and morels, which are held as gourmet delicacies. *Aspergillusoryzae* is used in the fermentation of rice to produce sake. Other ascomycetes parasitize plants and animals, including humans. For example, fungal pneumonia poses a significant threat to AIDS patients who have a compromised immune system.

Asexual reproduction is frequent and involves the production of conidiophores that release haploid conidiospores.

Basidiomycota: The Club Fungi

The fungi in the Phylum **Basidiomycota** are easily recognizable under a light microscope by their club-shaped fruiting bodies called **basidia** (singular, **basidium**), which are the swollen terminal cell of a hypha. The basidia, which are the reproductive organs of these fungi, are often contained within the familiar mushroom, commonly seen in fields after rain, on the supermarket shelves, and growing on your lawn (Figure 6). These mushroom-producing basidiomyces are sometimes referred to as "gill fungi" because of the presence of gill-like structures on the underside of the cap. The "gills" are actually compacted hyphae on which the basidia are borne. This group also includes shelf fungus, which cling to the bark of trees like small shelves. In addition, the basidiomycota includes smuts and rusts, which are important plant pathogens; toadstools, and shelf fungi stacked on tree trunks. Most edible fungi belong to the Phylum Basidiomycota; however, some basidiomycetes produce deadly toxins.

The lifecycle of basidiomycetes includes alternation of generations (Figure 7). Spores are generally produced through sexual reproduction, rather than asexual reproduction. The club-shaped basidium carries spores called basidiospores. In the basidium, nuclei of two different mating strains fuse (karyogamy), giving rise to a diploid zygote that then undergoes meiosis. The haploid nuclei migrate into basidiospores, which germinate and generate monokaryotic hyphae.

Deuteromycota: The Imperfect Fungi

Imperfect fungi—those that do not display a sexual phase—are classified in the form phylum **Deuteromycota**. Deuteromycota is a polyphyletic group where many species are more closely related to organisms in other phyla than to each other; hence it cannot be called a true phylum and must, instead, be given the name form phylum. Since they do not possess the sexual structures that are used to classify other fungi, they are less well described in comparison to other divisions. Most members live on land, with a few aquatic exceptions. They form visible mycelia with a fuzzy appearance and are commonly known as **mold**. Molecular analysis shows that the closest group to the deuteromycetes is the ascomycetes. In fact, some species, such as *Aspergillus*, which were once classified as imperfect fungi, are now classified as ascomycetes.

Glomeromycota

The **Glomeromycota** is a newly established phylum which comprises about 230 species that all live in close association with the roots of trees. Fossil records indicate that trees and their root symbionts share a long evolutionary history. It appears that all members of this family form **arbuscularmycorrhizae**: the hyphae interact with the root cells forming a mutually beneficial association where the plants supply the carbon source and energy in the form of carbohydrates to the fungus, and the fungus supplies essential minerals from the soil to the plant.

REPRODUCTION:

Asexual reproduction occurs in the fungi when spores form by mitosis. These spores can be conidia, sporangiospores, arthrospores (fragments of hyphae), or chlamydospores (spores with thick walls).

During sexual reproduction, compatible nuclei unite within the mycelium and form sexual spores. Sexually opposite cells may unite within a single mycelium, or different mycelia may be required. When the cells unite, the nuclei fuse and form a diploid nucleus. Several divisions follow, and the haploid state is reestablished.

Fungal spores are important in the identification of the fungus, since the spores are unique in shape, color, and size. A single spore is capable of germinating and reestablishing the entire mycelium. Spores are also the method for spreading fungi in the environment. Finally, the nature of the sexual spores is used for classifying fungi into numerous divisions.

CULTIVATION:

Fungal (mycotic) cultures are microbiology laboratory tests to detect or rule out the presence of fungi (plural of fungus) in specimens taken from patients, animals, and the environment. The laboratory uses optimal conditions to grow and identify any fungus present in the specimen while attempting to eliminate or identify contaminants. The specimen is cultured by spreading a small portion of it on various agar media (inoculation). The media are then incubated in a warm, moist environment and examined regularly to detect growth of any organisms. The isolated fungus is identified primarily by its colony morphology and microscopic structures.

Fungi differ from higher plants in that they do not contain chlorophyll and thus cannot manufacture their own carbohydrates. They must use preformed carbon and nitrogen compounds made by other organisms and are therefore either saprophytic (living on dead or decaying organic matter) or parasitic (living on or within other living organisms). Most fungi trace back to a soil origin. All are obligate aerobes (require oxygen to survive), and tend to thrive in a dark, moist, undisturbed atmosphere. They grow well at room temperature, but some of the pathogenic (disease causing) dimorphic fungi also grow well at body temperature.

Of the more than 50,000 species of fungi, only 100 to 150 species of yeast and molds cause disease in humans. The number routinely seen is much lower. Humans are generally resistant to fungi even when they become accidental hosts by inhaling spores or by having a cut or scrape exposed to a fungus. However, inhalation of spores of some of the dimorphic fungi produces illness ranging from mild cough and fever to severe disseminated disease. People with weakened immune systems (immunocompromised) are susceptible to illness from many normally harmless fungi. The characteristics of fungi that make them pathogenic to humans are:

- a small enough spore size to be able to reach the alveoli of the lungs
- the ability to grow at body temperature
- the ability of a dimorphic fungus to convert from a mold to a yeast form within the host
- toxin production

Purpose

The purpose of the fungal culture is to attempt to grow and identify any fungus originating from a patient's specimen when the medical staff of a hospital, clinic or doctor's office suspects fungal infection . Further, the goal is to determine whether the isolated fungus is clinically significant; that is, the causative agent of the patient's disease. While the physician makes the final decision regarding clinical significance, the laboratory may assist in this process by noting the presence of common contaminants, etc. Since the goal is also to provide information in a timely manner, fungal cultures will usually include smears and stains taken directly from the specimen for microscopic examination. The direct examination attempts to visually detect such fungal elements as hyphae, yeast, or spores.

VIRUS:

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.

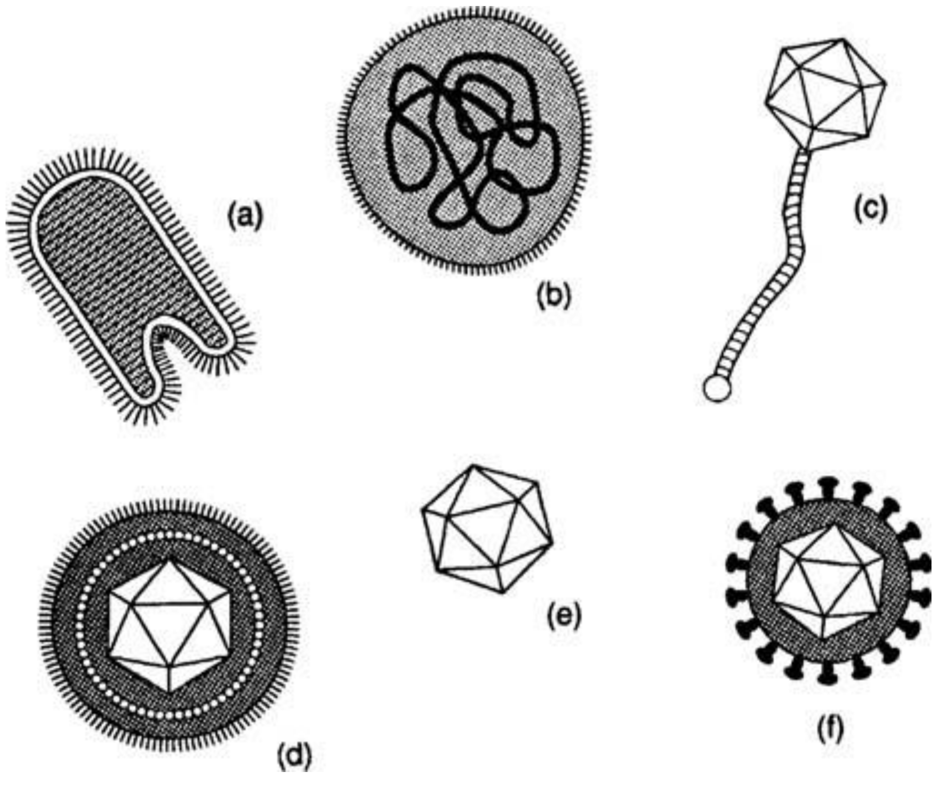
MORPHOLOGY:

Certain viruses contain ribonucleic acid (RNA), while other viruses have deoxyribonucleic acid (DNA). The nucleic acid portion of the viruses is known as thegenome. The nucleic acid may be single-stranded or double-stranded; it may be linear or a closed loop; it may be continuous or occur in segments.

The genome of the virus is surrounded by a protein coat known as a capsid, which is formed from a number of individual protein molecules called capsomeres. Capsomeres are arranged in a precise and highly repetitive pattern around the nucleic acid. A single type of capsomere or several chemically distinct types may make up the capsid. The combination of genome and capsid is called the viral nucleocapsid.

A number of kinds of viruses contain envelopes. An envelope is a membranelike structure that encloses the nucleocapsid and is obtained from a host cell during the replication process. The envelope contains viral-specified proteins that make it unique. Among the envelope viruses are those of herpes simplex, chickenpox, and infectious mononucleosis.

The nucleocapsids of viruses are constructed according to certain symmetrical patterns. The virus that causes tobacco mosaic disease, for example, has helical symmetry. In this case, the nucleocapsid is wound like a tightly coiled spiral. The rabies virus also has helical symmetry. Other viruses take the shape of an icosahedron, and they are said to have icosahedral symmetry. In an icosahedron, the capsid is composed of 20 faces, each shaped as an equilateral triangle (Figure 1). Among the icosahedral viruses are those that cause yellow fever, polio, and head colds.



An array of viruses. (a) The helical virus of rabies. (b) The segmented helical virus of influenza. (c) A bacteriophage with an icosahedral head and helical tail. (d) An enveloped icosahedral herpes simplex virus. (e) The unenveloped polio virus. (f) The icosahedral human immunodeficiency virus with spikes on its envelope.

The envelope of certain viruses is a lipid bilayer containing glycoproteins embedded in the lipid. The envelope gives a somewhat circular appearance to the virus and does not contribute to the symmetry of the nucleocapsid. Projections from the envelope are known as spikes. The spikes sometimes contain essential elements for attachment of the virus to the host cell. The virus of AIDS, the human immunodeficiency virus, uses its spikes for this purpose.

Bacteriophages are viruses that multiply within bacteria. These viruses are among the more complex viruses. They often have icosahedral heads and helical tails. The virus that attacks and replicates in *Escherichia coli* has 20 different proteins in its helical tail and a set of numerous fibers and "pins." Bacteriophages contain DNA and are important tools for viral research.

REPLICATION:

During the process of **viral replication**, a virus induces a living host cell to synthesize the essential components for the synthesis of new viral particles. The particles are then assembled into the correct structure, and the newly formed virions escape from the cell to infect other cells.

The first step in the replication process is **attachment.** In this step, the virus adsorbs to a susceptible host cell. High specificity exists between virus and cell, and the envelope spikes may unite with cell surface receptors. Receptors may exist on bacterial pili or flagella or on the host cell membrane.

The next step is **penetration** of the virus or the viral genome into the cell. This step may occur by phagocytosis; or the envelope of the virus may blend with the cell membrane; or the virus may "inject" its genome into the host cell. The latter situation occurs with the bacteriophage when the tail of the phage unites with the bacterial cell wall and enzymes open a hole in the wall. The DNA of the phage penetrates through this hole.

The **replication** steps of the process occur next. The protein capsid is stripped away from the genome, and the genome is freed in the cell cytoplasm. If the genome consists of RNA, the genome acts as a messenger RNA molecule and provides the genetic codes for the synthesis of enzymes. The enzymes are used for the synthesis of viral genomes and capsomeres and the assembly of these components into new viruses. If the viral genome consists of DNA, it provides the genetic code for the synthesis of messenger RNA molecules, and the process proceeds.

In some cases, such as in HIV infection (as discussed below), the RNA of the virus serves as a template for the synthesis of a DNA molecule. The enzyme reverse transcriptase catalyzes the DNA's production. The DNA molecule then remains as part of the host cell's chromosome for an unspecified period. From this location, it encodes messenger RNA molecules for the synthesis of enzymes and viral components.

Once the viral genomes and capsomeres have been synthesized, they are assembled to form new virions. This **assembly** may take place in the cytoplasm or in the nucleus of the host cell. After the assembly is complete, the virions are ready to be released into the environment (Figure 2).

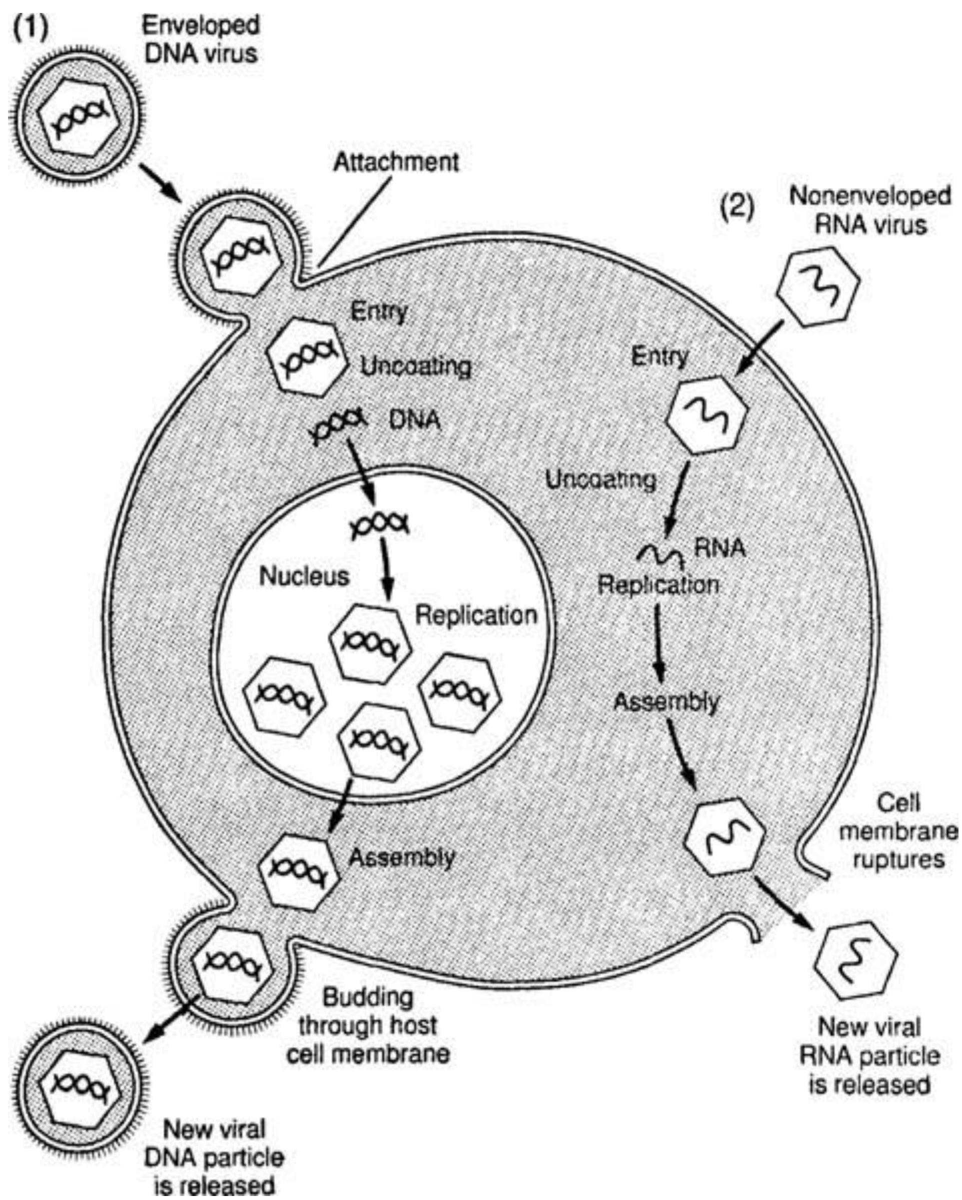


Figure: A generalized representation of the replication of two viruses. Replication of a DNA virus is shown in (1); replication of an RNA virus is displayed in (2).

For the **release** of new viral particles, any of a number of processes may occur. For example, the host cell may be "biochemically exhausted," and it may disintegrate, thereby releasing the virions. For enveloped viruses, the nucleocapsids move toward the membrane of the host cell, where they force themselves through that membrane in a process called **budding.** During budding, a portion of cell membrane pinches off and surrounds the nucleocapsid as an envelope. The replication process in which the host cell experiences death is called the **lytic cycle** of reproduction. The viruses so produced are free to infect and replicate in other host cells in the area.

Lysogeny. Not all viruses multiply by the lytic cycle of reproduction. Certain viruses remain active within their host cells for a long period without replicating. This cycle is called the **lysogenic cycle.** The viruses are called **temperate viruses**, or **proviruses**, because they do not bring death to the host cell immediately.

In lysogeny, the temperate virus exists in a latent form within the host cell and is usually integrated into the chromosome. Bacteriophages that remain latent within their bacterial host cell are called **prophages.** This process is a key element in the recombination process known as **transduction.**

An example of lysogeny occurs in **HIV infection.** In this case, the human immunodeficiency virus remains latent within the host T-lymphocyte. An individual whose infection is at this stage will not experience the symptoms of AIDS until a later date.

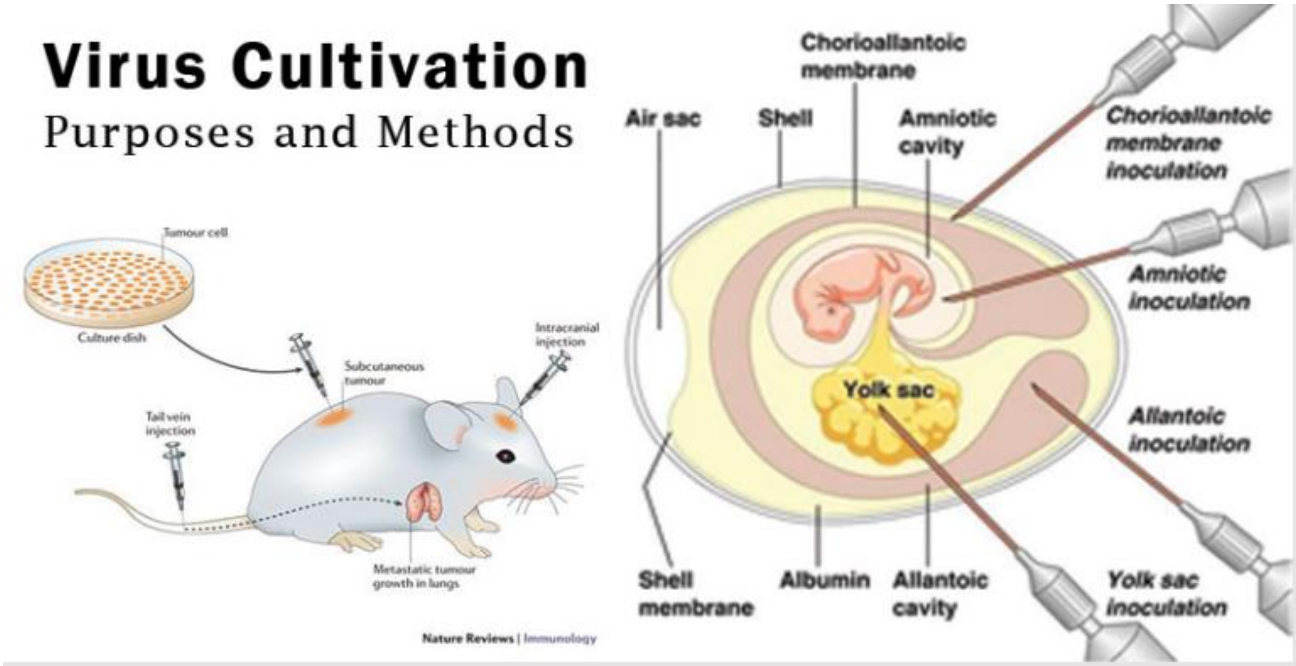
CULTIVATION:

Viruses are obligate intracellular parasites so they depend on host for their survival. They cannot be grown in non-living culture media or on agar plates alone, they must require living cells to support their replication. The primary purpose of virus cultivation is: **To isolate and identify viruses in clinical samples**.

Viruses can be cultivated within suitable hosts, such as a living cell. To study bacteriophages, for example, bacteria are grown in a suitable growth medium; then bacteriophages are added. The bacteriophages multiply within the bacteria and increase their numbers substantially.

Animal and plant viruses are cultivated in cell cultures. A **cell culture** is prepared by encouraging cell growth outside the animal or plant source. The cells are kept alive in a suspension of growth factors within a Petri dish. A thin layer of cells, or monolayer, is then inoculated with viruses, and replication takes place. Fertilized eggs and living animals can also be used to cultivate viruses.

For research study, viruses can be cultivated in large volumes by inoculations to tissue culture systems. After a time, the cells are degenerated, and viruses are harvested. The viral particles are concentrated by precipitation methods and purified by repeated centrifugations. Highly purified viruses can be obtained by crystallization and concentration under established conditions.



Viral measurements: Viruses are generally too small to be seen under the light microscope, and an electron microscope is usually necessary to make them visible. Although viruses can be quantified by observation, it is also possible to determine their number in terms of **virus infectious units**, each of which is the smallest unit that causes a detectable effect when viruses infect a susceptible host. Virus infectious units are expressed per volume of fluid.

One method for determining virus infectious units is by the **plaque assay.** The plaque assay is performed by cultivating viruses on a "lawn" of host cells and noting the presence of clear areas where viruses have replicated and destroyed the cells.

Another way of determining virus infectious units is by cultivating viruses in living animals and determining which dilution of virus is lethal to the animals. The **end-point dilution** can be calculated by this method.

Antiviral agents: The antibiotics normally used to treat bacterial disease cannot be used to inactivate viruses because viruses do not perform the biochemical functions that antibiotics interfere with. For example, penicillin is used to interrupt the synthesis of the bacterial cell wall, but viruses have no cell wall.

However, there are several nucleotide analog drugs that interfere with viral replication. **Acyclovir**, for example, is used against herpes viruses because this drug prevents the synthesis of DNA during viral replication. A drug called **azidothymidine (AZT)** is used for patients with HIV infection because this drug also prevents the synthesis of DNA. A drug called **ganciclovir** is used against cytomegaloviruses, and **amantadine** is useful against influenza viruses.

Interferon, a naturally produced antiviral agent approved for certain uses, is a group of proteins produced by host cells after they have been infected by viruses. The interferons do not protect the host cell, but they do provide protection to neighboring cells against viral replication. Interferons can be produced by genetic engineering methods.

Viral vaccines: Protection against viral disease can be rendered by using a **viral vaccine.** Viral vaccines can be composed of inactivated or attenuated viruses.**Inactivated viruses** ("dead viruses") are unable to replicate in host cells because of some chemical or physical treatment. The Salk vaccine against polio and the yellow fever vaccine are examples.

Attenuated viruses ("live viruses") are weakened viruses that replicate at a very slow rate in host cells and generally do not produce any symptoms of disease when inoculated to humans. Attenuated viruses are used in the Sabin polio vaccine and in the vaccines against measles and rubella. The most contemporary vaccines are composed of viral proteins produced by **genetic engineering methods.** The vaccine for hepatitis B is an example of this type of vaccine.

Viral inactivation: Virus particles are composed of nucleic acid, protein, and in some cases, a lipid envelope. As such, the viruses are susceptible to normal inactivation by chemical substances that react with any of these organic compounds. Such things as chlorine, iodine, phenol, detergents, and heavy metals rapidly inactivate viruses. In addition, viruses are destroyed by heating methods used for other microorganisms, and they are very susceptible to the effects of ultraviolet light. Filters can be used to remove viruses from fluids so long as the filter pores are small enough to trap viral particles.

CLASSIFICATION OF DISINFECTANTS:

Disinfectants are chemical agents designed to inactivate or destroy microorganisms on inert surfaces. Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores.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. Disinfectants are also different from biocides — the latter are intended to destroy all forms of life, not just microorganisms. Disinfectants work by destroying the cell wall of microbes or interfering with their metabolism.

TYPES:

- Air disinfectants
- Chemical disinfectants -
  - Organic

  – Inorganic
- Non- chemical disinfectants
- Oxidising disinfectants
- Home disinfectants
- Others disinfectants

Air disinfectants:

Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air. Disinfectants are generally assumed to be limited to use on surfaces, but that is not the case. In 1928, a study found that airborne microorganisms could be killed using mists of dilute bleach. An air disinfectant must be dispersed either as an aerosol or vapour at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

Organic disinfectant –

Alcohols: are most effective when combined with distilled water (concentration of 60% to 70% v/v) to facilitate diffusion through the cell membrane & have fairly rapid bactericidal action against vegetative bacteria. 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. Additionally, high-concentration mixtures

(such as 80% ethanol + 5% isopropanol) are required to effectivelyinactivate lipid-enveloped viruses (such as HIV, hepatitis B, andhepatitis C.The higher alcohols (propyl, butyl, amyl etc) are more germicidal than ethyl alcohol. Alcohols are used as preservatives in some vaccines.

Aldehyde: Formaldehyde (HCHO) is the main aldehyde used for disinfection, have a wide microbiocidalactivity and are sporicidaland fungicidal. Formaldehyde in solution is useful for sterilization of certain instruments. Sometimes, ortho-phthalaldehyde is also used.

Phenol and its derivatives: Phenol is the chief products obtained by the distillation of the coal tar. Phenol 1% has bactericidal action. Many derivatives of phenol are more effective and less costly. Phenolics are active ingredients in some household disinfectants. They are also found in some mouthwashes and in disinfectant soap and handwashes. Phenols are toxic to cats and newborn humans. o-Phenylphenol is often used instead of phenol, since it is somewhat less corrosive.

Hexachlorophene is a phenolic that was once used as a germicidal additive to some household products but was banned due to suspected harmful effects.

Chloroxylenol is the principal ingredient in Dettol, a household disinfectant and antiseptic.

Thymol, derived from the herb thyme, is the active ingredient in some "broad spectrum" disinfectants that often bear ecological claims. It is used as a stabilizer in pharmaceutic preparations. It has been used for its antiseptic, antibacterial, and antifungal actions, and was formerly used as a vermifuge.

Amylmetacresol is found in Strepsils, a throat disinfectant.

Although not a phenol, 2,4-dichlorobenzyl alcohol has similar effects as phenols, but it cannot inactivate viruses.

Quaternary ammonium compound : ("quats"), such as benzalkonium chloride, are widely used for the control of microorganisms like non-enveloped viruses such as norovirus, rotavirus, or polio virus, bacteria and pathogenic fungi within (3–5 minutes) on floors, walls, nursing homes and other public places. They are also used as skin antiseptics and as sanitizing agents in dairy, egg and fishing industries. Quats are biocides that also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth.

Terpenes : such as Pine oil and Thyme ; Pine oil is a disinfectant that is mildly antiseptic, effective against , the fungi ,bacteria, influenza virus etc.. It will kill the causative agents of typhoid, gastroenteritis (some agents), rabies, cholera several forms of meningitis, whooping cough, gonorrhea and several types of dysentery. It is not effective against spore related illnesses, such as tetanus or anthrax, or against non-enveloped viruses such as poliovirus, rhinovirus, hepatitis B, or hepatitis C.

Thymol can also be used as a medical disinfectant and general purpose disinfectant ; used in alcohol solutions and in dusting powders for the treatment of ringworm infections.

Lactic acid : is a registered disinfectant. Due to its natural and environmental profile, it has gained importance in the market

Inorganic disinfectants –

• **Acids and bases :** Sodium hydroxide, Potassium hydroxide,Calcium hydroxide,Magnesium hydroxide, Sulfurous acid, Sulfur dioxide

• **Metals**

•**Iodine** :Iodophors are prepared by mixing iodine with the solubilizing agent; Diluted iodophor is often used by brewers and winemakers to sanitize equipment and bottles; Its major advantage over other sanitizers is that when used in proper proportions, it does not require rinsing.

• **Chlorine** : This group comprises aqueous solution of chlorine, hypochlorite, or hypochlorous acid. Occasionally, chlorine-releasing compounds and their salts are included in this group. Frequently, a concentration of < 1 ppm of available chlorine is sufficient to kill bacteria and viruses, spores and mycobacteria requiring higher concentrations. Chlorine has been used for applications, such as the deactivation of pathogens in drinking water, swimming pool water and wastewater, for the disinfection of household areas and for textile bleaching.

Non-chemical disinfectants-

• **Ultraviolet Light** germicidal irradiation is the use of high-intensity shortwave ultraviolet light for disinfecting smooth surfaces such as dental tools, but not porous materials that are opaque to the light such as wood orfoam.

• Ultraviolet light is also used for municipal water treatment. Ultraviolet light fixtures are often present in microbiology labs, and are activated only when there are no occupants in a room(e.g., at night).

• The phrase "sunlight is the best disinfectant" was popularized in 1913 by United States. As sunlight's ultraviolet rays can act as a disinfectant, the Earth's ozone layerblocks the rays' most effective wavelengths. Therefore, ultraviolet light-emitting machines, such as those used to disinfect some hospital rooms, make for better disinfectants than sunlight.

• **Heat treatment** can be used for disinfection and sterilization.

Oxidising disinfectants-

Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysisand death. Chlorine and oxygenare strong oxidizers, so their large number of disinfectants operate in this way are as follows:

• **Electrolyzed water** or "Anolyte" is an oxidizing, acidic hypochlorite solution made by electrolysis of sodium chloride into sodium hypochlorite ;hypochlorous acid is the predominant oxychlorine species.

• **Hydrogen peroxide** is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergicreactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.

• Hydrogen peroxide vapour is used as a medical sterilant and as room disinfectant. Hydrogen peroxide has the advantage that it decomposes to form oxygen and water thus leaving no long term residues. Occupational Safety and Health Administration OSHA permissible exposurelimit is 1 ppm.

• Accelerated Hydrogen Peroxide , 2% solution (antimicrobial action of hydrogen peroxide can be enhanced by surfactants and organic acids), stabilized for extended use, achieves high-level disinfection in 5 minutes, and is suitable for disinfecting medical equipment made from hard plastic, such as in endoscopes. The evidence available suggests that products based on Accelerated Hydrogen Peroxide, apart from being good germicides, aresafer for humans and benign to the environment.

• **Ozone** is a gas used for disinfecting water, laundry, foods, air, and surfaces. It is chemically aggressive and destroys many organic compounds, resulting in rapid decolorization and deodorization in addition to disinfection. Regardless, ozone has a very wide range of applications from municipal to industrial water treatment due to its powerfulreactivity.

• **Potassium permanganate (KMnO4) is** a purplish-black crystalline powder that colours everything it touches, through a strong oxidizing action. It is used to disinfect aquariums and is used in some community swimming pools as a foot disinfectant before entering the pool.

Home disinfectants-

• The most cost-effective home disinfectant is chlorine bleach (typically a >10% solution of sodiumhypochlorite which is effective against most common pathogens, including disinfectant-resistant organisms such as tuberculosis (mycobacterium tuberculosis), hepatitis B and C, fungi, and antibiotic-resistant strains of staphylococcus and enterococcus. It has disinfectant action against some parasitic organisms.

• The benefits of chlorine bleach include its inexpensive and fast acting nature. However it is harmful to mucous membranes and skin upon contact, has a strong odour ; and combination with other cleaning products such as ammonia and vinegarcan generate noxious gases like chlorine.

Others-

• **Biguanidepolymer :**Polyaminopropylbiguanide is specifically bactericidal at very low concentrations (10 mg/l). It has a unique method of action: The polymer strands are incorporated into the bacterial cell wall, which disrupts the membrane and reduces its permeability, which has a lethal effect to bacteria. It is also known to bind to bacterial DNA, alter its transcription, and cause lethal DNA damage. It has very low toxicity to higher organisms such as human cells, which have more complex and protective membranes.

• **Detergents and Soaps**: They are widely used as surface active agents, wetting agents and emulsifiers. They are classified into four main groups such as anionic, cationic, non-ionic and amphoteric. 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 from unsaturated acids have greater action against gram positive. Nonionic detergents are not ionized. However these substances do not possessignificient anti-microbial activity. Amphoteric compounds have the detergent properties of anionic surfactants combined with disinfectant properties of cationic surfactants. Eg: Tegocompounds.

• **Sodium bicarbonate**: (NaHCO3) has antifungal properties, and some antiviral and antibacterial properties, though those are too weak to be effective at a home environment.

• **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 agent.

Evaluation of bactericidal and Bacteriostatic

Antibiotics

Antibiotics are the substances which are derived from one microorganism in order to kill another microorganism. Antibiotics are effective against bacterial, fungal and parasitic infections. But, antibiotics are not helpful against viral infections.

The development of chemical synthesis has helped to produce the synthetic components which act as an antimicrobial agent against the pathogenic bacteria. These synthetic components are also called as antibiotics. Pathogenic bacteria can be killed by synthetic components at low concentrations. Examples: Ampicillin and amoxicillin.

In 1908, a German bacteriologist, Paul Ehrlich had developed a synthetic component from an arsenic-based structure for the treatment of syphilis, which is called as arsphenamine or salvarsan.

Then, in 1929, Alexander Fleming discovered Penicillin from the fungus *Penicilliumnotatum*. Penicillin is used to treat different types of bacterial infections.

Two types of antibiotics are commonly available. These are as follows:

1. **Bactericidal antibiotics** – These antibiotics had killing effects on bacteria. Example: Penicillin, Aminoglycosides, Ofloxacin.
2. **Bacteriostatic antibiotics** – These antibiotics have an inhibitory effect on bacteria. Example: Erythromycin, Tetracycline, Chloramphenicol.

Evaluation of bactericidal and Bacteriostatic Disinfectants:

The ***phenol coefficient*** is one of the methods to determine the effectiveness of a disinfectant.The phenol coefficient can be defined as:

"The number obtained by dividing the degree of dilution of test disinfectant by the degree of dilution of phenol in a certain span of time."

If the number is greater than 1, it means that for given dilution, the test disinfectant is more powerful and can kill germs better than phenol. If the number obtained is less than 1, it means that for the given dilution, phenol is better at controlling germs.

Phenol is recognized as one of the oldest antiseptic agents with excellent antifungal and antibacterial properties. At concentrations of 0.1 % to 1 %, it is bacteriostatic (i.e. stops the reproduction). At higher concentrations (from 1% to 2%), phenol is *fungicidal* and *bactericidal* (i.e. destroys the fungus or bacteria). Phenol can kill *Anthrax* spores (which cause severe skin lesions, lung infections, and intestine diseases) at 5% concentration within 48 hours.

Although phenol has excellent antiseptic properties, it is not used as a common antiseptic due to its systemic toxicity on the skin. Death can result from oral ingestion in significant quantities. Thus phenol is used for comparison of the power of disinfection of other disinfectants such as chlorine, ozone, hydrogen peroxide, etc.

Phenol coefficient is a number obtained by dividing dilution ratio test disinfectant with the dilution ratio of phenol under predetermined conditions.

For instance, suppose phenol diluted to 1 part in 100 parts of diluent (1/100) is able to kill an organism in 10 minutes. Another disinfectant is diluted 1 part in 500 parts diluent (1/500) is able to kill organisms at the same time. So the phenol coefficient can be calculated as follows:

$$Phenol\ coefficient = \frac{dilution\ ratio\ of\ disinfectant}{dilution\ ratio\ of\ phenol}$$

$$Phenol\ coefficient = \frac{500}{100} = 5$$

Thus, the phenol coefficient is 5. It means that the test disinfectant is stronger than phenol in terms of disinfection.

Two types of phenol coefficient tests are done:

1. **Rideal Walker method for phenol coefficient determination**
2. **Chick Martin test**

1. Rideal Walker Method for Phenol coefficient determination-

In 1903, Rideal Walker proposed a method to determine the power of a disinfectant in comparison with phenol. Let's take a sample experimental procedure as follows.

1. The broth with a test organism (*Salmonella typhi*) is prepared using microbiological grade meat extract (20g), sodium chloride (10g), microbiological grade peptone (20g), and distilled water 1000ml.

2. The solids are mixed in water and boiled, sterilized, and then brought to room temperature.
3. The pH of the solution is maintained between 7.3 and 7.5 using hydrochloric acid. The test organism *Salmonella typhi* culture is maintained by weekly sub-culture on nutrients. The sub-culture is added to the broth.
4. The phenol solutions are prepared by dissolving 1 gram phenol in i.e. 95, 100, 105, 155 ml of water.
5. Similarly, the desired concentrations of test disinfectant are prepared.
6. Subcultures are tested on plates for intervals of 2.5, 5, 7.5, and 10 minutes

The following table shows sample empirical data:

Disinfectant	Dilution ratio	Time			
		2.5	5.0	7.5	10
Phenol	1:95	+	–	–	–
	1:100	+	+	–	–
	1:105	+	+	+	–
	1:155	+	+	+	+
Test disinfectant	1:100	+	–	–	–
	1:200	+	–	–	–
	1:250	+	+	–	–
	1:300	+	+	+	+
	1:350	+	+	+	+
	1:400	+	+	+	+

The limitation of the Rideal Walker method is that it does not account for the presence of any organic matter. Moreover, the time for disinfection testing is too short. *This test is used only to determine the power of phenolic type disinfectants only*.

2. **Chick Martin test-**

Chick Martin Test also determines the phenol coefficient of the test disinfectant like RW test. Unlike in the RW test method where the test is carried out in the water, the disinfectants are made to act in the presence of yeast suspension (or 3% dried human feces) to simulate the presence or organic matter.

The time for subculture is fixed at 30 minutes and the organism used to test efficacy is **S.typhi as well as S.aureus**.

IMPORTANT QUESTIONS

10 marks

1. Classify disinfectants. Write the mechanism of action and uses of phenolic disinfectants.
2. Explain different factors affecting disinfection.
3. Explain different methods for evaluation of bacteriostatic disinfectant
4. Discuss in detail about Redial Walker's test.
5. Enlist various methods of evaluation of bacteriostatic and bactericidal disinfectant.ExplainanyMethod of bacteriostatic disinfectant.
6. Write classification, mechanism of action and uses of phenolic and aldehyde disinfectants.

5 marks

1. Write a note on cultivation of virus.
2. Discuss about merits and demerits of viral cultivation techniques.
3. Describe steps involved in replication of virus.
4. Write about classification of virus.

2 Marks

1. Mention media for cultivation of fungi
2. Mention pharmaceutical uses of fungi.
3. Write structure of typical virion.
4. What is mycelium?
5. Why are virus described as obligate parasites?
6. Give examples for disinfectants with viricidal activity
7. Give two examples for alcoholic disinfectants.
8. Differentiate between disinfection and antisepsis.
9. Write the ideal properties of disinfectant
10. Define disinfection.
11. Mention the factors affecting disinfectant activity
12. Difference between bacteriostatic and bactericidal agents.
13. What is direct inoculation method?
14. Write the significance of positive control in sterility testing.
15. What is direct inoculation method of sterility testing?
16. Write the principle of sterility testing.
17. Write the principle of membrane filtration method of sterility testing