MICROBIOLOGY

UNIT 3 NOTES

FUNGI AND VIRUSES

- MORPHOLOGY, CLASSIFICATION
- REPLICATION AND CULTIVATION

DISINFECTANTS

- CLASSIFICATION
- MODE OF ACTION
- FACTORS INFLUENCING DISINFECTION
- EVALUATION OF DISINFECTION
- STERILITY TESTING OF PRODUCTS

IMPERFECT PHARMACY

FUNGI

- Fungi are eukaryotic microorganism that includes microbes such as Yeasts, Moulds, Mushrooms etc.
- The cell wall of fungi is mainly composed of chitins, glucans etc.
- They are heterotrophic in nature, means they obtain nutrients by absorbing organic materials.
- They can be either unicellular (Yeasts) or multicellular (moulds). Fungi reproduce both sexually & asexually through spores.
- Study of fungi is generally known as Mycology.

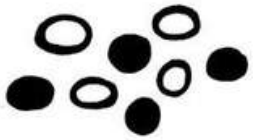
MORPHOLOGICAL CLASSIFICATION OF FUNGI

According to Morphology, fungi are mainly classified into 4 types:

- Yeast
- Yeast like Fungi
- Molds
- Dimorphic Fungi

YEAST

- They are unicellular in nature.
- They are round to oval shaped.
- They generally reproduce asexually by budding.
- They generally grow at 37°C.
- Example: Cryptococcus Neoformans



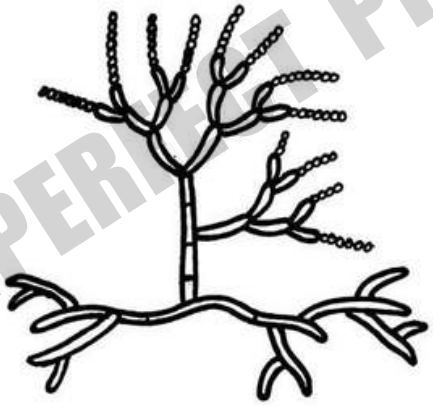
YEAST

② MOLDS

- They are multicellular in nature.
- They composed of filamentous structure called Hyphae.
- They grow at 25°C.
- They reproduce both sexually & asexually.
- Based on Hyphae, they are of two types :

ⓐ Septate Hyphae
ⓑ Aseptate Hyphae

- Examples : Rhizopus



MOLD

③ Yeast Like Fungi

- They are similar to Yeast but have pseudohyphae
- They are unicellular in nature.
- They grow at 37°C.
- They are oval to round shaped.
- Example : Candida Albicans.

④ Dimorphic Fungi

- These are those fungi that can switch between Yeast and Mold forms depending on environmental conditions :

ⓐ Yeast form (Typically at 37°C, Body Temperature)
ⓑ Mold Form (Typically at 25°C, room temperature)

- They are mostly pathogenic (disease causing) in nature.
- Example : Histoplasma capsulatum
  Blastomyces dermatitidis

REPRODUCTION IN FUNGI

- Fungi Reproduce through both sexual & asexual methods:
- The type of reproduction depends on the species and environmental conditions.
- Reproduction in Fungi is a complex process that mainly involves production of spores, which are similar to seeds of plants.

① ASEXUAL PRODUCTION

- Asexual reproduction in Fungi is the most common form, occuring without the fusion of gametes.
- It leads to the formation of genetically identical offspring.
- There are following methods of asexual reproduction:

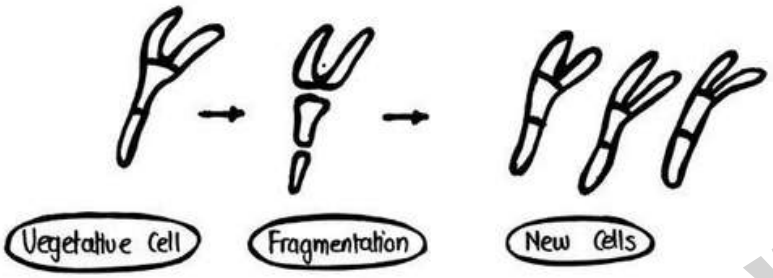
@ Budding

- In this a small outgrowth (bud) forms on the parent cell, which detaches to become a new individual.
- Example: Saccharomyces Cerevisiae (Yeast)

BUDDING

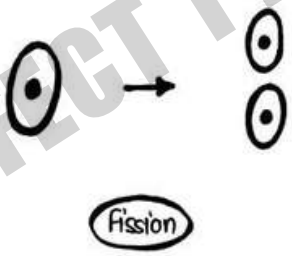
ⓑ Fragmentation

- In this hyphae of the parent fungus break into fragments, each of which grows into a new individual.
- Example: Rhizopus



ⓒ Fission

- In this parent cell divides into two equal cells.
- Example: Some yeast species like Schizosaccharomyces.



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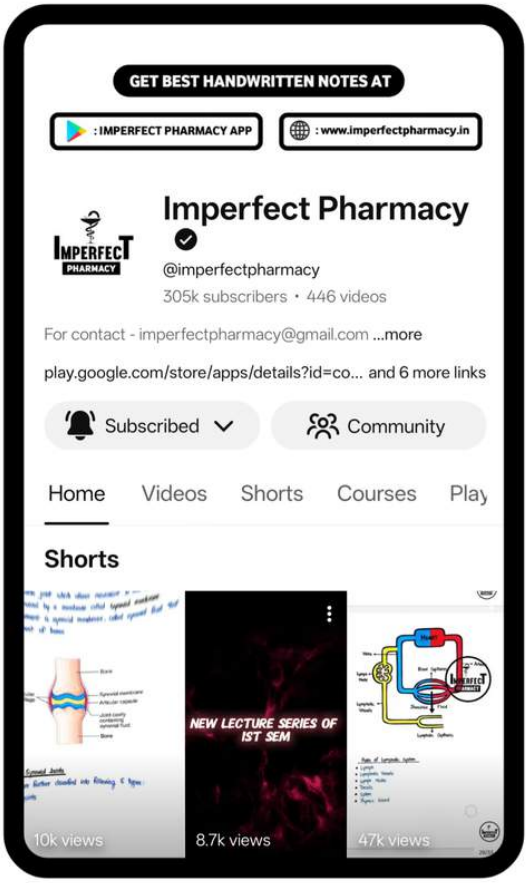

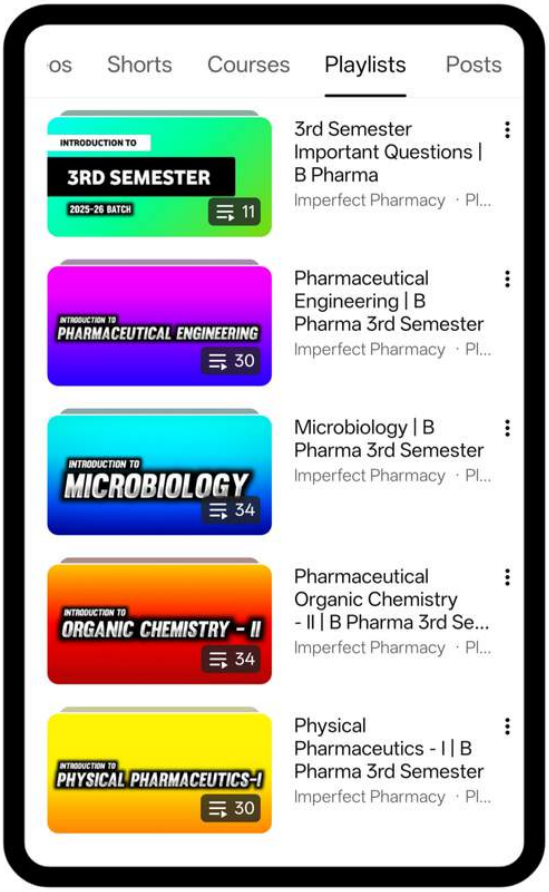

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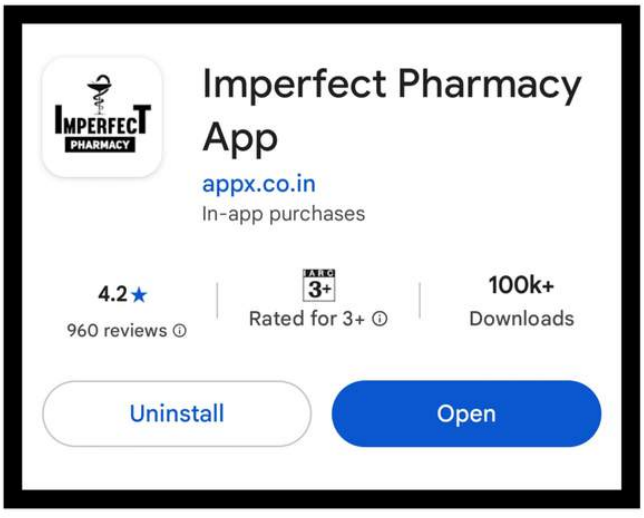
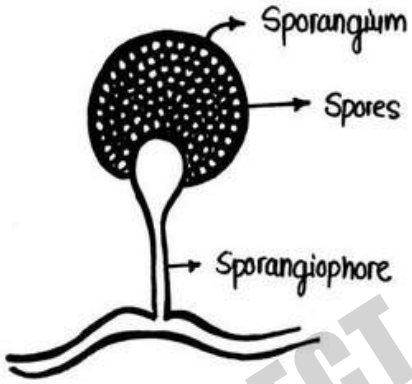


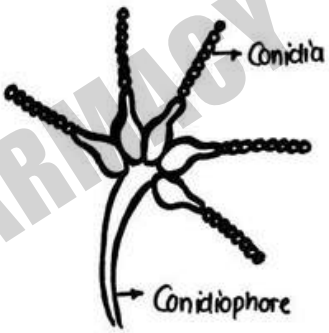
ⓓ Spore formation

- In this fungi reproduces through spore formation
- Some examples include

(1) Sporangiospores
(2) Conidiospores
(3) Chlamydospores
(4) Arthrospores
(5) Blastospores



Sporangiospores



Conidiospores

② Sexual Reproduction

- Sexual Reproduction in Fungi is a process that involves the fusion of two compatible haploid nuclei, resulting in the formation of genetically diverse offspring.
- This process is crucial for maintaining genetic variation and adapting to changing environmental conditions.

Stages Of Sexual Reproduction

There are mainly 3 stages of sexual reproduction in Fungi:

① Plasmogamy
② Karyogamy
③ Meiosis

Plasmogamy

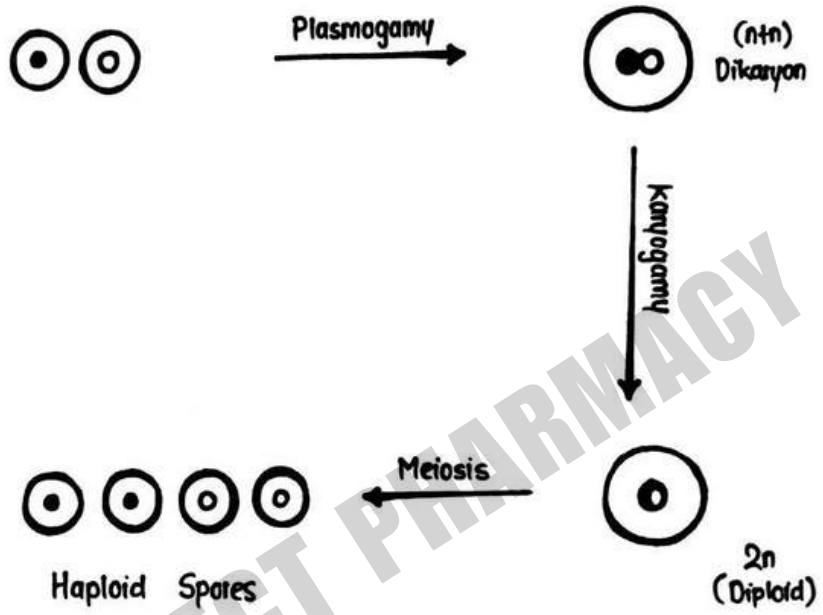
- It is the fusion of the cytoplasm from two genetically different mating types (+ & -).
- Results in a dikaryotic stage, where two nuclei coexist in the same cell without fusing.

karyogamy

- In this two haploid nuclei fuse to form a diploid (2n) nucleus.
- This stage may be delayed leading to prolonged dikaryotic phase in some fungi.

Meiosis

The diploid nucleus undergoes meiosis, producing haploid spores, which can germinate into new fungal organisms.



Examples Of Sexual Spores

- Zygospores
- Ascospores
- Basidiospores
- Oospores

Zygospore

CULTIVATION OF FUNGI

Cultivation of Fungi refers to the process of growing Fungi in a controlled environment, such as laboratory, greenhouse or industrial settings for various purposes such as :

- Food Production
- Medicinal Application
- Industrial Application
- Research etc.

CULTURE MEDIA

- The culture medium mainly used for cultivation of Fungi is Sabouraud Dextrose Agar (SDA).
- It contains :
  - Dextrose : 4 g
  - Peptone : 1 g
  - Agar : 2 g
  - H_2O : 100 ml
- The pH of the medium is adjusted to 5.6 to enhance growth of Fungi.

PROCESS

- ISOLATION : Obtaining a fungal isolate from natural or laboratory sources.
- INOCULATION : Transferring fungal isolate to growth medium.
- INCUBATION : Providing optimal conditions for fungal growth.
- MAINTAINANCE : Subculturing & Monitoring.
- SCALE UP : Large scale production.

VIRUSES

- Viruses are microscopic infectious agents that can only replicate inside the living cells of an organism.
- They are not living organisms rather they are considered obligate intracellular parasites, means they rely on a host cell's machinery to reproduce
- They contain either DNA or RNA as their genetic material, but never both.
- They typically range from 20 nm to 300 nm, much smaller than bacteria.
- They can infect all forms of life, including animals, plants and microorganisms.

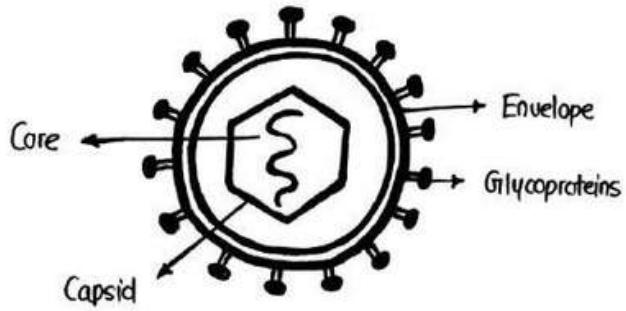
STRUCTURE OF A VIRUS

A fully assembled infectious virus is known as virion & consist of:

① Core: It is the genetic material of the virus & can be either DNA or RNA, but not both.

② Capsid: It is the protein shell surrounding the genetic material.
- It can be either icosahedral, helical or complex shaped.

③ Envelope: Some viruses have an additional lipid envelope surrounding the capsid, derived from host cell membrane.
- This envelope contains viral glycoproteins embedded on it.



VIRUS STRUCTURE

CLASSIFICATION OF VIRUS

Virus can be classified on the basis of two types :

- Based on Host
- Based on Morphology

① BASED ON HOST

Based on host, virus can be classified into following 4 types

ⓐ Animal Viruses
ⓑ Plant Viruses
ⓒ Bacteriophages

ⓐ Animal Viruses

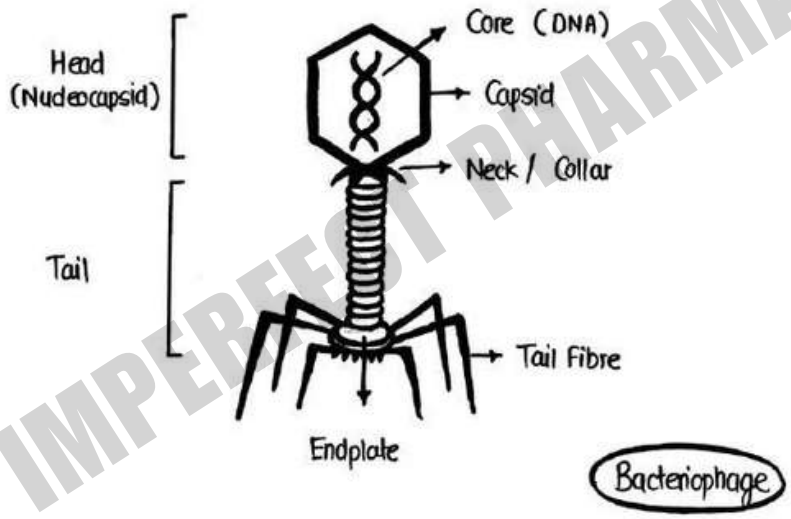
- They infect animals and humans.
- Example : Rabies virus , HIV, Influenza virus .

ⓑ Plant Viruses

- They usually have RNA & infect plants.
- Example: Tobacco mosaic virus (TMV) .

ⓒ Bacteriophages

- They usually have DNA & infect bacteria
- They consist of two parts : Head & Tail.
- Example: T4 bacteriophage.



Bacteriophage

② Based On Morphology

On the basis of morphology, virus can be classified into following types:

- Icosahedral Viruses
- Helical Viruses
- Complex Viruses
- Enveloped Viruses
- Non- Enveloped Viruses

ⓐ Icosahedral Viruses

- They are characterized by their highly symmetrical, spherical shape.
- Example: Adenovirus, Poliovirus.

ⓑ Helical Viruses

- They are characterized by their rod like or filamentous shape.
- Example: Tobacco mosaic virus, Rabies virus.

ⓒ Complex Viruses

- They have complex structure with additional features, such as tail fibres.
- Example: T4 bacteriophage.

ⓓ Enveloped Viruses

- They are surrounded by a lipid membrane derived from host cell.
- Example: HIV, Influenza virus.

⑥ Non- Enveloped Viruses

- They lack an envelope, also known as naked viruses.
- Example: Norovirus

VIRAL REPLICATION

The viral replication also known as life cycle of virus is complex process that involves several stages:

① Attachment

- In this, virus binds to specific receptors on host cell using proteins or glycoproteins on its surface.

② Entry

- The virus or its genetic material enters the host cell.
- This can happen through direct fusion or endocytosis.

③ Uncoating & Replication

- After entry, the viral capsid is removed, exposing the viral genome.
- Viral genome starts replicating, using host cell machinery.

④ Biosynthesis

- Viral proteins are synthesized using host ribosomes.

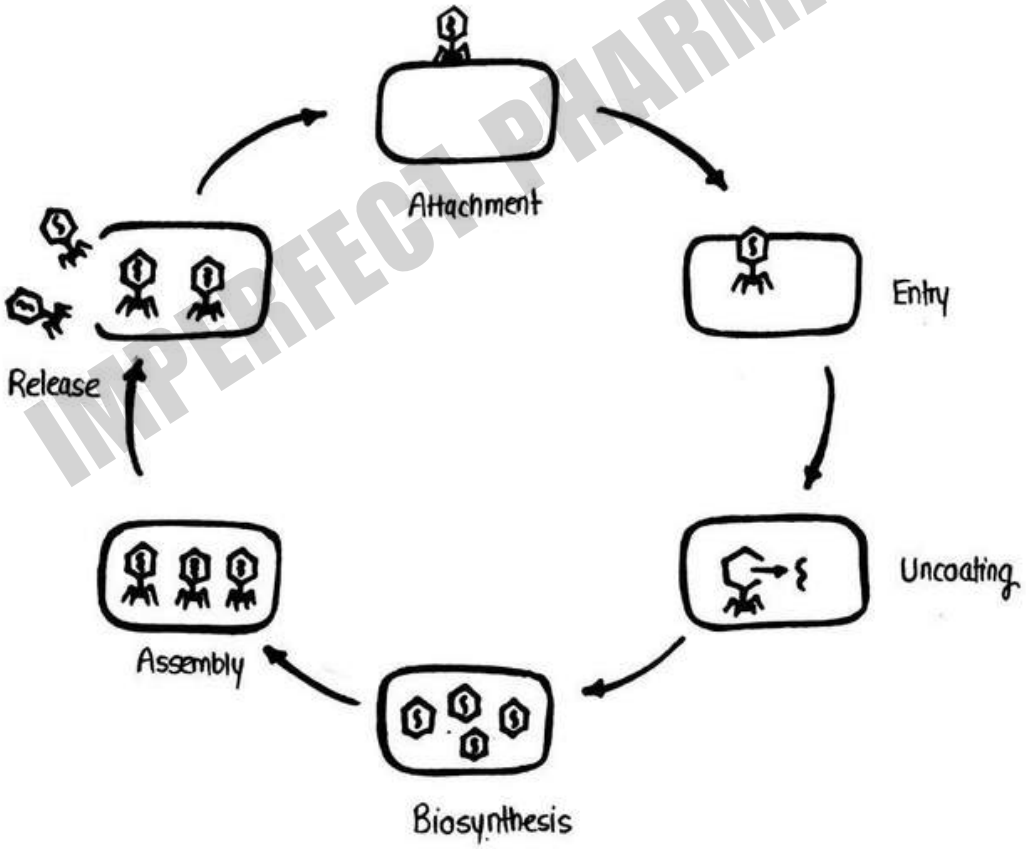
⑤ Assembly / Maturation

- Newly synthesized viral genomes and proteins are assembled into new viral particles (virions)

⑥ Release

- The newly synthesized viruses exit the host cell to infect other cells by two ways.

(a) Budding : Enveloped Viruses
(b) Cell lysis : Non- Enveloped Viruses



CULTIVATION OF VIRUSES

- Viruses are obligate intracellular parasites, means they require living host cells to grow and replicate.
- Cultivating viruses involves providing suitable host systems such as living organisms, tissues or cell cultures.
- This process is essential for studying viral properties, producing vaccines and diagnosing viral diseases.

Methods Of Viral Cultivation

Viruses are cultivated by following methods :

- Cultivation in Animal
- Cultivation in Embryonated Egg
- Cultivation in Cell Culture

① CULTIVATION IN ANIMAL

- In this viruses are injected into specific organisms or tissues of animals like mice, rabbits or guinea pigs etc.
- Example: Rabies virus is grown in mice.
- It is mainly used for pathogenic studies & vaccine development.

② CULTIVATION USING CELL CULTURE

- It involves growing viruses in artificial environments like cell cultures derived from tissues.
- Example: Monkey kidney cells for polio virus.
- They are short lived & difficult to maintain.

③ Cultivation In Embryonated Eggs

- The cultivation of viruses in embryonated eggs is a widely used method for isolating, growing and studying viruses, especially for vaccine production.
- It involves using a fertilized chicken egg (typically around 7 - 10 days old)
- Viruses are inoculated into different parts of the egg, depending on virus type :

(1) Chorioallantoic Membrane (CAM) : Herpesvirus
(2) Amniotic Sac : Influenza Virus
(3) Allantoic Cavity : Newcastle disease virus
(4) Yolk Sac : Yellow fever Virus

Advantages

- Cost effective & easily available.
- Suitable for large scale vaccine production.

DISINFECTION

- Disinfection is the process of eliminating or reducing harmful microorganisms such as bacteria, viruses, fungi from surfaces objects or living beings.
- Disinfection does not necessary kill all microorganisms, but it reduces their number to a level that is considered safe based on public health standards.
- Disinfection is mainly performed using these two chemical agents:

① Disinfectant
② Antiseptic

① DISINFECTANTS

- Disinfectants are chemicals used to kill or reduce harmful microorganisms on inanimate (lifeless) objects or surfaces.
- Disinfectants tends to be stronger & can be too harsh for use on living tissues.
- Example: Bleach, Hydrogen peroxide, alcohol based cleaners etc.

② ANTISEPTICS

- Antiseptics are chemicals used to prevent or stop the growth of microorganisms on living tissues, such as skin or mucous membrane.
- They are less harsh than disinfectants to avoid damaging living tissue.
- Example: Hand sanitizers, Iodine solutions etc.

Ideal Characteristics Of Disinfectants

- It should have a broad spectrum of activity.
- It should be fast aching.
- It should be non- toxic.
- It should be active at all pH.
- It should be stable.
- It should have a long shelf life.
- It should have a high penetration power.
- It should be inexpensive and easily available.
- It should have a pleasant colour.

Classification & Mode Of Action Of Disinfectants

Different type of disinfectants shows different mode of action.
Here is classification of disinfectants along with their moa.

- Phenols & Phenolic compounds
- Alcohols
- Aldehydes
- Halogens
- Heavy Metals
- Oxidising Agents
- Dyes
- Detergents

① PHENOLS & PHENOLIC COMPOUNDS

- Mode of Action : They act by disrupting the cell membrane, precipitating the proteins and inactivating microbial enzymes.
- Examples : Phenol, Cresol, triclosan etc.
- Use : commonly used in hospitals and homes.

② ALCOHOLS

- Mode of Action : Denature proteins and disrupt cell membranes of microorganisms.
- Examples : Ethanol, Isopropanol.
- Use : They are widely used as hand sanitizers and surface disinfectants.

③ ALDEHYDES

- Mode of Action : Cross-link proteins and inactivate nucleic acids.
- Examples : Formaldehyde, Glutaraldehyde
- Use : For high level disinfection and sterilization.

④ HALOGENS

- Mode of Action : They act by oxidizing cellular components of microorganisms.
- Examples : Chlorine, Iodine
- Use : They are used in water treatment & wound care.

⑤ HEAVY METALS

- Mode of Action: They binds to cellular proteins and inactivate them
- Examples: Mercuric Chloride, silver nitrate, copper sulphate etc.
- Uses: They are mainly used in water purification and as surface disinfectant

⑥ OXIDISING AGENT

- Mode of Action: Oxidize cellular components.
- Examples: Hydrogen peroxide, peracetic acid.
- Uses: Used in medical and food processing settings.

⑦ DYES

- Mode of Action: They act by interference with cell membrane integrity and protein denaturation.
- Examples: Crystal violet, acriflavine, malachite green etc.
- Uses: They are mainly used as wound antiseptic.

⑧ DETERGANTS

- Mode of Action: They act by disrupting cell membranes.
- Examples: Quaternary Ammonium Compounds (Quats)
- Uses: commonly used as household cleaners.

FACTORS INFLUENCING DISINFECTION

Several factors influence the effectiveness of disinfection. Understanding these factors is essential for optimizing disinfection processes and ensuring microbial control

- Concentration of disinfectants
- Temperature
- Contact Time
- pH of the environment
- Surface condition
- Characteristics of Microorganism
- Water Hardness

① Concentration Of Disinfectants

- Concentration of the disinfectant directly influences the rate of killing microorganisms.
- However too high concentration can lead to decrease in effectiveness.

② Temperature

- Most disinfectants are more effective at higher temperatures.
- However, extremely high temperatures can cause evaporation or degradation of some disinfectants.

③ Contact Time

- Disinfectants required adequate time of contact to kill or inhibit microorganisms.

④ pH Of The Environment

- Some disinfectants are more effective in acidic or alkaline environments.

⑤ Surface Condition

- Rough or damaged surfaces can harbor microorganisms and make disinfection less effective.

⑥ Characteristics Of Microorganisms

- The efficacy of disinfection greatly depends on nature and number of contaminating microorganisms.
- Bacterial spores and Non-enveloped viruses are generally more resistant.

⑦ Water Hardness

- Hard water can reduce the effectiveness of some disinfectants, such as quaternary ammonium compounds.

EVALUATION OF DISINFECTANT

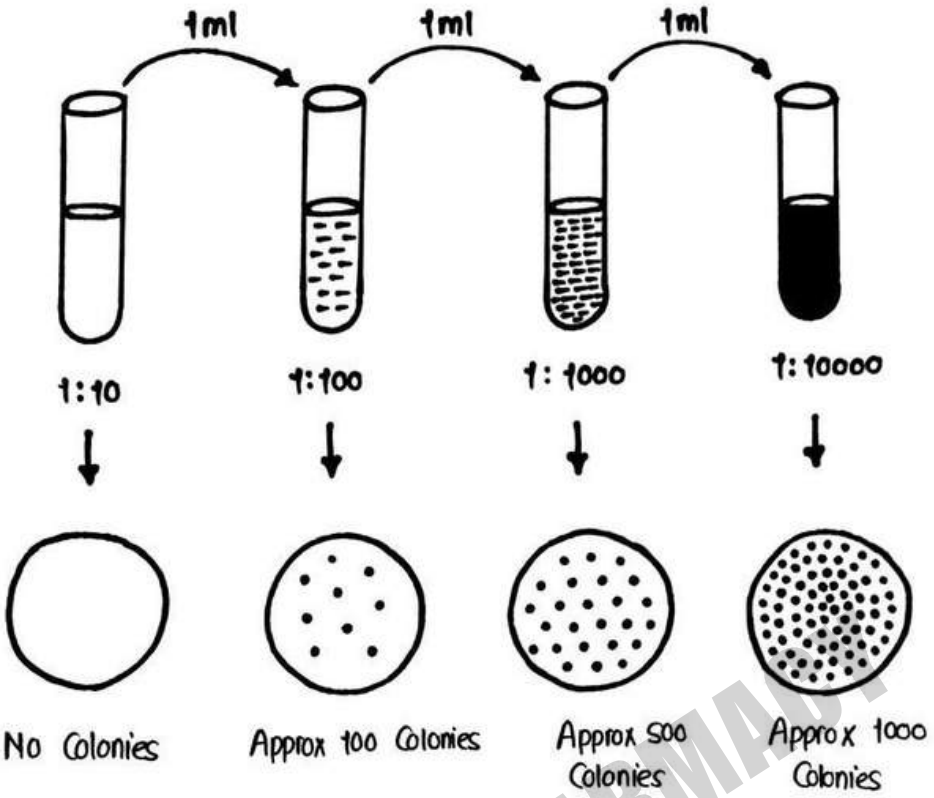
Evaluation of disinfectant assess their ability to kill or inhibit the growth of microorganisms . It involves following methods :

- Tube dilution and agar plate method
- Cup - Plate Method
- Ditch - Plate Method
- Phenol coefficient Method
- kelsey Sykes Method

① TUBE DILUTION & AGAR PLATE METHOD

- Tube dilution and agar plate method is a laboratory technique used to evaluate the effectiveness of disinfectants against microorganisms
- It involves following steps :

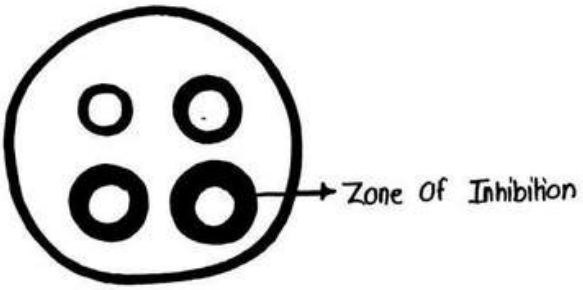
* Prepare a series of dilutions of disinfectant typically ranging from 1:10 to 1:1000 .
* Inoculate each dilution with a known number of microorganism .
* Now incubate the test tubes at a specific temperature (around 37°C) for specific period of time , typically around 24-48 hour .
* Observe the test tubes or agar plates for visible growth & turbidity and record the results .



② Cup Plate Method

- The cup plate method, also known as cylinder plate method, is a technique used to evaluate effectiveness of disinfectants
- It involves following steps:

* Pour agar medium into petri dishes and allow to solidify.
* Spread the microbial suspension onto the agar surface.
* Use a sterile borer to punch small wells into agar plate
* Carefully add a specific volume of disinfectant to each well of different concentration.
* Incubate the plate at 37°C for 24-48 hour.
* Measure the zone of inhibition around each well.



CUP PLATE METHOD

③ DITCH PLATE METHOD

- The ditch plate method is a laboratory technique used to evaluate the antimicrobial efficacy of disinfectants.
- It involves following simple steps
* Agar plates are prepared with microorganisms streaked on it.
* A ditch or groove is made in the agar.
* Disinfectant solution is placed in the ditch.
* Measure the zone of inhibition around the ditch

④ PHENOL COEFFICIENT METHOD

- The Phenol Coefficient method evaluates disinfectant efficacy by comparing it to phenol, a standard disinfectant
- The phenol coefficient is a number that represents the relative effectiveness of disinfectant compared to phenol.
- It involves two major test :

ⓐ Rideal - Walker Test
ⓑ Chick- Martin Test

ⓐ Rideal Walker Test

Rideal - Walker test is a historical method used to evaluate the effectiveness of disinfectant by comparing it to phenol, which is used as a standard.
It involves following steps:

- Dilutions of both phenol & the disinfectant to be tested are prepared in sterile distilled water, the dilutions are typically made in a series, with each dilution being a fraction of previous one.
- A specific volume of standardized suspension of Salmonella typhi bacteria is added to each dilution of disinfectant and phenol.
- The tubes containing the dilutions and bacteria are incubated at a specific temperature
- After the incubation period, samples from each dilution are transferred to tubes containing sterile nutrient broth.
- These subcultures are then incubated for a further period to determine if any bacterial growth has occurred.

DISINFECTANT	DILUTION	TIME INTERVAL			
		2·5	5	7·5	10
Test Disinfectant	1:1000	+	−	−	−
	1:2000	+	+	−	−
	1:3000	+	+	+	−
	1:4000	+	+	+	+
Phenol	1:80	+	−	−	−
	1:100	+	+	−	−
	1:120	+	+	+	−
	1:140	+	+	+	+

$$\text{R·W· Coefficient} = \frac{\text{Dilution of disinfectant killing in 7·5 min· but not in 5m·}}{\text{Dilution of phenol killing in 7·5 min· but not in 5 mm.}}$$

$$= \frac{2000}{100}$$

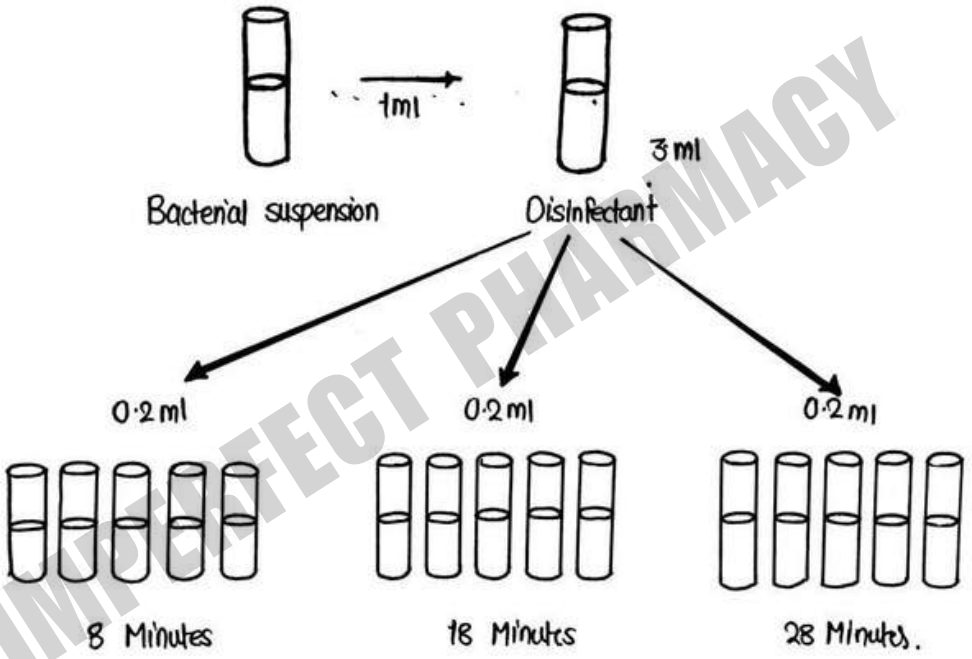
$$= 20$$

Conclusion

- If the rideal - walker test for given disinfectant is 1, the disinfectant has same effectiveness as phenol.
- If RW· coefficient is 20, then disinfectant is 20 times more active than phenol

⑤ KELSEY-SYKES TEST

- The kelsey-sykes test is a method used to evaluate the bactericidal effectiveness of disinfectant.
- It involves mixing the disinfectant with bacterial suspension and then subjected to three successive challenges.
- The number of surviving bacteria is counted to assess the disinfectant's efficacy.



STERILITY TESTING

- Sterility Testing is defined as a testing which confirms that products are free from presence of microorganisms.
- It is a critical quality control process used in pharmaceutical, microbiology, biotechnology and medical device industries to ensure that product are free from viable microorganisms.

PRINCIPLE OF STERILITY TESTING

- The principle of sterility testing is based on the concept of providing optimal conditions for growth of microorganisms
- If a product is contaminated with viable microorganism, they will multiply and grow and their presence can be indicated by turbidity in the original ~~colour~~ clear medium

CULTURE MEDIA USED FOR STERILITY TESTING

Following culture medias are mainly used for Sterility Testing

1. Fluid Thioglycolate Medium
2. Alternative Thioglycolate Medium
3. Soyabean Casein Digest Medium

① Fluid Thioglycolate Medium

It is mainly used for clear fluid products and suitable for growth of anaerobic bacteria. It composed of :

- L- Cystine
- Sodium Chloride
- Dextrose Monohydrate
- Yeast Extract
- Pancreatic digest of casein
- Sodium Thioglycolate
- Dipotassium hydrogen Phosphate
- Resazurin
- Distilled Water

② Alternative Thioglycolate Medium

This medium is mainly used for turbit and viscid medium and mainly consist of :

- L- Cystine
- Sodium Chloride
- Dextrose Monohydrate
- Yeast Extract
- Pancreatic digest of casein
- Sodium thioglycollate
- Distilled Water

Soyabean Casein Digest Medium

It is suitable for culturing both Fungi and anaerobic bacteria and mainly consist of :

- Pancreatic digest of casein
- Papaic digest of soyabean meal
- Sodium chlorride
- Dibasic potassium phosphate
- Dextrose monohydrate
- Distilled water

MEDIA SUITABILITY TEST

The test is perform prior to sterility testing for culture media to make sure that :

- Media is sterile
- Media supports growth of microorganisms

It involves two test

① Media Sterility Test
② Growth Promotion Test

① Media Sterility Test

- Media sterility test is a quality control test used to verify that microbial growth medium is sterile and free from contaminants.
- In this medium is incubate for 14 days.
- The medium should show no visible sign of microbial growth.

② Growth Promotion Test

- A growth promotion test is a quality control test used to verify that a microbiological culture medium is capable of supporting the growth of microorganisms.
- The test involves inoculating the medium with a small amount of standardized microbial suspension and then incubating it for a specific period.
- The medium should show visible sign of microbial growth.

METHODS FOR STERILITY TESTING

Sterility Testing is performed using following 2 methods:

- Membrane Filtration
- Direct Inoculation

① MEMBRANE FILTRATION

Membrane filtration method is a widely used technique for sterility testing of products, especially in microbiology.

Principle

The method relies on filtering a liquid sample through a membrane filter that retains microorganisms, allowing for their subsequent growth in suitable culture medium.

Procedure

- Sample Collection
- Filtration setup using a filtration apparatus equipped with a sterile membrane filter paper, typically with pore size of 0.45 µm.
- Pour the sample onto membrane filter ensuring process is conducted under aseptic conditions.
- After filtration, place the membrane filter into culture medium.
- Regularly check for microbial growth.

Result

- No growth indicates sterile sample.
- Growth indicates presence of microorganism.

② Direct Inoculation Method

Direct Inoculation method of sterility testing involves adding a sample directly to a sterile growth medium, such as Agar broth to detect presence of microorganisms

Poinciple

- Microorganism, if present in the sample will grow in nutrient rich medium.
- Sterile sample will not support microbial growth.

Procedure

- Prepare the sample
- Inoculate the sample directly into sterile growth medium
- Incubate the medium at specified temperature for required time
- Observe the microbial growth

Result

- No growth indicates sterile sample.
- Growth indicates presence of microorganisms.

THANK YOU

FOR CHOOSING IMPERFECT PHARMACY AS YOUR STUDY PARTNER

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