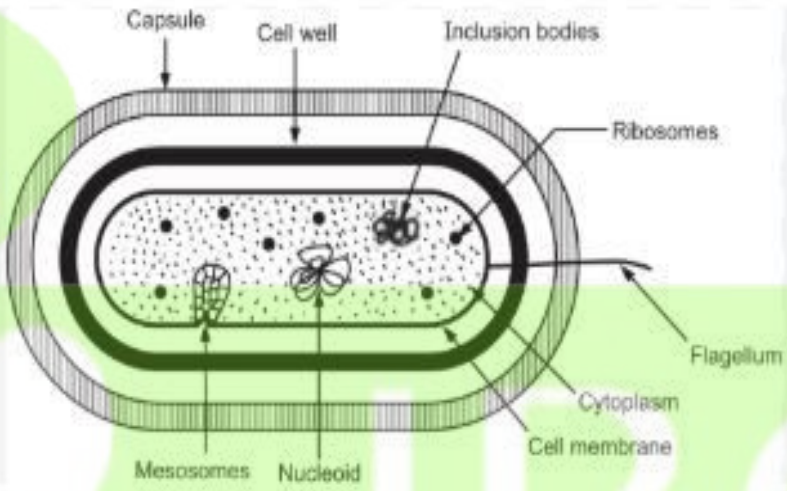
Model Question and Answer
B. Pharmacy, 3rd Semester
Sub: Pharmaceutical Microbiology, Sub. Code: BP303T
UNIT-I & II

Long Answer Type **(10 marks)**

Q1. Explain the ultra-structure of bacteria with neat labeled diagram.

Ans:



Capsules:

Capsule is the outer most layer of the bacteria (extra cellular). It is a condensed well defined layer closely surrounding the cell. It is a viscous material that essentially forms a covering layer or a sort of envelope around the cell wall. It is visualized by the aid of light microscopy employing highly sophisticated and specialized staining techniques.

Flagella

(i) Monotrichous – Single flagella on one side
(ii) Lophotrichous – tuft of flagella on one side
(iii) Amphitrichous – single or tuft on both sides
(iv) Peritrichous – surrounded by lateral flagella Various types of mobility is observed because of the presence of the flagella as Serpentine motility is seen with Salmonella, darting motility with Vibrio and Tumbling motility with Listeria monocytogenes.

Functioning of Flagella: The modus operandi of flagella are as given under:

(1) Flagella are fully responsible for the bacterial motility.
(2) Deflagellation by mechanical means renders the motile cells immotile.

On the basis of distribution of flagella, bacteria are classified as follows:
Atrichous: Flagella are absent.
Monotrichous: There is a single flagellum only.
Amphitrichous: There are two flagella, one at both the ends of bacterium.
Cephalotrichous: There are many flagella that occur at one end only.
Lophotrichous: There are many flagella that occur at both the ends of the bacterium.
Peritrichous: There are many flagella that occur equally distributed all over the surface of bacterium.

Pili / Fimbriae

- Hair-like proteinaceous structures that extend from the cell membrane to external environment are pili which are otherwise known as fimbriae.
- They are thinner, shorter and more numerous than flagella and they do not function in motility. The fimbriae is composed of a subunit called pilin.
- There are two types pili namely Non-sex pili (Common pili) eg. fimbriae or type IV and the sex pili.
- The fimbriae are antigenic and mediate their adhesion which inhibits phagocytosis. The sex pili help in conjugation.

Cell wall

- Beneath the external structures is the cell wall. It is very rigid & gives shape to the cell.
- Its main function is to prevent the cell from expanding & eventually bursting due to water uptake.
- Cell Wall constitutes a significant portion of the dry weight of the cell and it is essential for bacterial growth & division.
- Chemically the cell wall is composed of peptidoglycan.
- Mucopeptide (peptidoglycan or murien) formed by N acetyl glucosamine & N acetyl muramic acid alternating in chains, cross linked by peptide chains.
- Embedded in it are polyalcohol called Teichoic acids.

Outer Membrane

- Outer membrane is found only in Gram-negative bacteria, it functions as an initial barrier to the environment and is composed of lipopolysaccharide (LPS) and phospholipids Lipopolysaccharide (LPS).
- The LPS present on the cell walls of Gram-negative bacteria account for their endotoxic activity and antigen specificity.

Cytoplasmic Membrane

- Just inside the peptidoglycan layer of the cell wall lies the cytoplasmic membrane, which is composed of a phospholipid bilayer similar in microscopic appearance to that in eukaryotic cells.
- They are chemically similar, but eukaryotic membranes contain sterols, whereas prokaryotes generally do not.
- The only prokaryotes that have sterols in their membranes are members of the genus Mycoplasma.

The cytoplasm has two distinct areas when seen in the electron microscope:
(1) An amorphous matrix that contains ribosomes, nutrient granules, metabolites, and plasmids.
(2) An inner, nucleoid region composed of DNA.

Ribosomes

- Bacterial ribosomes are the site of protein synthesis as in eukaryotic cells, but they differ from eukaryotic ribosomes in size and chemical composition.
- Bacterial ribosomes are 70S in size, with 50S and 30S subunits, whereas eukaryotic ribosomes are 80S in size, with 60S and 40S subunits.
- The differences in both the ribosomal RNAs and proteins constitute the basis of the selective action of several antibiotics that inhibit bacterial, but not human, protein synthesis.

Nucleoid

The nucleoid is the area of the cytoplasm in which DNA is located. The DNA of prokaryotes is a single, circular molecule that has a molecular weight (MW) of approximately.

Q2. Describe in detail the principle and procedure of differential staining.

Ans:

Gram Staining

Principle

- Gram staining and differentiation are based on the differences in cell wall structure and composition of bacteria.
- Bacteria having cell walls with a thick layer of peptidoglycan will resist decolorization of primary stain and appear violet or purple.
- Bacteria having a thin peptidoglycan layer with lesser cross-linkage lose primary stain during decolorizing and gain counter stain appearing pink or red.
- When Gram's Iodine is added as mordant, the iodine (I– or I-3 ion) interacts with CV+ ion and forms CV-I complex within cytoplasm and cell membrane and cell wall layers.
- When decolorizing solution (ethanol or a mixture of ethanol and acetone) is added it interacts with lipids in the cell wall.
- The outer membrane of the Gram-Negative bacterial cell wall is dissolved exposing the peptidoglycan layer.
- The peptidoglycan layer is thin with less cross-linking in the Gram-Negative cell wall, hence becoming leaky.
- This causes cells to lose most of the CVI complexes. Whereas in Gram-Positive bacteria, there is no outer membrane, and the peptidoglycan layer is also thick with higher cross-linkage.
- So, the decolorizing solution dehydrates the peptidoglycan layer trapping all the CVI complexes inside the cell wall and bacteria retain the purple or violet color of crystal violet.
- When counterstain, positively charged safranin, is added, it interacts with the free negatively charged components in Gram-Negative cell wall and membrane and bacteria becomes pink/red. Whereas, there is no space to enter inside the dehydrated Gram-Positive cell wall due to CVI complex and dehydration. Hence, safranin can't stain them red or pink and Gram-Positive bacteria reveal the purple or violet color.

Procedure:

- Flood crystal violet solution over fixed smear
- After 30 – 60 seconds, pour off the CV solution and rinse with gentle running water.
- Flood the Gram's Iodine solution over the smear
- Leave the iodine solution for 30 – 60 seconds and pour off the excess iodine and rinse with gentle running water
- Shake off the excess water over the smear
- Decolorize the smear by passing the decolorizing solution till the solution runs down in clear form. Alternatively, add a few drops of decolorizing solution and shake gently and rinse with distilled water after 5 seconds.
- Rinse with distilled water to wash decolorizer
- Shake off the excess water over the smear
- Pour counter stain over the smear
- Leave for 30 – 60 seconds and wash with gentle running water.
- Air dry or blow-dry the smear.

ZN staining (Ziehl-Neelsen staining)

Principle:

- The Ziehl-Neelsen stain uses basic fuchsin and phenol compounds to stain the cell wall of Mycobacterium species.
- Mycobacterium does not bind readily to simple stains and therefore the use of heat along with carbol-fuschin and phenol allows penetration through the bacterial cell wall for visualization.
- Mycobacterium cell wall contains high lipid content made up of mycolic acid on its cell wall making it waxy, hydrophobic, and impermeable. These are ß-hydroxycarboxylic acids made up of 90 carbon atoms that define the acid-fastness of the bacteria.
- Use of Carbol-fuschin which is basic strongly binds to the negative components of the bacteria which include the mycolic acid and the lipid cell wall. addition of acid alcohol along with the application of heat forms a strong complex that can not be easily washed off with solvents.
- The acid-fast bacilli take up the red color of the primary dye, carbol-fuschin.
- While non-acid-fast bacteria easily decolorize on the addition of the acid-alcohol and take up the counterstain dye of methylene blue and appear blue
- This technique has been used in the identification of Mycobacterium tuberculosis and Mycobacterium leprae.

Procedure:

- On a clean sterile microscopic slide, make the smear of the sample culture and heat fix the smear over blue heat.
- Over the smear, pour and flood the smear with carbol-fuschin and heat gently until it produces fumes.
- Allow it to stand for 5 minutes and wash it off with gently flowing tap water.
- Add 20% sulphuric acid and leave it for 1-2 minutes. Repeat this step until the smear appears pink in color.
- Wash off the acid with water.
- Flood the smear with methylene blue dye and leave it for 2-3 minutes and wash with water.
- Air dry and examine the stain under the oil immersion lens.

Q3. Give a suitable illustration of Dark-field Microscope and discuss the functioning of its essential parts.

Ans:

Principle

- A dark field microscope is arranged so that the light source is blocked off, causing light to scatter as it hits the specimen.
- This is ideal for making objects with refractive values similar to the background appear bright against a dark background.
- When light hits an object, rays are scattered in all azimuths or directions. The design of the dark field microscope is such that it removes the dispersed light, or zeroth order, so that only the scattered beams hit the sample.
- The introduction of a condenser and/or stop below the stage ensures that these light rays will hit the specimen at different angles, rather than as a direct light source above/below the object.
- The result is a "cone of light" where rays are diffracted, reflected and/or refracted off the object, ultimately, allowing the individual to view a specimen in dark field.
- The dark-ground microscopy makes use of the dark-ground microscope, a special type of compound light microscope.
- The dark-field condenser with a central circular stop, which illuminates the object with a cone of light, is the most essential part of the dark-ground microscope.
- This microscope uses reflected light instead of transmitted light used in the ordinary light microscope.
- It prevents light from falling directly on the objective lens.
- Light rays falling on the object are reflected or scattered onto the objective lens with the result that the microorganisms appear brightly stained against a dark background.

Applications:

- It is useful for the demonstration of very thin bacteria not visible under ordinary illumination since the reflection of the light makes them appear larger.
- This is a frequently used method for rapid demonstration of Treponema pallidum in clinical specimens.
- It is also useful for the demonstration of the motility of flagellated bacteria and protozoa.
- Dark-field is used to study marine organisms such as algae, plankton, diatoms, insects, fibers, hairs, yeast and protozoa as well as some minerals and crystals, thin polymers and some ceramics.
- Dark-field is used to study mounted cells and tissues.
- It is more useful in examining external details, such as outlines, edges, grain boundaries and surface defects than internal structure.

Advantages

- Dark-field microscopy is a very simple yet effective technique.
- It is well suited for uses involving live and unstained biological samples, such as a smear from a tissue culture or individual, water-borne, single-celled organisms.
- Considering the simplicity of the setup, the quality of images obtained from this technique is impressive.
- Dark-field microscopy techniques are almost entirely free of artifacts, due to the nature of the process.
- A researcher can achieve a dark field by making modifications to his/her microscope.

Limitations

- The main limitation of dark-field microscopy is the low light levels seen in the final image.
- The sample must be very strongly illuminated, which can cause damage to the sample.

Q3. Describe in detail the isolation and preservation of pure culture.

Ans:

ISOLATION TECHNIQUE OF PURE CULTURE

- Cultures composed of cells arising from a single progenitor

•Progenitor is termed a CFU

•Aseptic technique prevents contamination of sterile substances or objects

•Common isolation techniques

-Streak plate method

-Pour plate method

-Spread plate method

-Stab culture

1.Streak plate method

• Streaking is the process of spreading the microbial culture with an inoculating needle on the surface of the media.

• Sterilize the inoculating needle by flame to make red hot and allow it to cool for 30 seconds.

• The sample is streaked in such a way to provide series of dilution.

• purpose-thin out inoculum to get separate colonies.

• sub-culturing can be done by streaking well isolated colonies from streak plate to new plate.

2. Pour plate method

• The bacterial culture and liquid agar medium are mixed together.

• After mixing the medium, the medium containing the culture poured into sterilized Petri dishes (Petri plates), allowed solidifying and then incubated.

• After incubation colonies appear on the surface.

DISADVANTAGES

1.Microorganism trapped beneath the surface of medium hence surface as well as subsurface colonies are developed which makes the difficulties in counting the bacterial colony.

2. Tedious and time consuming method, microbes are subjected to heat shock because liquid Medium maintained at45°C.

3. Unsuitable-Psychrophilic

3. Spread plate method

• This is the best method to isolate the pure colonies.

• In this technique, the culture is not mixed with the normal saline and serially diluted.

• 0.1 ml of sample taken from diluted mixture, which is placed on the surface of the agar plate and spread evenly over the surface by using L-shaped glass rod called spreader.

• Incubate the plates

• After incubation, colonies are observed on the agar surface.

ADVANTAGES

1. It is a simple method.
2. In this method only surface colonies are formed.
3. Micro-organisms are not exposed to higher temperature.

4. Stab Culture method:

1. Prepare and autoclave 0.7% LB agar (standard LB medium containing 7 g/L agar).
2. Cool the LB agar to below 50°C (when you can hold it comfortably) and add the appropriate antibiotic(s). While still liquid, add 1 ml agar to a 2 ml screw-cap vial under sterile conditions, then let solidify.
3. Using a sterile toothpick, pick a single colony from a freshly grown plate and stab it deep into the soft agar several times.
4. Incubate the vial at 37°C for 8–12 h leaving the cap slightly loose.
5. Seal the vial tightly and store in a dark place, preferably at 4°C.

Preservation Methods:

Refrigeration:

Storing cultures at low temperatures (e.g., 4°C) can slow down metabolic activity and extend the viability of the culture.

Cryopreservation (Liquid Nitrogen):

Freezing cultures in liquid nitrogen provides extremely long-term preservation by slowing down metabolic processes to a near standstill.

Mineral Oil Overlay:

Overlaying a culture with sterile mineral oil can prevent drying out and can be used for short-term storage.

Lyophilization (Freeze-drying):

This method involves removing water from the culture under vacuum, preserving the cells in a dormant state for long periods.

Storage in Sterile Soil:

Some microorganisms can be stored in sterile soil, which can provide a protective environment and slow down growth.

Short Answer Type **(5marks)**

Q1. Define Bacterial growth. Write different phases of growth with growth curve.

Ans:

Bacterial Growth

Bacteria are unicellular organisms that tend to reproduce asexually by the means of binary fission. Bacterial growth is the increase in the number of bacterial cells rather than the increase in their cell size. The growth of these bacterial cells takes place in an exponential manner, i.e., one cell divides into 2, then 4, then 8, 16, 32 and so on.

The time taken for a bacterial cell to double is called generation time. The generation time varies among different species of bacteria based on the environmental conditions they grow in. Clostridium perfringens is the fastest growing bacteria that has a generation time of 10 minutes while Escherichia coli has a doubling time of 20 minutes. Mycobacterium tuberculosis is one of the slowest growing bacteria, taking about 12 to 16 hours to double.

Growth Curve

In a closed system with enough nutrients, a bacteria shows a predictable growth pattern that is the bacterial growth curve. It consists of four different phases. Read on to learn about the phases in detail.

Phases of the Bacterial Growth Curve

Upon inoculation into a new nutrient medium, the bacteria shows four distinct phases of growth. Let us dive into each of the phases in detail –

Lag Phase

The bacteria upon introduction into the nutrient medium take some time to adapt to the new environment. In this phase, the bacteria does not reproduce but prepares itself for reproduction. The cells are active metabolically and keep increasing in size. The cells synthesize RNA, growth factors and other molecules required for cell division.

Log Phase

Soon after the lag phase, i.e., the preparation phase, the bacterial cells enter the log phase. The log phase is also known as the exponential phase. This phase is marked by the doubling of the bacterial cells. The cell number increases in a logarithmic fashion such that the cell constituent is maintained. The log phase continues until there is depletion of nutrients in the setup. The stage also comes to a stop if toxic substances start to accumulate, resulting in a slower growth rate. The cells are the healthiest at this stage and researchers prefer to use bacteria from this stage for their experimental processes.

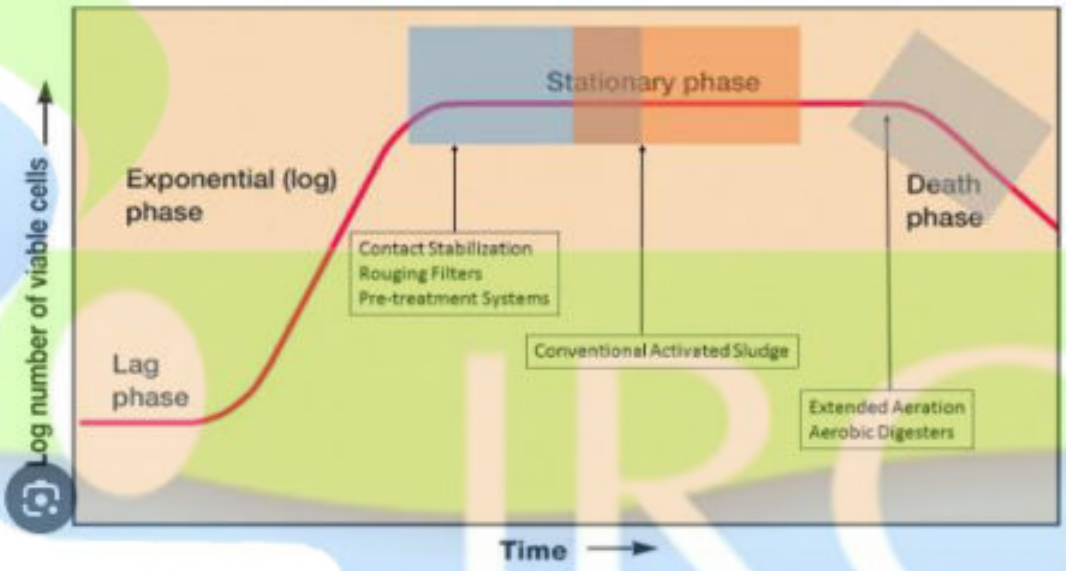
Plotting this phase on the bacterial growth curve gives a straight line. Upon calculation of the slope of this line, the specific growth rate of the organism is obtained. It is the measure of divisions per cell per unit of time.

Stationary Phase

In the stationary phase, the rate of growth of the cells becomes equal to its rate of death. The rate of growth of the bacterial cells is limited by the accumulation of toxic compounds and also depletion of nutrients in the media. The cell population remains constant at this stage. Plotting this phase on the graph gives a smooth horizontal linear line.

Death Phase

This is the last phase of the bacterial growth. At this stage, the rate of death is greater than the rate of formation of new cells. Lack of nutrients, physical conditions or other injuries to the cell leads to death of the cells.



Q2. Write about the different methods of measurement of bacterial growth.

Ans:

Measurements of microbial growth

For the measurement of growth either mass or population number is followed because growth leads to increase in both.

Growth can be measured by one of the following types of measurements:

Cell count this method involves the measurement of growth either by microscopy or by using an electronic particle counter or indirectly by a colony count.

Cell mass in this growth can be measured directly by weighing or by a measurement of nitrogen concentration in cells or indirectly by the determination of turbidity using spectrophotometer.

Cell activity in this growth can be measured indirectly by analysis of the degree of biochemical activity to the size of population.

Methods

Direct microscopic count
Electronic enumeration of cell numbers
The plate count method
Turbidity estimation of bacterial numbers
Determination of nitrogen content
Determination of dry weight of cells
Membrane Filtration method

Direct microscopic count

- The most obvious way to count microbial numbers is through direct counting.
- Petroff-hausser counting is one of the easiest and accurate way to count bacteria.
- Side view of the chamber showing the cover glass and the space beneath it that holds a bacterial suspension.
- A top view of the chamber. The grid is located in the center of the slide.
- An enlarged view of the grid. The bacteria in several of the central squares are counted, usually at X400 to X500 magnification.
- Concentration of the cells can be calculated by using the average no. of bacteria the avg. number of bacteria in these squares.

Electronic enumeration of cell numbers

- In this method of microbial growth measurement, bacterial suspension is kept inside an electronic particle counter, within which the bacteria are passed through tiny orifice 10 to 30 μm in diameter.
- This orifice is then connected to the two compartments of the counter which contains an electrically conductive solution.
- The electrical resistance between two compartments will increases momentarily, when bacterium passes through the orifice. This generates an electrical signal which is automatically counted.

The plate count method

- This method allows the determination of the number of cells that will multiply under certain defined conditions.
- Plate count method can be done in two ways either by spread plate method or by pour plate method.
- This method of bacterial counting is most commonly used with satisfactory results for the estimation of bacterial populations in milk, water, foods and many other materials.

Turbidity estimation of bacterial numbers

- As bacteria multiply in a liquid medium, the medium becomes turbid, or cloudy with cells.
- Turbidity is the Cloudiness or haziness of a media or fluid caused by large no. of individual particles.
- The instrument used to measure turbidity is a spectrophotometer (or colorimeter).
- Microbial mass can be determined by determination of absorption of light.
- In the spectrophotometer, a beam of light is transmitted through a bacterial suspension to a light-sensitive detector, as the bacterial numbers increase, less light will reach the detector.
- As the population increases, absorbance of the light increases by the cells, so the turbidity also increases. Turbidity can be measured by using an instrument spectrophotometer.
- The absorbance is used to plot bacterial growth.

Determination of nitrogen content

- The major constituents of cell material are protein, and since nitrogen is characteristics part of proteins.
- Bacterial population or cell crop can measure in terms of bacterial nitrogen.
- In this growth can be measured by first harvesting the cells and wash them free of medium and then perform a quantitative chemical analysis of nitrogen.

Determination of dry weight of cells

- For filamentous bacteria and molds, the usual measuring methods are less satisfactory.
- A plate count would not measure this increase in filamentous mass.
- In plate counts of actinomycetes and molds, it is mostly the number of asexual spores that is counted instead.
- One of the better ways to measure the growth of filamentous organisms is by dry weight.
- In this procedure, the fungus is removed from the growth medium, filtered to remove extraneous material, and dried in a desiccator, it is then weighed.
- Growth measurement by measuring cell mass is one of the easiest ways, a known volume of culture sample from the ferment or withdrawn and centrifuged.

Membrane Filtration method

- When the number of bacteria is extremely few, as in lakes or relatively pure streams, bacteria are often counted by filtration methods.
- During this technique, a minimum of 100 ml of water are passed through a thin membrane filter whose pores are too tiny to permit bacteria to pass.
- After filtration bacteria are filtered out and present on the surface of the filter. Then filter is transferred to a Petri plate containing a in liquid nutrient medium, where colonies grow from the bacteria on the filter's surface.
- This method is applied frequently to detection and enumeration of coliform bacteria, which are indicators of fecal contamination of food or water.

Q3. Explain different types of nutrition in bacteria.

Ans:

Nutritional Types of Bacteria

On the basis of energy source organisms are designated as:

Phototrophs:

The organisms which can utilize light as an energy source are known as phototrophs. These bacteria gain energy from light.

Chemotrophs:

These bacteria gain energy from chemical compounds. They cannot carry out photosynthesis.

On the basis of electron source organisms are designated as:

Lithotrophs:

Some organisms can use reduced inorganic compounds as electron donors and are termed as Lithotrophs. They can be Chemolithotrophs and Photolithotrophs

Organotrophs:

Some organisms can use organic compounds as electron donors and are termed as organotrophs.
Some can be Chemoorganotrophs and Photoorganotrophs.
Thus, bacteria may be either:

Photo-lithotrops:

These bacteria gain energy from light and use reduced inorganic compounds such as H_2S as a source of electrons. eg: Chromatium okeinii.

Photo-organotrophs:

These bacteria gain energy from light an d use organic compounds such as Succinate as a source of electrons.eg; Rhodospirillum.

Chemo-lithotrophs:

These bacteria gain energy from reduced inorganic compounds such as NH_3 as a source of electron eg; Nitrosomonas.

Chemo-organotrophs:

These bacteria gain energy from organic compounds such as glucose and ammino acids as a source of electrons.eg; Pseudomonas pseudoflora.
Some bacteria can live ether chemo-lithotrophs or chemo-organotrophs like Pseudomonas pseudoflora as they can use either glucose or H_2S as electron source.

On the basis of carbon source bacteria may be:

All organisms require carbon in some form for use in synthesizing cell components.
All organisms require at least a small amount of CO_2.
However, some can use CO_2 as their major or even sole source of carbon; such organisms are termed as Autotrophs (Autotrophic bacteria).
Others require organic compounds as their carbon source and are known as Heterotrophs (Heterotrophic bacteria).

Autotrophic Bacteria
These bacteria synthesize all their food from inorganic substances (H2O, C02, H2S salts).
The autotrophic bacteria are of two types:
(i) Photoautotrophs
These bacteria capture the energy of sunlight and transform it into the chemical energy.
In this process, CO2 is reduced to carbohydrates.
The hydrogen donor is water and the process produce free oxygen.
e.g., Cyanobacteria.
(ii) Chemoautotrophs
These bacteria do not require light (lack the light phase but have the dark phase of photosynthesis) and pigment for their nutrition.
These bacteria oxidize certain inorganic substances with the help of atmospheric oxygen.
This reaction releases the energy (exothermic) which is used to drive the synthetic processes of the cell.
e.g Sulphomonas (Sulphur bacteria).
Heterotrophic Bacteria
The heterotrophic bacteria obtain their-readymade food from organic substances, living or dead.
Most of pathogenic bacteria of human beings, other plants and animals are heterotrophs.
Heterotrophic bacteria are of three types:
a. Photoheterotrophs
These bacteria can utilize light energy but cannot use CO2 as their sole source of carbon.
They obtain energy from organic compounds to satisfy their carbon and electron requirements. Bacteriochlorophyll pigment is found in these bacteria.
g., Purple non-sulphur bacteria (Rhodospirillum, Rhodomicrobium, Rhodopseudomonas palustris).
b. Chemoheterotrophs
Chemoheterotrophs obtain both carbon and energy from organic compounds such as carbohydrates, lipids and proteins.

Q4. Write notes on SEM and TEM.
Ans:
SEM (Scanning Electron Microscopy):

- Image Formation: Scans the surface of the sample with a focused electron beam, collecting scattered electrons to create an image.
- Sample Preparation: Does not require ultrathin sections, allowing for the analysis of various samples, including solid materials, powders, and polished surfaces.
- Information Provided: Primarily reveals the surface morphology, topography, and elemental composition of the sample.
- Magnification: Can magnify samples up to 2 million times.
- Resolution: Generally, has a resolution of 0.5 to 4nm.
- Applications: Commonly used for analyzing surfaces, powders, polished microstructures, and for imaging IC chips.

TEM (Transmission Electron Microscopy):

- Image Formation: Passes a broad beam of electrons through a thin sample, revealing the internal structure by capturing transmitted electrons.
- Sample Preparation: Requires ultrathin sections to allow electrons to pass through.
- Information Provided: Provides information about the internal structure, morphology, composition, and crystal structure of the sample.
- Magnification: Can magnify samples up to 50 million times.
- Resolution: Has a higher resolution than SEM, typically 0.1 to 0.3nm.
- Applications: Used for imaging dislocations, small precipitates, grain boundaries, and other defect structures in solids.

Q5. Differentiate between Prokaryotes and Eukaryotes.

Ans:

Characteristics	Prokaryotes	Eukaryotes
Type of Cell	Always unicellular	Unicellular and multi-cellular
Cell size	Ranges in size from 0.2 µm – 2.0 µm in diameter	Size ranges from 10 µm – 100 µm in diameter
Cell wall	Usually present; chemically complex in nature	When present, chemically simple in nature
Nucleus	Absent. Instead, they have a nucleoid region in the cell	Present
Ribosomes	Present. Smaller in size and spherical in shape	Present. Comparatively larger in size and linear in shape
DNA arrangement	Circular	Linear
Mitochondria	Absent	Present
Cytoplasm	Present, but cell organelles absent	Present, cell organelles present
Endoplasmic reticulum	Absent	Present
Plasmids	Present	Very rarely found in eukaryotes
Ribosome	Small ribosomes	Large ribosomes
Lysosome	Lysosomes and centrosomes are absent	Lysosomes and centrosomes are present
Cell division	Through binary fission	Through mitosis
Flagella	The flagella are smaller in size	The flagella are larger in size
Reproduction	Asexual	Both asexual and sexual
Example	Bacteria and Archaea	Plant and Animal cell

Q6. Write about different types of culture media used in microbiology laboratory.

Ans:

In microbiology laboratories, various types of culture media are employed to cultivate, isolate, and identify microorganisms. These media are classified based on their composition, consistency, and purpose. Here are some commonly used types:

Basal (General Purpose) Media:

These are simple media that support the growth of non-fastidious organisms. Examples include Nutrient Agar and Tryptic Soy Agar, which provide essential nutrients for a wide range of bacteria.

Enriched Media:

Formed by adding blood, serum, or other nutrients to basal media, enriched media support the growth of fastidious organisms. Blood Agar, for instance, is used to cultivate Streptococcus species and to observe hemolytic reactions.

Selective Media:

These media contain agents that inhibit the growth of certain microbes while allowing others to grow. MacConkey Agar, for example, contains bile salts and crystal violet to suppress Gram-positive bacteria, facilitating the isolation of Gram-negative enteric bacteria.

Differential Media:

Designed to distinguish between different types of bacteria based on their biological characteristics. Mac-Conkey Agar also serves this purpose by differentiating lactose fermenters, which appear pink, from non-fermenters, which remain colorless.

Transport Media:

Used to preserve specimens during transport to the laboratory, ensuring that the viability of pathogens is maintained without overgrowth. Examples include Cary-Blair and Stuart's media.

Q7. Write brief notes on IMViC test.

Ans:

The IMViC test is a series of four biochemical tests (Indole, Methyl Red, Voges-Proskauer, and Citrate) used to identify and differentiate between coliform bacteria, particularly members of the Enterobacteriaceae family. These tests help determine if a bacterium can produce indole from tryptophan, produce acid from glucose, produce acetoin, and utilize citrate as a sole carbon source.

Indole Test:

Detects the ability of bacteria to produce indole from the amino acid tryptophan. A positive result is indicated by the formation of a red or pink ring when Kovac's reagent is added after incubation.

Methyl Red Test:

Measures the ability of bacteria to produce acid from glucose fermentation. A positive result is indicated by a red color change in the Methyl Red reagent.

Voges-Proskauer Test:

Identifies acetoin production, a byproduct of glucose fermentation, after 24-48 hours. A positive result is indicated by the formation of a red color when Voges-Proskauer reagents are added.

Citrate Utilization Test:

Tests the ability of bacteria to utilize citrate as a sole carbon and energy source. A positive result is indicated by a change in color from green to blue and the presence of growth on Simmon's citrate agar.

By combining the results of these four tests, microbiologists can differentiate between various species of Enterobacteriaceae, like E. coli and Enterobacter. The acronym "IMViC" is used as a mnemonic device to remember the four tests.

Mr. Biswajit Biswal

Model Question & Answer(MQA)
Sub-Pharmaceutical Microbiology
Class-B.Pharm 3rd Semester

Unit-III

1.Long Question-Describe in detail about Evaluation of Disinfectant.

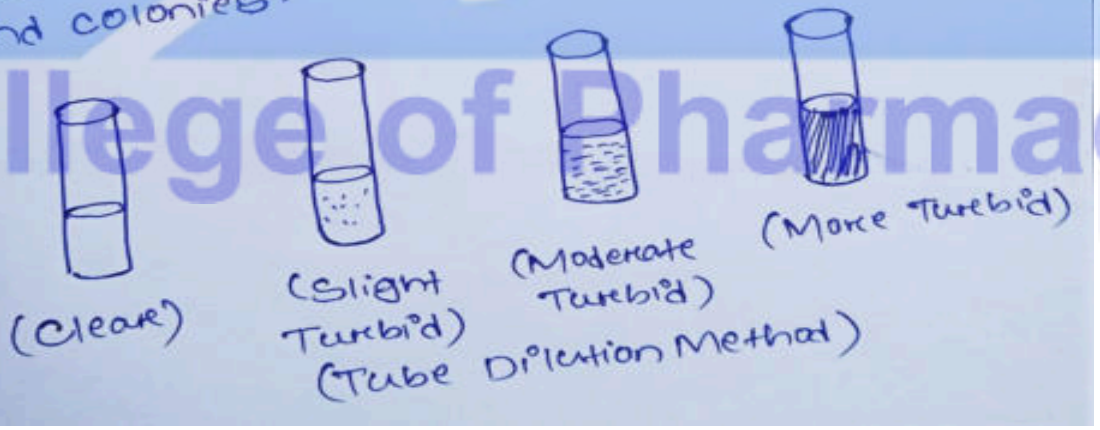
Evaluation of Disinfectant-

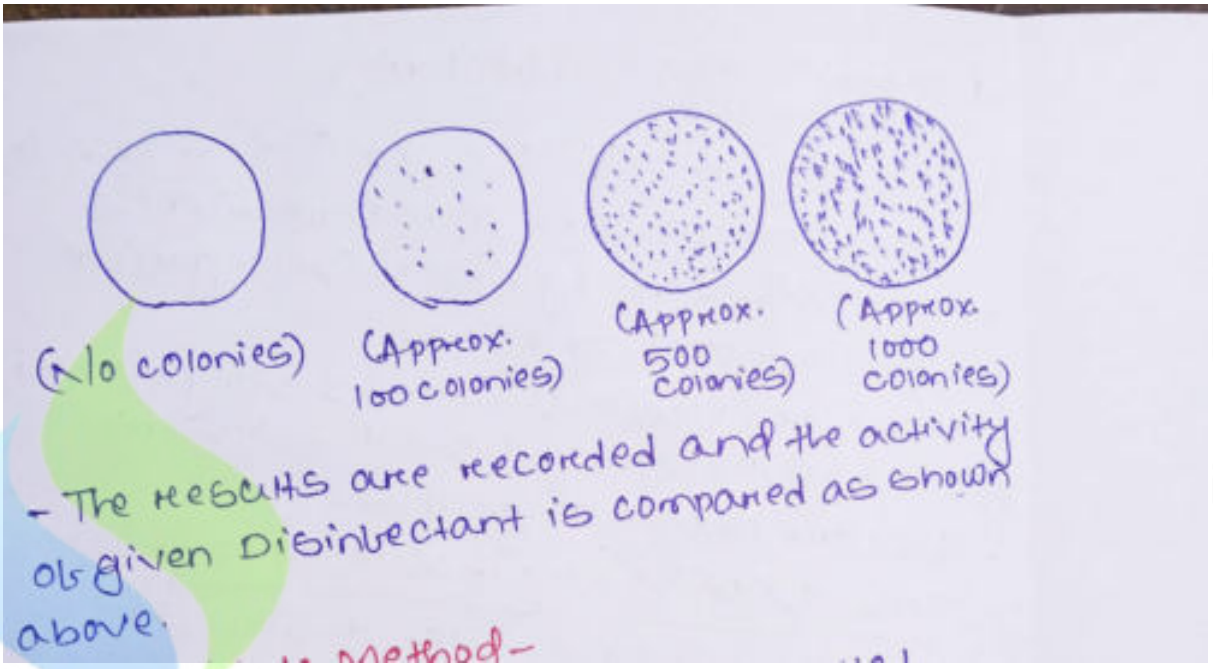
Evaluation of Disinfectant is the process of testing and measuring the effectiveness of a disinfectant against Microorganism under controlled conditions.

- There are various methods being employed to evaluate the potency of the disinfectant.
- These are of following types.

1) Tube Dilution & Agar plate Method-

- In this method the test disinfectant is incorporated into Nutrient broth or Agar Medium and inoculated with the test microorganism
- The tubes & plates are incubated at 30-35°C for 2-3 days and after incubation the results are observed in the form of turbidity and colonies.

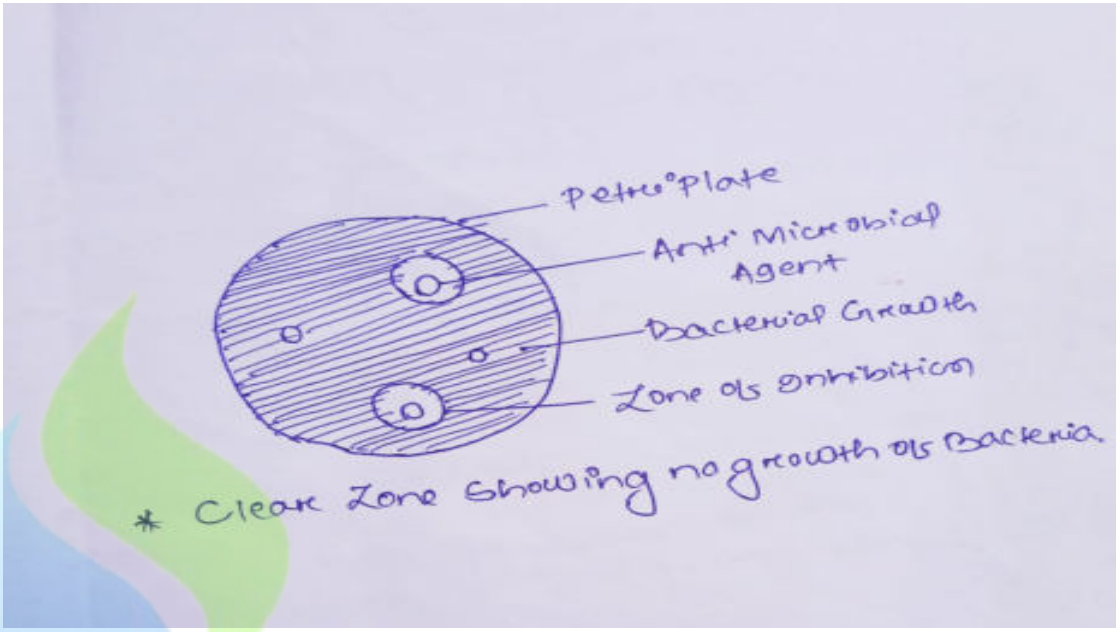




- The results are recorded and the activity of given Disinfectant is compared as shown above.

2) Cup plate Method-

- In this method, the agar is melted and cooled to about 45°C, inoculated with the test microorganism and poured into a sterile petri plate.
- When the inoculated agar has solidified holes of about 9 mm in diameter are cut in the medium with a sterile cup.
- The anti microbial agent is directly placed in the holes & then the zone of inhibition is observed after incubation at 35°-37°C for 2-3 days
- The diameter of the zone of inhibition gives an indication of the relative activities of different antimicrobial substances against the test microorganism



* Clear Zone Showing no growth of Bacteria.

3) Phenol coefficient method-

- In this method, a test chemical is rated for its antimicrobicidal property with reference to phenol under identical conditions.
- Organisms used in this method are Salmonella typhi, Staphylococcus aureus, pseudomonas aeruginosa.

Phenol coefficient test includes-

i) Rideal Walker Test (RW Test)
ii) Chick Martin Test (CM Test)

Rideal Walker Test-

- The phenol coefficient of test disinfectant may be calculated by Rideal Walker Test that uses Rideal Walker broth and Salmonella typhi as the sensitive anicreo organism.

Procedure-

- Different dilutions of the test disinfectant and phenol are prepared and 5 ml of each dilution is inoculated with 0.5 ml of the 24 hours broth culture of the organism.

- All tubes (disinfectant + organism and phenol + organism) are placed in a 17.5°C water bath.
- Subcultures of each reaction mixture is taken and transferred to 5 ml sterile broth after 2.5, 5, 7.5 and 10 min.
- The broth tubes are incubated at 37°C for 48 to 72 hours and are examined for the presence or absence of growth.

Disinfectant	Dilution	Time interval for Subculture			
		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	+	+	+	+

(+) = it shows growth (-) = it shows no growth

- Now, calculate Rideal Walker co-efficient of the disinfectant

R.W coefficient = Dilution of test disinfectant killing in 7.5 but not in 5 min / Dilution of phenol killing in 7.5 but not in 5 min

$$R.W = \frac{2000}{100} = 20$$

- If the phenol co-efficient (RW coefficient) of given test disinfectant is

1 → Same effectiveness as Phenol

Less than 1 → Less effectiveness than phenol

More than 1 → More effective than phenol

* Here is 20, that means the test disinfectant is 20 times more active than phenol.

*Chick Martin Test-

- This type of test is done in controlled amount of organic matter in the form of standardization suspension of Yeast cell.

- In this test all the conditions are like Rideal walker test but the temperature is 20°C and the exposure here is 30 minutes

- For example

Disinfectant	Dilution	Time interval for Subculture			
		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	+	+	+	+

(+) = it shows growth (-) = it shows no growth

Chick Martin Co-efficient = Dilution of Test Disinfectant killing in 7.5 but not in 5 min / Dilution of Phenol killing in 7.5 but not in 5 min

$CM = \frac{2000}{100} = 20$

- of the Chick Martin co-efficient of the given disinfectant is

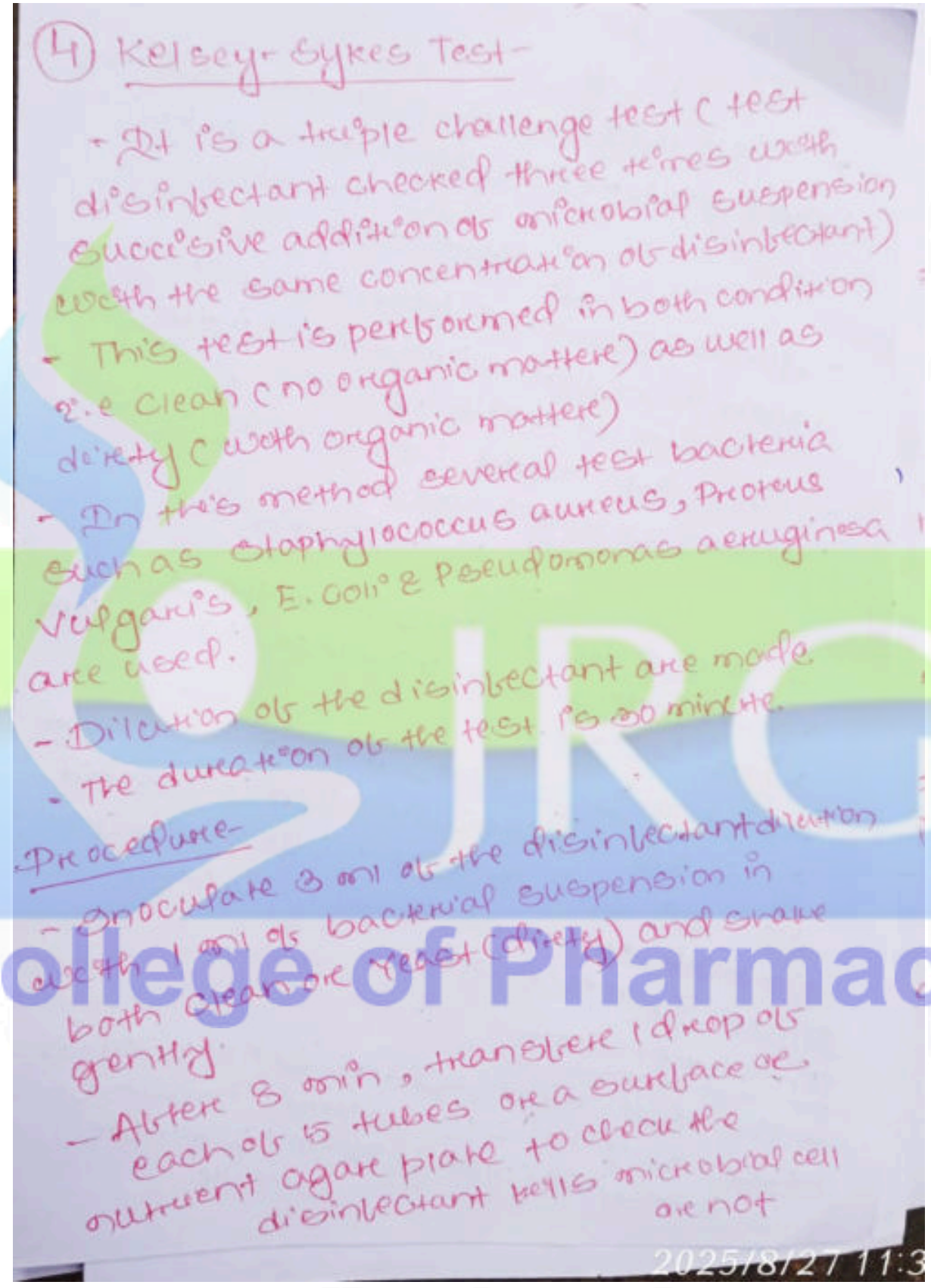
of given test

1 → same effectiveness as phenol

Less than 1 → Less effectiveness than phenol

More than 1 → More effective than phenol

* Here is 20, that means the test disinfectant is 20 times more active than phenol.



(4) Kelsey-Sykes Test-

- It is a triple challenge test (test disinfectant checked three times with successive addition of microbial suspension with the same concentration of disinfectant)
- This test is performed in both condition i.e clean (no organic matter) as well as dirty (with organic matter)
- In this method several test bacteria such as Staphylococcus aureus, Proteus vulgaris, E. coli & Pseudomonas aeruginosa are used.
- Dilution of the disinfectant are made.
- The duration of the test is 30 minute.

Procedure-

- Inoculate 3 ml of the disinfectant dilution with 1 ml of bacterial suspension in both clean or yeast (dirty) and shake gently.
- After 8 min, transfere 1 drop of each of 5 tubes on a surface of nutrient agar plate to check the disinfectant kills microbial cell are not

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↓

- Now, add 1 ml of microbial cell into same mixture (Disinfectant + Bacterial suspension)

↓ After 10 Min

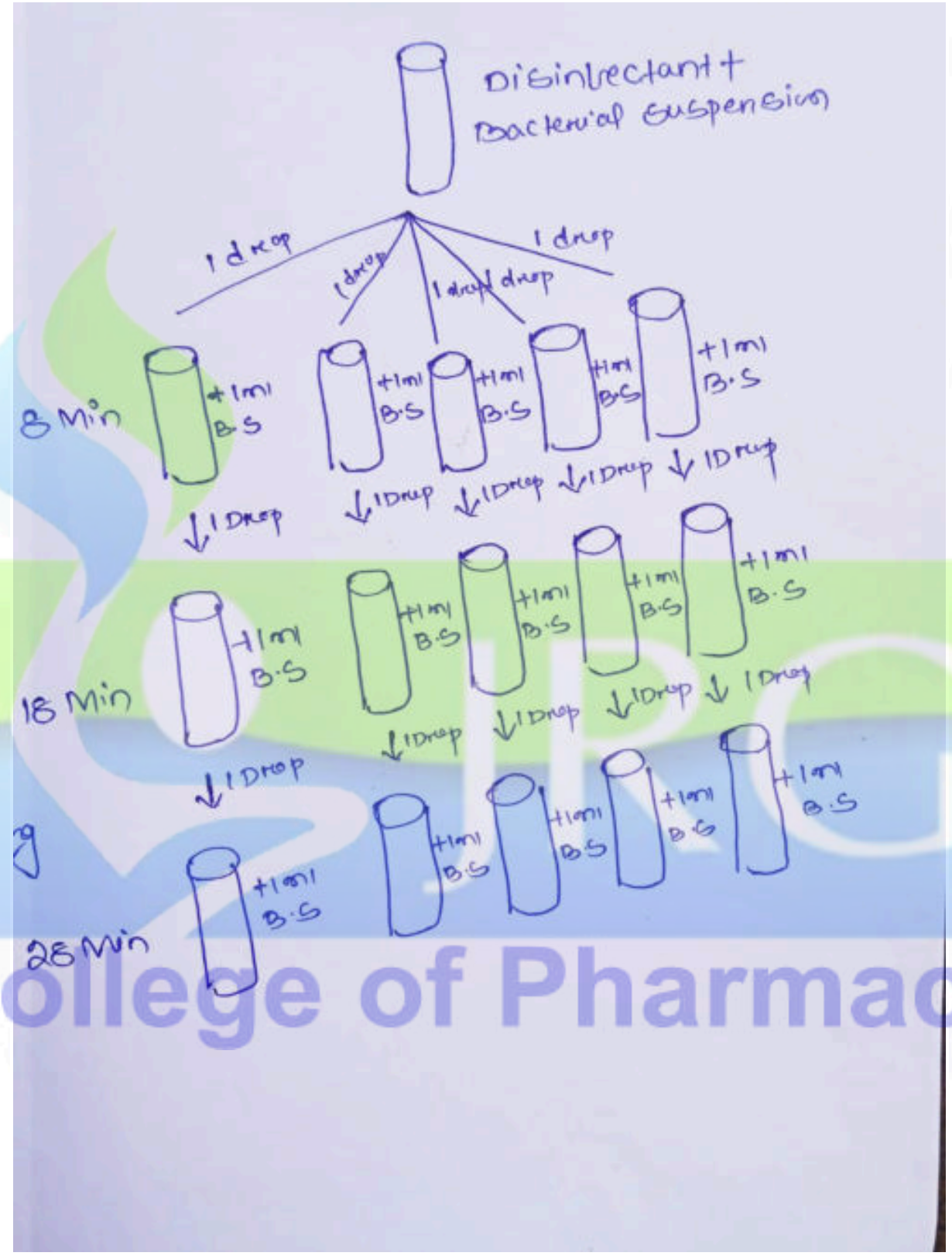
- After 18 minute from the starting, again transfere microbial cell into nutrient media for checking.
- Then again add 1 ml of microbial cell into same mixture

↓ After 10 Min

- After 28 minutes from the starting, again transfere microbial cell to nutrient agare to check.
- After this, samples that are taken at 8, 18, 28 minutes are then incubated at 30-37°C and the no. of tubes showing Growth or the no. of colonies on the plate is recorded.

Interpretation:-

- The test disinfectant is reported as PASS if it kills the bacteria in atleast 2 tubes/petri plates of the 18 minute sample.



2.Short Notes-Write a short note on Cultivation of Viruses.

Cultivation of Virus

Viruses are obligate intracellular parasites, which means they cannot grow on artificial media like bacteria or fungi. They require living host cells for their growth and replication. Therefore, the cultivation of viruses involves growing them inside suitable host systems.

Purpose of Virus Cultivation

- To study viral structure, replication, genetics, and life cycle.
- To produce vaccines (e.g., polio, measles).
- For diagnosis of viral infections.
- For research and development of antiviral drugs.

Methods of Virus Cultivation

There are three main methods of virus cultivation:

1. Animal Inoculation

- Description: Virus is injected into a live animal (e.g., mice, rabbits, monkeys).
- Uses:
  - Study of pathogenicity and immune response.
  - Isolation of viruses that don't grow in cell cultures.
- Drawbacks:
  - Expensive, time-consuming.
  - Ethical concerns.
  - May not show symptoms if the virus isn't pathogenic to the animal.

2. Embryonated Egg Inoculation

- Description: Virus is injected into specific sites of a fertilized hen's egg (6–12 days old).

- **Common Sites:**
  - Chorioallantoic membrane (CAM) – for poxviruses.
  - Allantoic cavity – for influenza virus.
  - Amniotic cavity – for mumps virus.
  - Yolk sac – for certain arboviruses**.**
- **Advantages:**
  - Inexpensive and sterile.
  - Used for vaccine production (e.g., flu vaccine).
- **Disadvantages:**
  - Not suitable for all viruses.
  - Requires skilled handling.

3. Tissue (Cell) Culture

- Description: Viruses are cultivated in monolayers of host cells in laboratory vessels.
- Types:
  - Primary cell culture – from fresh tissues (e.g., monkey kidney).
  - Diploid cell lines – can be subcultured ~50 times (e.g., human embryonic lung).
  - Continuous cell lines – immortal (e.g., HeLa, Vero).
- Detection:
  - Cytopathic effect (CPE) – visible changes or cell death.
  - Hemadsorption – attachment of red blood cells.
- Advantages:
  - Most commonly used method.

- Allows detailed study of viral replication.

- Disadvantages:
  - Needs strict aseptic conditions.
  - Some viruses do not grow well in culture.

Summary Table

Method	Host Used	Example Virus	Use
Animal Inoculation	Live animal (e.g., mice)	Rabies virus	Pathogenicity studies
Embryonated Egg	Fertilized hen egg	Influenza, Poxvirus	Vaccine production
Cell Culture	Cell lines in lab	Herpes, Polio	Routine virus cultivation

3.Short type Questions

a)Which Fungal spore only helps in growth & development?
Ans-Vegetative Spore

b)The envelope of all viruses are made up of which substance?
Ans-Lipids

c)Which antimicrobial agent only prevents the growth & multiplication of microorganisms?
Ans-Bacteriostatic Agents

d)Which culture media is mostly used for the isolation of Fungi?
Ans-Sabraoud Dextrose Agar(SDA)

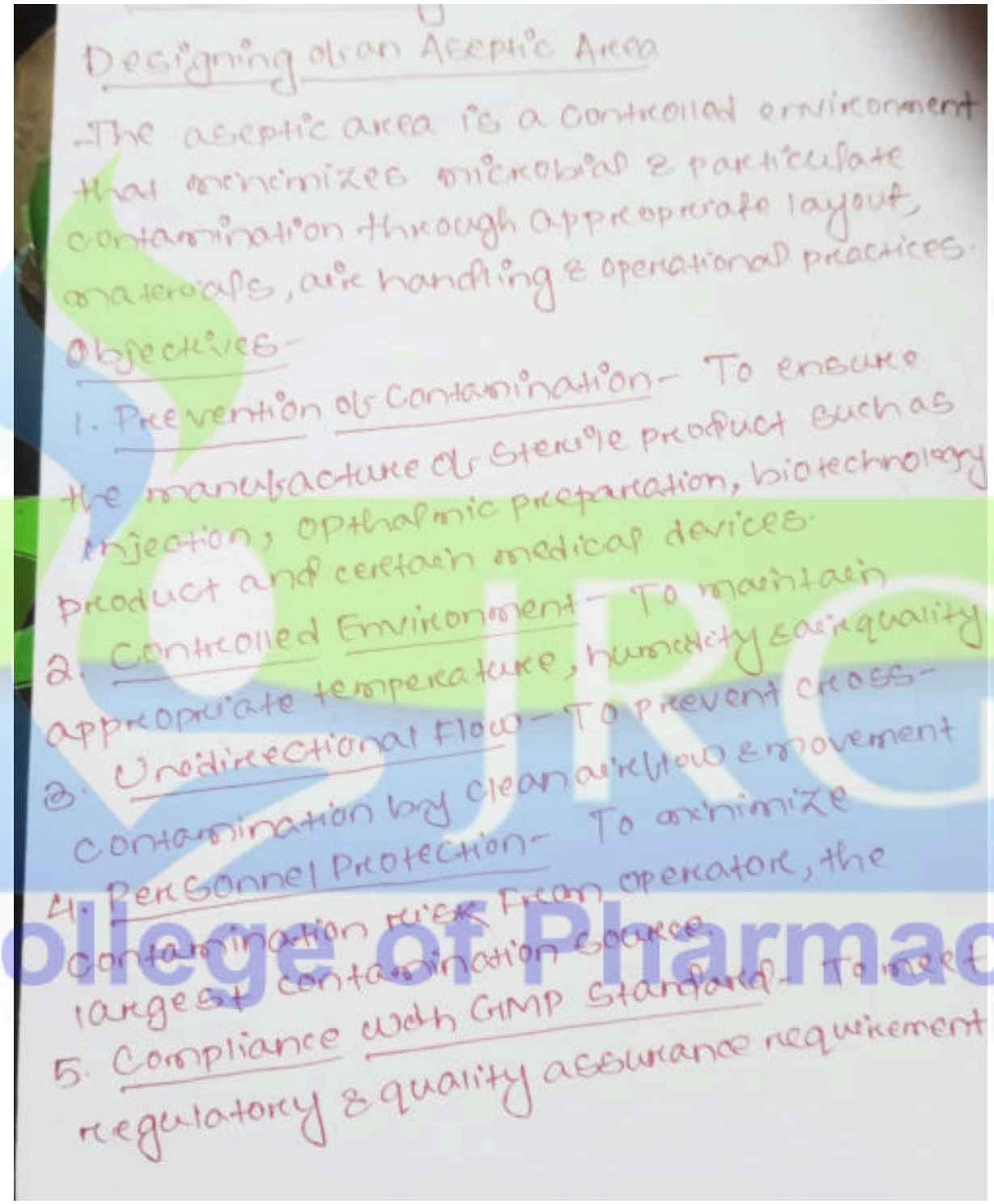
e)In which evaluation method organic matter is added to check the effectiveness of Disinfectant?
Ans-Chick Martin Method

f)The minimum concentration of antibactericidal agent that prevents 99.97% of bacterial growth is called as?
Ans-Minimum Bactericidal Concentration(MBC)

g)Which disinfectant have high level of activity?
Ans-Aldehydes

Unit-IV

4.Short Notes-Write a short note on designing of an Aseptic area.



Designing of an Aseptic Area

The aseptic area is a controlled environment that minimizes microbial & particulate contamination through appropriate layout, materials, air handling & operational practices.

Objectives-

1. Prevention of Contamination- To ensure the manufacture of Sterile product such as injection, opthalmic preparation, biotechnology product and certain medical devices.
2. Controlled Environment- To maintain appropriate temperature, humidity & air quality
3. Unidirectional Flow- To prevent cross-contamination by clean air flow & movement
4. Personnel Protection- To minimize contamination risk from operator, the largest contamination source.
5. Compliance with GMP Standard- To meet regulatory & quality assurance requirement

- The pore size of the filter should be 0.2 micron.

Layout & Zoning of an Aseptic Area-

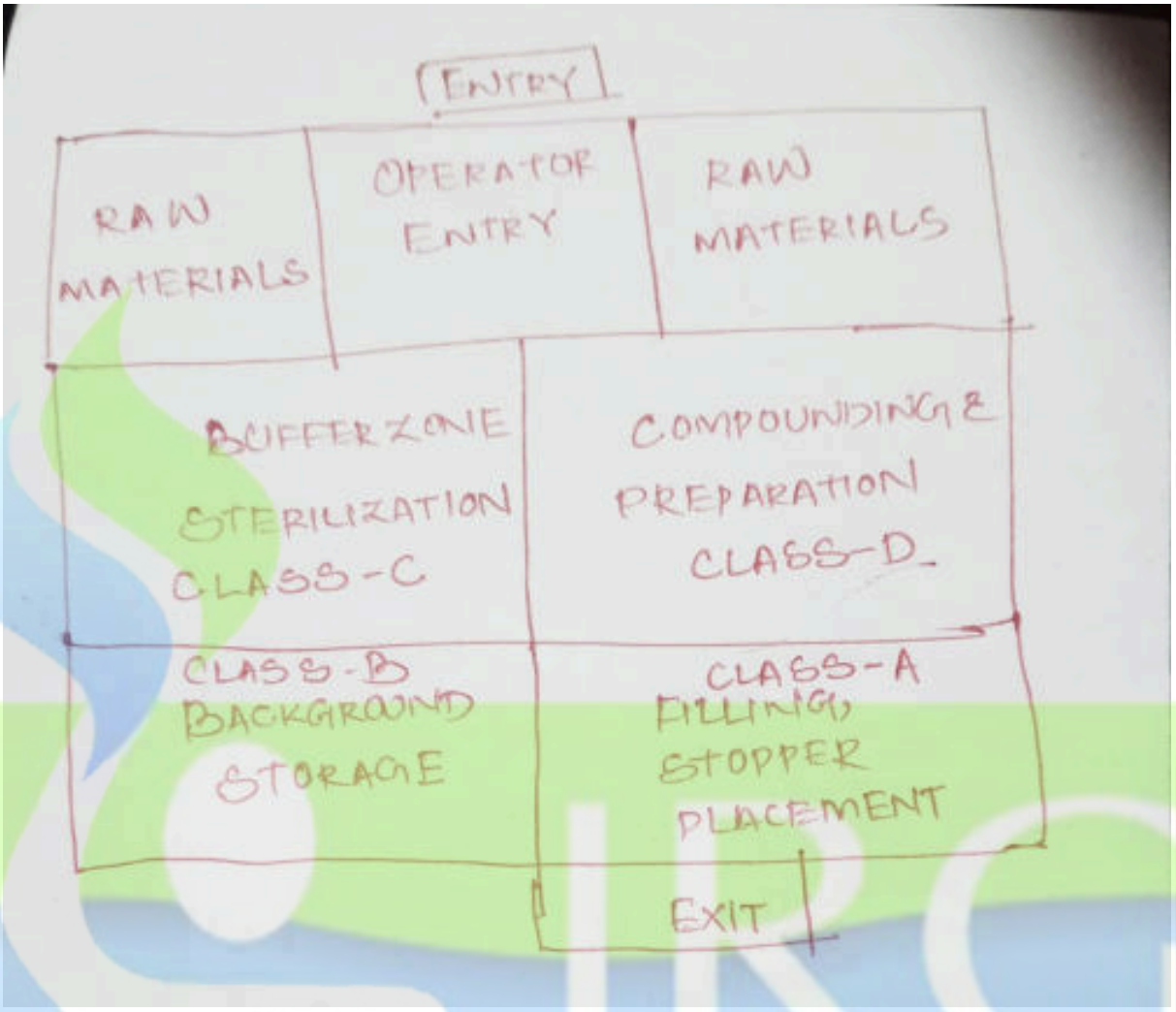
- The Aseptic Area is devided into multiple zones, each with decreasing microbial and particulate tolerance levels as moves inward.

1. Uncontrolled Area (General Area):-
→ Normal working environment outside the cleanroom.
→ Personnel enter here before changing into cleanroom Garments

2. Cleanroom (Support Area)-
Class C' - This Area act as a buffere zone between uncontrolled & critical Areas
Class b' - Used For storage of materials, Preparation & initial Processing.

3. Critical Areas
Class A- Laminar Air Flow workstation or isolator where filling, stopper placements capping
Class B- Background environment are For Aseptic Filling & storage.
- This Area must maintain ISO class 5 particulate level.

carried out



5.Short type Question

a)What is the normal pore size of a HEPA filter?
Ans-0.2-0.4micron

b)Which type of door must be installed in an aseptic area?
Ans-Airlocked Door

c)Which part of the laminar air flow is is used for sterilization before & after use?
Ans-UV Light

d)Which part of the aseptic area acts as a buffer zone between General & Clean Area?
Ans-Class-C

Model Questions & Answers
B. Pharmacy, 3rd Semester
Sub: Ph. Microbiology, Sub. Code: BP303T
Unit-II

Long Answer Type Questions **(10 marks)**

Q1. Define and classify Sterilization. Explain various methods of Sterilization.

Ans.

Definition: -

In microbiology, sterilization can be defined as the complete removal of all forms of microorganisms, both vegetative and spore forms, from a surface or an object. Sterilization is carried out by various physical and chemical methods such that it eliminates around 106 log colony-forming units. Sterilization is done to avoid the growth of microorganisms which may grow on the surface of an object if left without killing the germs. It is, however, different from disinfection or sanitization where only reduction of the microorganisms takes place, rather than total elimination. After sterilization, an object becomes sterile or aseptic.

Classification of Sterilization: -

Sterilization is achieved by different physical and chemical methods in microbiology. Sterilization is classified into 2 types – physical sterilization and chemical sterilization.

Physical Methods of Sterilization

Physical sterilization includes the following methods:

Heat Sterilization

Heat sterilization is the most effective method of sterilization, where the elimination of microbes is achieved by the destruction of cell constituents and enzymes. It is done by two methods:

Moist Heat Sterilization:

It is one of the best methods of sterilization. Moist heat sterilization is done with the help of an instrument called an autoclave. An autoclave works on the principle of producing steam under pressure. Thus moist heat sterilization is also known as steam sterilization. The water is boiled in an autoclave at 121-134°C at a pressure of 15psi. This leads to coagulation of proteins in the microorganism, and they are effectively killed.

Dry Heat Sterilization:

This method is used on objects that are sensitive to moisture. Moisture-free heat or dry heat is applied on the surface or objects such that there is denaturation and lysis of proteins which leads to oxidative damage, and ultimately the microbial cell dies out or may even burn. Some methods of dry heat sterilization include incinerators, hot air ovens and flaming techniques.

Filtration

This is a mechanical method of sterilization in microbiology. This method uses membranous filters with small pores to filter out the liquid so that all the bigger particles and microbes cannot pass through. The three steps of filtration are sieving, adsorption and trapping.

Irradiation

Irradiation is the process of exposing surfaces or objects to different kinds of radiation for sterilization. It is of two types:

Non-ionizing Radiation:

Ultraviolet radiation is exposed to the object, which is absorbed by nucleic acids of the microorganisms. This leads to the formation of pyrimidine dimers in the DNA strand, which causes the replicative error, and eventually, the microbe dies.

Ionizing Radiation:

Upon exposure to ionizing radiations such as gamma rays and X-rays, reactive oxygen species such as hydrogen peroxide and superoxide ions are formed that oxidize the cellular components of the microbe, and they die.

Sound Waves Vibration

Sonic sound waves ranging from 20-40 kHz in frequency are applied across the fluid to be sterilized. These ultrasonic waves produce an alternation of compressive and tensile forces forming cavities in the solution. These cavities suddenly collapse, which creates submicroscopic voids and removes microorganisms from the container.

Fractional Sterilization

Fractional sterilization or tyndallization is a method used for media containing gelatin or sugar. Typically, exposure to 100°C for 20 minutes on 3 successive days is required. The principle is that the first exposure kills all spores and vegetative bacteria. If they germinate, they will be killed in the subsequent exposures. However, this method may fail to kill spores of certain thermophiles and anaerobes.

Chemical Methods of Sterilization

Chemical methods of sterilization are used in microbiology for biological specimens and plastic equipment. In this method, several chemicals work as bactericidal agents. They can be of two types: gaseous or liquid.

Gaseous Sterilization

Gaseous sterilization is the method where the object is exposed to gas in a closed, heated and pressurized chamber. The gaseous chemical agents used for sterilization include ethylene oxide, formaldehyde, nitrogen dioxide and ozone.

Liquid Sterilization

Liquid sterilization is the process of immersing the object in a liquid such that it kills all the viable microorganisms and their spores. This method is less effective than gaseous sterilization and is used to remove low levels of contamination. Common liquid chemical agents that are used for sterilization include hydrogen peroxide, glutaraldehyde and hypochlorite solution.

Q2. What is Moist Heat Sterilization. Explain construction, working principle, and application of Autoclave. (10marks)

Ans.

Moist Heat Sterilization:

It is one of the best methods of sterilization. Moist heat sterilization is done with the help of an instrument called an autoclave. An autoclave works on the principle of producing steam under pressure. Thus moist heat sterilization is also known as steam sterilization. The water is boiled in an autoclave at 121-134℃ at a pressure of 15psi. This leads to coagulation of proteins in the microorganism, and they are effectively killed.

Construction:

a. Pressure Chamber

The pressure chamber is the main component of a steam autoclave consisting of an inner chamber and an outer jacket.

The inner chamber is made up of stainless steel or gunmetal, which is present inside the out chamber made up of an iron case.

The autoclaves used in healthcare laboratories have an outer jacket that is filled with steam to reduce the time taken to reach the sterilization temperature.

The inner chamber is the case where the materials to be sterilized are put.

The size of the pressure chamber ranges from 100 L to 3000 L.

b. Lid/ Door

The next important component of an autoclave is the lid or door of the autoclave.

The purpose of the lid is to seal off the outside atmosphere and create a sterilized condition on ht inside of the autoclave.

The lid is made airtight via the screw clamps and asbestos washer.

The lid consists of various other components like:

Pressure gauge

A pressure gauge is present on the lid of the autoclave to indicate the pressure created in the autoclave during sterilization.

The pressure gauge is essential as it assures the safety of the autoclave and the working condition of the operation.

Pressure releasing unit/ Whistle

A whistle is present on the lid of the autoclave is the same as that of the pressure cooker.

The whistle controls the pressure inside the chamber by releasing a certain amount of vapor by lifting itself.

Safety valve

A safety valve is present on the lid of the autoclave, which is crucial in cases where the autoclave fails to perform its action or the pressure inside increases uncontrollably.

The valve has a thin layer of rubber that bursts itself to release the pressure and to avoid the danger of explosion.

c. Steam generator/ Electrical heater

An electrical steam generator or boiler is present underneath the chamber that uses an electric heating system to heat the water and generate steam in the inner and the outer chamber.

The level of water present in the inner chamber is vital as if the water is not sufficient; there are chances of the burning of the heating system.

Similarly, if the water is more than necessary, it might interfere with the trays and other components present inside the chamber.

d. Vacuum generator

In some types of autoclaves, a separate vacuum generator is present which pulls out the air from the inside of the chamber to create a vacuum inside the chamber.

The presence of some air pockets inside the chamber might support the growth of different microorganisms. This is why the vacuum chamber is an important component of an autoclave.

e. Wastewater cooler

Many autoclaves are provided with a system to cool the effluent before it enters the draining pipes.

This system prevents any damage to the drainage pipe due to the boiling water being sent out of the autoclave.

Working Principle

The autoclave works on the principle of moist heat sterilization where steam under pressure is used to sterilize the material present inside the chamber.

The high pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.

Water usually boils at 100°C under normal atmospheric pressure (760 mm of Hg); however, the boiling point of water increases if the pressure is to be increased.

Similarly, the high pressure also facilitates the rapid penetration of heat into deeper parts of the material, and moisture present in the steam causes the coagulation of proteins causing an irreversible loss of function and activity of microbes.

This principle is employed in an autoclave where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg.

Uses of Autoclave

- Autoclaves are important devices to ensure the sterilization of materials containing water as they cannot be sterilized by dry heat sterilization. Besides, autoclaves are used for various other purposes.
- They are used to decontaminate specific biological waste and sterilize media, instruments, and labware.
- Regulated medical waste that might contain bacteria, viruses, and other biological materials is recommended to be inactivated by autoclaving before disposal.
- In medical labs, autoclaves are used to sterilize medical equipment, glassware, surgical equipment, and medical wastes.
- Similarly, autoclaves are used for the sterilization of culture media, autoclavable containers, plastic tubes, and pipette tips.

Q3. What is Dry Heat Sterilization. Explain construction, working principle, and application of Hot Air Oven. (10marks)

Ans.

Dry Heat Sterilization:

This method is used on objects that are sensitive to moisture. Moisture-free heat or dry heat is applied on the surface or objects such that there is denaturation and lysis of proteins which leads to oxidative damage, and ultimately the microbial cell dies out or may even burn. Some methods of dry heat sterilization include incinerators, hot air ovens and flaming techniques.

Principle of Hot Air Oven

Hot air oven works on the principle of the dry air sterilization process through convection, conduction, and radiation. The heating elements heat the air inside the chamber, which may be circulated evenly within it with the help of fans such that the sample surfaces are exposed to hot and dry air. This exposure causes the heating of the external surface of items, and by the conduction process, the heat is transferred toward the center of the item. Likewise, in microorganisms, heat causes water inside them to evaporate, causing oxidative damage of cellular constituents, denaturation of proteins, and toxic effect of elevated levels of electrolytes and, ultimately, the death of the microorganisms.

Construction:

A hot air oven is an essential laboratory equipment that uses to dry heat (hot air) to sterilize laboratory objects and samples. This type of sterilization is also known as dry heat sterilization. This mechanism of heat treatment was introduced by French scientist Louis Pasteur in the late 1800s where he used dry heat for a brief period of time to kill off harmful microorganisms from the wine without altering its taste.

Hot air oven comprises mechanical and electrical parts.

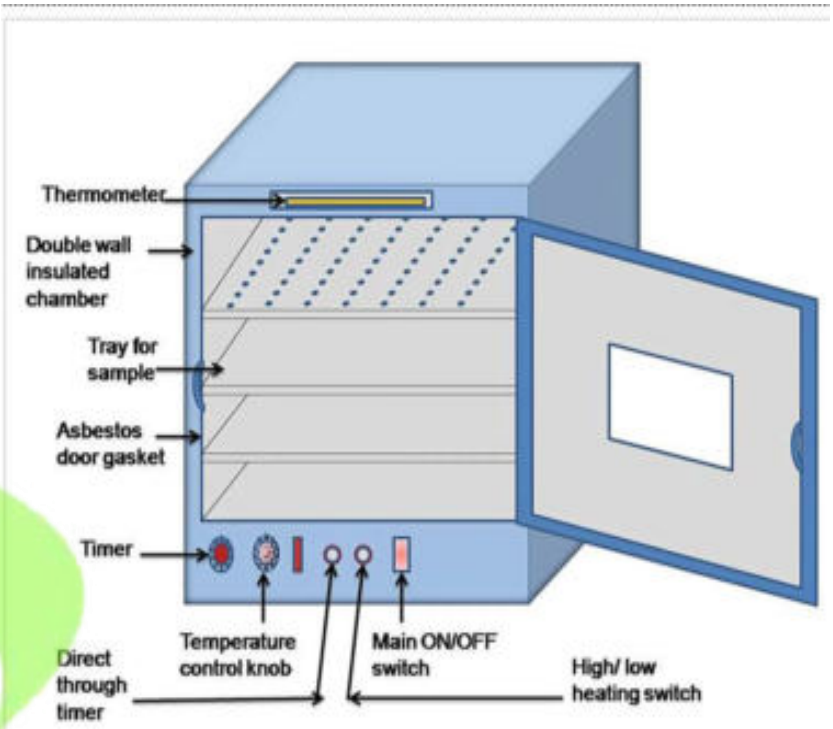
Mechanical parts

Coat/Cabinet: The external shield is built of aluminum or stainless steel, which resists mechanical shocks and oxidation. It also insulates the internal environment from the external surroundings and prevents heat loss.

Fiberglass:
The space between the outer cabinet and inner chamber is filled with thick glass wool insulation. Two types of fiberglass are present, namely, brown fiberglass and yellow fiberglass. The latter is less dangerous compared to the former. Brown glass causes inflammation in the respiratory system, while yellow glass causes skin sensitivity. Thus it is preferred to use hand gloves while dealing with it. It also functions to prevent heat loss from the inside of the device to the outside.

Chamber: The rectangular-shaped chamber is made of aluminum or stainless steel, which has space for ribs to keep shelves at the desired levels.
Shelves (Mesh): These are objects holding plates and are made of aluminum. Depending upon the number and size of objects, as well as the oven capacity, their number may vary. When they are placed on the ribs, the movement of air is facilitated by lifting some areas. Also, some shelves may contain openings for aeration.
Motorized fans/ blower: The fan is driven by the motor and is used to distribute hot air inside the chamber evenly.
Door: A single door on one side is fitted on the heavy hinges. The presence of an asbestos door gasket on the side is used to decrease heat loss during the operation.

Electric parts
Power supply: The power supply is done with the use of a 220V-50Hz transformer and rectifier.
Heater: With the passage of electric current through a conductor, heat is generated following the rise of temperature. The heating element has three main features: high resistance, electrical insulation, and high thermal conductivity. The different types of heaters used in hot air ovens are One side circular type heater, One side U type heater, One side wave type heater, One side square type heater, Three sides type heater, and Four sides type heater. The heater operates at temperatures from 50 to 300 degrees Celsius.
Thermostat: It is a heat sensor connected directly to a heater and can resist extreme heat with a high negative temperature coefficient. It facilitates users to obtain the desired temperature in the hot air oven and prevent temperature overshoot.
Temperature indicator: Either a thermometer or thermocouple can be used to determine the internal temperature of the oven.
Timer: There may be two types of timers: Electrical or Mechanical, which can operate for 5-60 minutes given the time period for sterilization.
Fuse: Fuse functions to prevent electrical damage due to high current during short circuits or high loads.
Control Panel: It is the region that allows the user to control different parameter settings such as temperature, time, etc., as well as has an indicator power lamp (usually green), indicator heater lamp (usually red), and switch knob.



Applications of Hot Air Oven

- It is used for sterilization of laboratory equipment such as glassware (flasks, pipettes, Petri-plates, and test tubes), culture media, metal items (forceps, spatula, scalpel, scissors), non-volatile compounds (zinc and starch powder, sulfonamide), and other materials that contain oils.
- It can be employed for testing food items, pharmaceutical products and other consumable materials in order to ensure their temperature stability during the shelf life.
- It can be used in research settings in the field of biology, chemistry and material science.
- It can be used in heat treatment and drying of samples, such as metals, alloys, soil, and other materials.

Q4. What is Sterilization. Explain construction, working principle, and application of Air filter. (10marks)

Ans.

Sterilization is done to avoid the growth of microorganisms which may grow on the surface of an object if left without killing the germs. It is, however, different from disinfection or sanitization where only reduction of the microorganisms takes place, rather than total elimination. After sterilization, an object becomes sterile or aseptic.

The quality of indoor air is crucial for ensuring a healthy living environment. With rising pollution levels and increased awareness of airborne contaminants, investing in an air purifier has become a necessity for many households.

A HEPA filter is an advanced air filter designed to capture at least 99.97% of airborne particles as small as 0.3 microns. These include dust, pollen, pet dander, and bacteria. The efficiency of HEPA filters makes them an essential component in air purifiers used in homes, hospitals, and laboratories.

Working Principle of HEPA Filters

HEPA filters function through multiple filtration mechanisms that trap airborne particles effectively. The working principle involves:

Interception: As air passes through the filter, larger particles (like dust and pollen) collide with the fibers and get trapped.

Impaction: Medium-sized particles are unable to navigate around the filter fibers due to airflow patterns, causing them to stick to the fibers.

Diffusion: Smaller particles, such as bacteria and smoke, move erratically and eventually settle within the filter fibers due to Brownian motion.

This combination of mechanical filtration ensures that Forbes air purifiers provide highly efficient air cleaning, making them ideal for homes with allergies, asthma concerns, or high pollution levels.

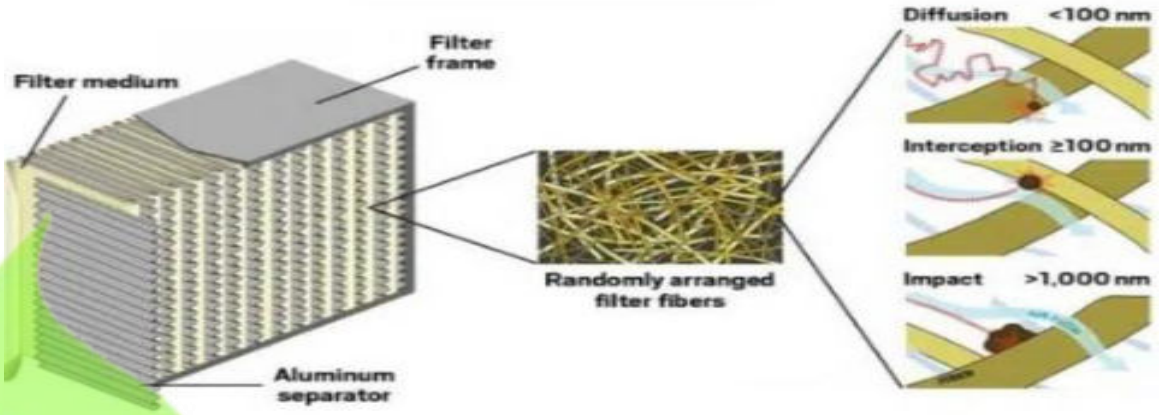
Effectiveness and Efficiency
The efficiency of a HEPA filter is measured by its ability to remove airborne particles of various sizes. True HEPA filters, like those in Forbes air purifiers, can filter out:

- Dust and pollen: Reducing common allergens in indoor air.
- Smoke and fine particles: Capturing harmful microscopic pollutants.
- With advanced filtration technology, Forbes air purifiers ensure that even the finest particles are removed, significantly improving air quality.

Advantages:

- Improves Respiratory Health: By eliminating airborne allergens and pollutants, HEPA filters help prevent respiratory issues, especially for individuals with asthma or allergies.
- Eliminates Unpleasant Odor's: Many HEPA-based air purifiers include activated carbon filters to neutralize odours from cooking, pets, and smoke.
- Reduces Indoor Pollutants: Whether it's dust, pet dander, or mould spores, HEPA filtration ensures your indoor air is fresh and clean.
- Enhances Overall Well-being: Breathing clean air can lead to better sleep, increased focus, and a healthier immune system.
- For households looking for a long-term air quality solution, Forbes air purifiers provide an ideal balance of efficiency and affordability.

HEPA Filter Diagram



Model Questions & Answers
B. Pharmacy, 3rd Semester
Sub: Ph. Microbiology, Sub. Code: BP303T
Unit-II

Short Answer Type Questions **(05 marks)**

Q1. Write a notes on Evaluation of efficiency of Sterilization methods.

Ans.

Evaluation of efficiency of Sterilization Methods :-

→ Evaluation of sterilization methods is carried out to ensure that the product being sterilized should be of best quality.

→ Evaluation of sterilization methods provides a high degree of assurance.

→ Evaluation of sterilization methods can be done by means of microbial destruction.

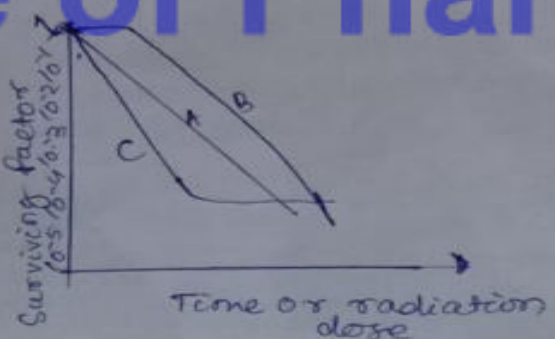
Calculation for evaluation :-

1. Death rates or Survivor Curves.
2. D-values.
3. Z-values.
4. F-values.
5. Q10 values or Temp^{n} co-efficient.
6. Inactivation factor.

1. Survivor Curves :-

→ Death in a microbial population is determined by assessing the reduction of no. of viable microbes resulting from a sterilization method.

→ This can be represented with a Survivor Curve by a plot of log of fraction of survivors against exposure time or dose.



portance of z-value :-
this value is used in the validation of heat sterilization process.

④ F-value :-
→ In food industry a unit of lethality has been devised called the F-value.
→ It is the time in minutes at specific temp°. is needed to kill a population of cells or spores.

$$F = D\,(\log N_0 - \log N)$$

where, D = D-value of organism at 121°C.
N_0 = Initial population numbers /unit volume.
N = Final population number /unit volume.

⑤ Q10-values or Temp°. Co-efficient :-
→ The Q10 value is most frequently employed for chemical disinfection than for heat sterilization.
→ It gives a measure of the relative resistance of different microorganism and describe the change in the death rate over a 10°C change in temp°.
→ Mathematically represented as

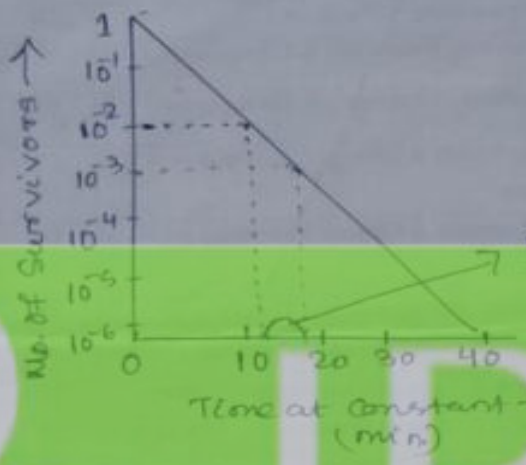
$$Q_{10} = \frac{K_T + 10}{K_T}$$

K_T = Initial temp°.

⑥ Inactivation factor :-
→ It is the degree to which the viable population of organisms is reduced by the sterilizing method by dividing the initial viable count by the final viable count.
→ It can be expressed as $IF = 10^{t/D}$
→ where t = holding time at sterilizing temp°.
D = D-value for microorganism.

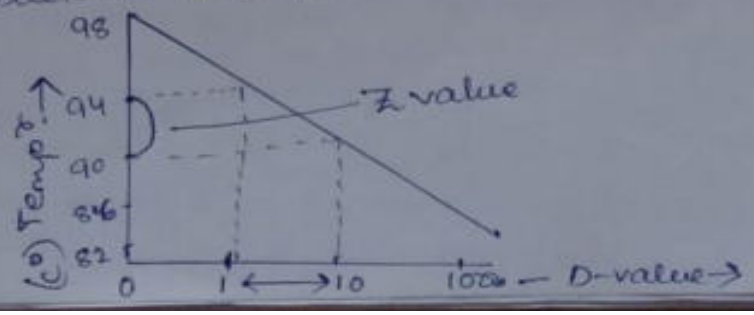
portance:-
Helps to compare the resistance of same microorganism at different temperature, chemicals or radiation.

② D-value
→ The resistance of an microorganism to a sterilizing agent can be described by D-value.
→ It is also known as decimal reduction time.
→ It determines the time required to reduce the microbial population by one decimal point.
→ It is the time required for 90% reduction in microbial population.

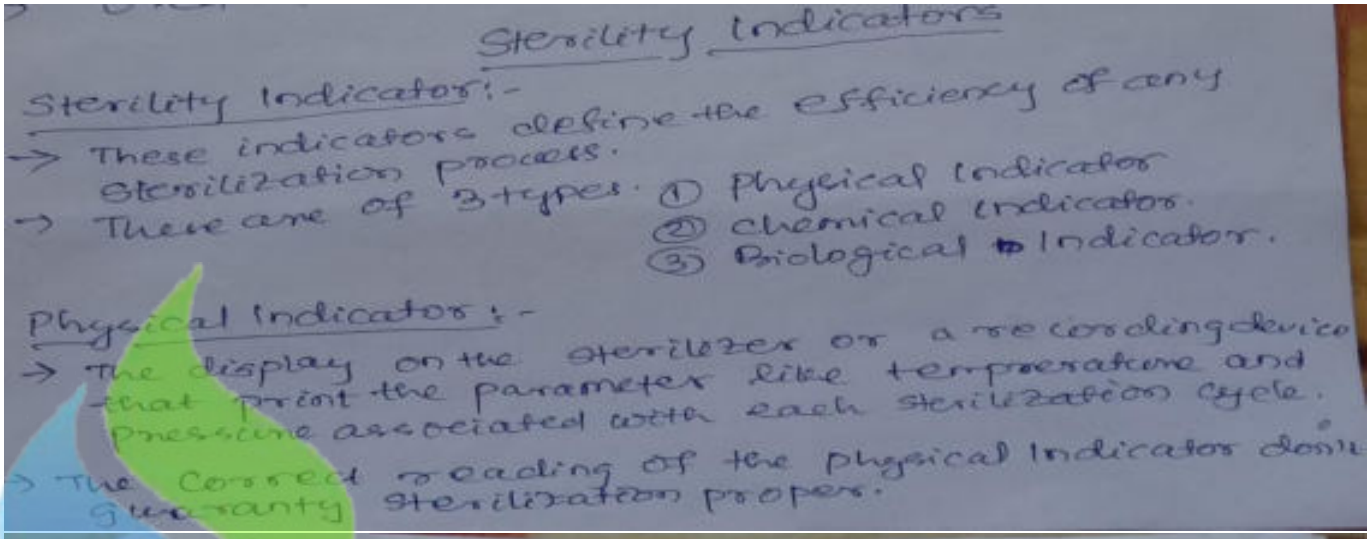


Importance:-
-Helps in determination of Z and F-value

③ Z-value
→ This value is defined as the no. of degrees of temp° required to produce one log change in D-value.
→ This relates the heat resistance of a microbe to change in temperature.
→ Bacterial spores have Z value 10-15°C
Non-sporing bacteria have Z value 4-6°C



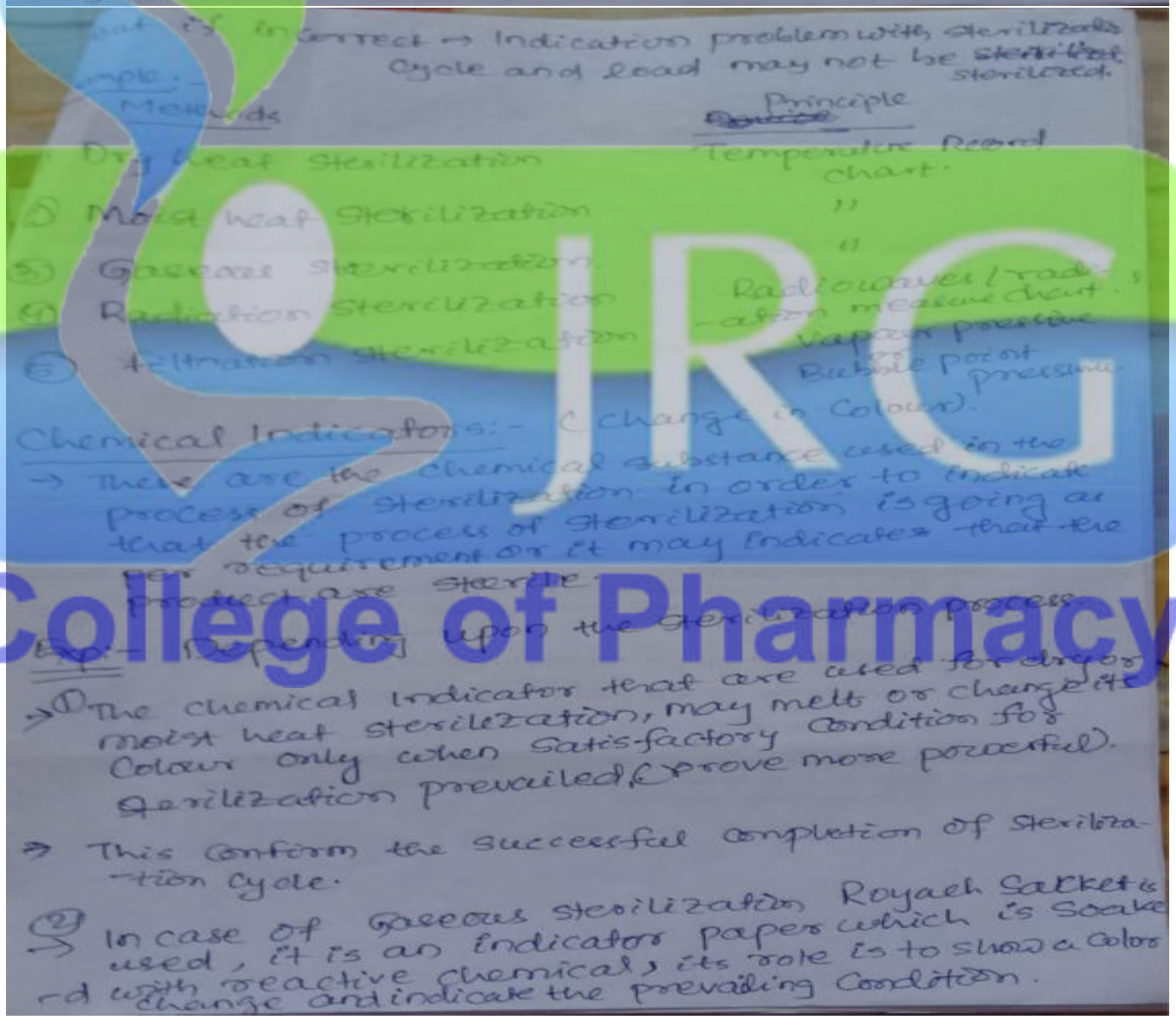
Q2. Write a notes on Sterility indicators.
Ans.



Sterility Indicators

Sterility Indicator:-
→ These indicators define the efficiency of any sterilization process.
→ There are of 3 types. ① Physical Indicator
② Chemical Indicator.
③ Biological Indicator.

Physical Indicator:-
→ The display on the sterilizer or a recording device that print the parameter like temperature and pressure associated with each sterilization cycle.
→ The correct reading of the physical Indicator does not guaranty sterilization proper.



→ Indication problem with sterilizer cycle and load may not be sterilized.

Principle
Temperature Record chart.
"
"
Radiowave / radiation measure chart.
Vapour pressure
Bubble point pressure
change in Colour).

Chemical Indicators:-
→ These are the chemical substance used in the process of sterilization in order to indicate that the process of sterilization is going as per requirement or it may indicates that the product are sterile.

→ The chemical indicator that are used for dry heat sterilization, moist heat sterilization, may melt or change its Colour only when satisfactory condition for sterilization prevailed & prove more powerful).

→ This conform the successful completion of sterilization cycle.

② In case of gaseous sterilization Royach Sarkar is used, it is an indicator paper which is soaked with reactive chemical, its role is to show a color change and indicate the prevailing condition.

Biological Indicator :-

Here we use standardized Bacterial spore used in the form of suspension in water or Culture media or its spore dried on paper or Plastic and kept in sterilizer.

→ The Bacterial spore are selected as per the process being used for sterilization like spore of Bacillus subtilis is used as biological indicator in dry heat sterilization process to determine D-value.

→ And Bacillus thermophilus (used as an indicator) in moist heat sterilization.

Method	Principle	Microorganism	Parameters
① Dry heat ster.	Temp. sensitive microbes	Bacillus subtilis	D-value.
② Gaseous ster.	"	"	D-value.
③ Moist heat ster.	"	Bacillus Stearothermophilus	D-value
④ Radiation ster.	Radiation sensitive microbes	Bacillus [illegible]	D-value
⑤ Filtration ster.	Retention of microbes	Pycnogonella dimineta	Size of microorganism

Q3. What is Tyndallization and Pasteurization.

Ans.

Tyndallization

Tyndallization is a process from the nineteenth century for sterilizing substances, usually food, named after its inventor John Tyndall, that can be used to kill heat-resistant endospores. Although now considered dated, it is still occasionally used.

- A simple and effective sterilizing method commonly used today is autoclaving: heating the substance being sterilized to 121 °C (250 °F) for 15 minutes in a pressured system.
- If autoclaving is not possible because of lack of equipment, or the need to sterilize something that will not withstand the higher temperature, unpressurized heating for a prolonged period at a temperature of up to 100 °C (212 °F), the boiling point of water, may be used.
- The heat will kill any bacterial cells; however, bacterial spores capable of later germinating into bacterial cells may survive. Tyndallization can be used to destroy the spores.

Pasteurization

In food processing, Pasteurization (also Pasteurization) is a process of food preservation in which packaged foods (e.g., milk and fruit juices) are treated with mild heat, usually to less than 100 °C (212 °F), to eliminate pathogens and extend shelf life. Pasteurization either destroys or deactivates microorganisms and enzymes that contribute to food spoilage or the risk of disease, including vegetative bacteria, but most bacterial spores survive the process.

Pasteurization is named after the French microbiologist Louis Pasteur, whose research in the 1860s demonstrated that thermal processing would deactivate unwanted microorganisms in wine. Spoilage enzymes are also inactivated during pasteurization. Today, pasteurization is used widely in the dairy industry and other food processing industries for food preservation and food safety.

By the year 1999, most liquid products were heat treated in a continuous system where heat was applied using a heat exchanger or the direct or indirect use of hot water and steam. Due to the mild heat, there are minor changes to the nutritional quality and sensory characteristics of the treated foods. Pascalization or high-pressure processing (HPP) and pulsed electric field (PEF) are non-thermal processes that are also used to pasteurize foods.

Efficacy against pathogenic bacteria

During the early 20th century, there was no robust knowledge of what time and temperature combinations would inactivate pathogenic bacteria in milk, so several different pasteurization standards were in use. By 1943, both HTST pasteurization conditions of 72 °C (162 °F) for 15 seconds, as well as batch pasteurization conditions of 63 °C (145 °F) for 30 minutes, were confirmed by studies of the complete thermal death (as best as could be measured at that time) for a range of pathogenic bacteria in milk.

Model Questions & Answers
B. Pharmacy, 3rd Semester
Sub: Ph. Microbiology, Sub. Code: BP303T
Unit-V

Short Answer Type Questions **(05 marks)**

Q1. Describe the sources and assessment of microbial contamination.

Ans.

Sources and Types of Contaminants

Contaminants can get entry into a production process. From several sources such as -

→ Raw material and ~~Semi finished material~~

→ ~~Packaging material~~

→ ~~Utilities~~.

① Atmosphere.
② Water.
③ Raw material.
④ Process operators.
⑤ Equipment.
⑥ Packaging.
⑦ Buildings.

Atmosphere :-

→ The no. of microorganisms in the atmosphere depends on the activity in the environment and the amount of dust which is present.

→ Microorganisms are carried into the atmosphere suspended on particles of dust, skin, clothing, droplets of moisture, sputum from coughing and sneezing

E.g. → Microorganisms like bacteria and fungus like-
Bacillus sps.
Streptococcus sps.
Penicillium sps.

(2) Water:-
→ Water is one of the main constituents of many products and it is used for washing and cooling process.
→ Different types of microorganisms are present in fresh water and some microbes may contaminate the water for sewage
E.g. → E.Coli, Clostridium spp.

(3) Raw materials:-
→ These are potentially rich source of microorganisms.
→ Synthetic raw materials are usually free from microbial contamination may occur

→ Raw materials mainly contain bacteria and fungi e.g. Bacillus spp., Salmonella spp etc. produced from animal sources such as gelatin, pancreas etc. may be contaminated with different pathogens.

(4) Process operators:-
→ Microorganisms may be transferred to pharmaceutical formulations from process operators as a result of poor personal hygiene.
E.g.:→ Sarcina sps.
Staphylococcus sps.

(5) Equipments:-
→ It should be simple as possible for the purposes required with minimum of junctions, valves and pumps to allow cleaning in place by circulation of detergents or other chemical

antimicrobial agents. The type and growth depend on source of the Contamination, environmental Conditions and nutrients.

(6) Packaging materials:-

→ Packaging material have a dual role and act both to contain the product and to prevent the entry of microorganisms or moisture

(7) Buildings:-

Different molds and a few bacterial species are the most common flora of ceilings and walls. To reduce microbial growth, all walls, floors and ceilings.

Assessment of microbial Contamination and spoilage:-

1. Physical and chemical changes.
2. Sterility Test.
3. Assessment of viable microorganisms in non-sterile products.
4. Estimation of Pathogens.

1. Physical and chemical changes:-

→ Physical and chemical changes of different pharmaceutical formulations indicate microbial spoilage.

→ Measurement of oxygen consumption of the product can indicate the microbial growth.

2. Sterility Testing:-

→ It is important that materials which are to be tested for sterility are not subject to contamination from the operators

during the course of test for its it should be Conducted by experienced personnel in an adequately cleanroom with laminar air flow cabinet.

③ Assessment of viable microorganism in non sterile products:-

→ This involves the testing for both total number of microbial cell and viable cells and the presence of specific objectionable pathogens using established protocol like -

→ Microbial Limit Test (MLT)

→ Colony forming Unit (CFU)

④ Detection of Pyrogen:-

What is Pyrogen:-

→ These are the microbial metabolic byproducts and specifically endotoxins produced by bacteria

yeasts, fungus and viruses. These are Lipopolysaccharides (LPS) in nature.

→ When they get enters into the body suddenly elevate the temperature causing Fever (Pyrexia).

(I) Exogenous Pyrogens.
(II) Endogenous Pyrogens.

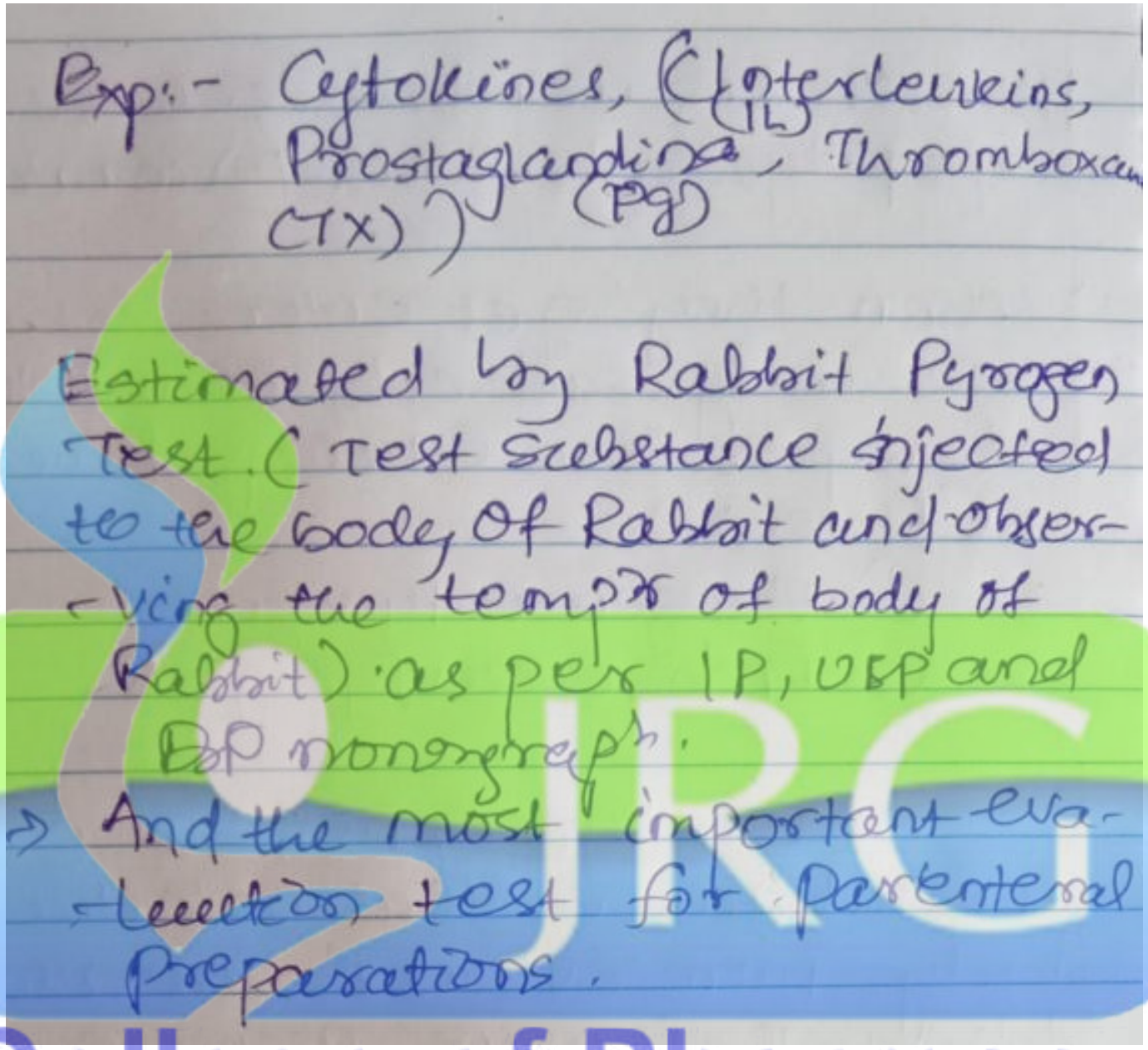
Exogenous Pyrogens:- These are the substances that enter the body from external sources and triggers the immune response. (Antigen-antibody Reaction).

Microbial — Non-microbial - Plastics, rubber and metal components.

Exp:- Endotoxins lipopolysaccharides.

Endogenous Pyrogens:-

→ These are the substances produced by the body's own immune cells in response to Exogenous Pyrogens.



Exp:- Cytokines, (Interleukins (IL), Prostaglandins (Pg), Thromboxane (TX))

Estimated by Rabbit Pyrogen Test. (Test substance injected to the body of Rabbit and observing the temp of body of Rabbit) as per IP, USP and BP monographs.

⇒ And the most important evaluation test for parenteral preparations.

Q1. Describe about types of Pharmaceutical spoilage.
Ans.

Pharmaceutical Spoilages

Definition

→ Pharmaceutical products are considered microbiologically spoiled when even low levels of pathogenic microbes or toxic microbial metabolites are present and physical or chemical change have been noted in the product.

→ These microbes not only spoil the pharmaceuticals but also foods.

→ These spoilage of pharmaceutical products can result in serious health hazards.

Types of spoilages:- (Microbial Contamination)

① Infection induced due to contaminated Pharmaceutical product.

→ Pharmaceutical products may get infected from many sources like raw materials, manufacturing and storage etc.

→ such infected or contaminated products

Common Pathogens Contaminating / Spoiling Pharmaceuticals

Dosage form	Contaminants or spoiling microbes	Outcome
(1) Tablets/Capsules	Salmonella species (Non typhoidal species) → Salmonellosis	Food poisoning
	Salmonella ser. servovars (Bacteria)	
(2) Antiseptic Solutions	Pseudomonas species (Bacteria)	Septi-caemia (Blood infection)
(3) Eye Products	Pseudomonas aeruginosa (Bacteria)	Eye infections
(4) Ointments and Creams	Gram (+ve) microbes	Dermal infections (Skin infection)

(5) IV medicines Candida Septi-baemia species (fungus)

(B) (2) Physicochemical spoilages:-

→ There are following types of Physico-chemical spoilages -

1. Viable growth.
2. Gas Production
3. Decolorization
4. Odour formation
5. Taste change.

Viable growth

→ The growth of microbes may occurs inside the containers. These growths may be visible in form of turbidity.

Exp:- Contaminants like fungus i.e Asperigilles sps. cause this type of spoilage.

Gas production:-

→ Some microbes may produce gas inside the Containers. There gases may be visible in the form of bubbles, flocculates etc.

Exp:- Contaminants like baeteria E.Coli may produce gas if the product contains sugars as ingredients.

Decolouration:-

Some microbes may decolorize formulation or it may produce unique colour which is different from the normal colour of the product.

Exp:- Contaminants like fungus penicillium and Aspergillus may decolorize the contents in the container.

Odour formation:-

→ Some microbial growth in the finished product may produce bad odour or foul odours or rotten smell.

Exp:- Contaminants like E.coli, and Bacillus sps. bacteria and like Aspergillus and Penicillium fungus.

Taste change:-

→ Microbial spoilage may change the taste of oral formulation, and produce bitter or obnoxious taste (extremely unpleasant taste)

Exp:- Pseudomonas, Bacillus (Bacteria)
Candida (yeast).

③ Physical Spoilages:-

→ These changes in physical appearance are caused by microbial activities in the formulation.

Example:-

① Cracking of Emulsion:-

→ Microbes eat off the emulsifying agent causing instability of emulsions resulting separation of phases. (Microbes Exp:- Micro:- -coccus sps., Bacillus sps. bacteria)

② Odour changes:-

- Microbes due to their activity in the pharmaceutical product produce bad smell.

Example:- Toluene smells due to spoilage of Balsam of Tolu by Penicillium sps. (Fungus)

④ Biological spoilages:-

→ Microbial contamination of the products result in formation of ~~chemicals~~ due to their metabolic activity, called biological spoilages.

→ mainly microbial ~~toxins~~ chemicals are two types ① Microbial metabolites ② Microbial toxins.

Microbial metabolites:-
Different types of amines and organic acids.

Microbial toxins:- Endotoxins produced by some Gram-ve bacteria like - E. Coli.

⑤ Chemical Spoilage:-
→ This occurs due to various types of chemical reactions, mediated by contaminating microorganisms.

Ingredients	Microorganism	Action
① Active ingredients		
1. Atropin	Pseudomonas	Hydrolysis
2. Aspirin	Clostridium/Bacillus	Hydrolysis
② Diluents		
① Starch	Bacterial amylase	Depolymerization
② Cellulose	Bacterial cellulase	Depolymerization

Long Question-Describe in detail about microbiological assay of Antibiotics

Microbiological Assay -

A microbiological assay is a biological method used to measure the potency (strength), concentration or activity of a compound (usually an Antibiotic, Vitamin or Amino Acid) by observing its effect on the growth of specific microorganisms.

- Instead of using chemical analysis, this method relies on the response of living microbes to the substance being tested.

Applications -

* Standardization of Antibiotics
* Determination of Vitamins
* Testing Amino Acids or Growth Factors.

Assay of Antibiotics -

- The microbiological assay of Antibiotics is a bioassay method that estimates the potency or concentration of an antibiotic sample by measuring its inhibitory effect on the growth of suitable microorganism under standardized condition.

- Unlike chemical methods, microbiological assay measure biological activity which is directly related to therapeutic effect.
- There are two methods which are generally employed for the Assay.

(1) Cylinder Plate or Cup Plate Method
(2) Turbidimetric or Tube Assay Method

Preparation for Antimicrobial Assay-

→ Preparation of Culture Media - Mullere Hinton Agar (MHA)
→ Selection of Test Microorganism - Bacillus pumilus, Staphylococcus epidermidis.
→ Preparation of Test & Standard Solutions of Antibiotic.

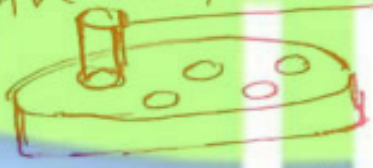
(1) Cylinder Plate or Cup Plate Method-

- This method depends upon diffusion of an Antibiotic from a vertical cavity or cylinder through a solidified agare layer in petri Plates.
- The growth of test microorganism is inhibited entirely in a circular area or zone around the cavity containing a solution of Antibiotic.

- Three layered plates are prepared for this method.

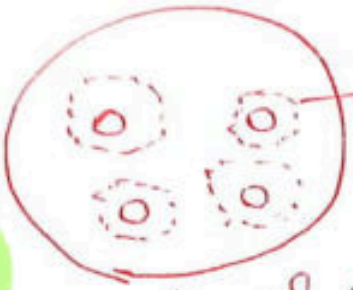
* First & Third Layers is prepared of Agar Medium (Without Microorganism).
* Second Layer (With Microorganism) (Thickness-4 mm)

- Now holes (cavities) are made in the Agar media by using cork borer and antibiotic solutions are filled in those holes with the help of micropipettes.

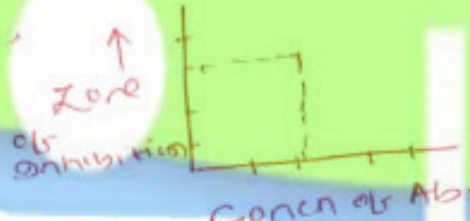


- Solutions of known conch of standard and test Antibiotic are prepared in appropriate manner (According to IP).
- Then these solutions are added into Sterile cavities in the Agar medium separately (separate For Test & Standard)
- These plates are left standing For 1 to 2 hours at room temperature.
- All plates are then incubated For about 18-24 hours.

- The diameter/Areas of circular inhibition zones produced by standard & Test Antibiotics solution are accurately measured.



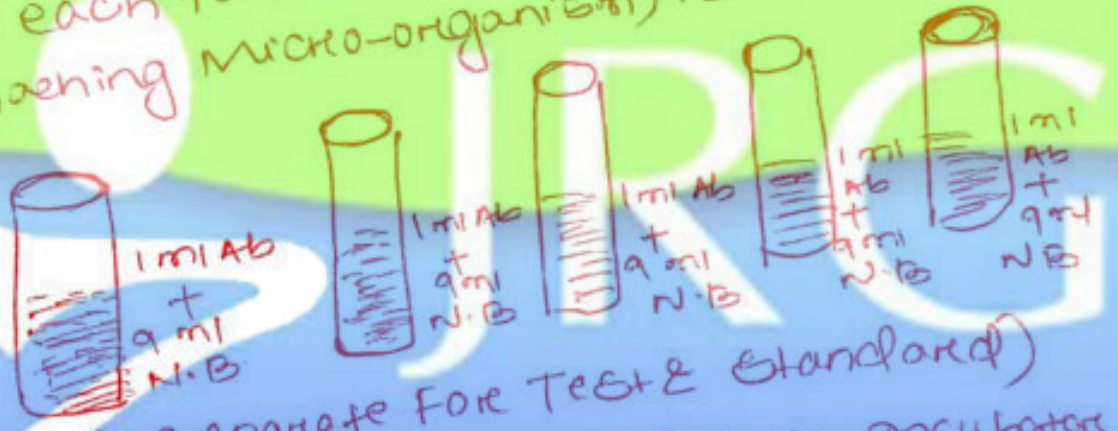
- Then a standard curve representating conch of Antibiotic in the X-axis and diameter of Zone of inhibition in Y-axis is plotted.



(2) Turbidimetric or Tube Assay Method-

- Here a liquid medium is inoculated with the test microorganism and different conch of test & standard antibiotics are added to it.
- Then the growth of the microorganism is inhibited in proportion to Antibiotic is measured spectrophotometrically in the form of optical density.

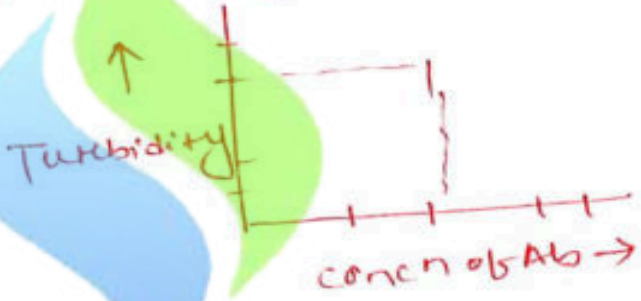
- First a nutrient broth medium is inoculated with a standardized microbial suspension.
- Then 5 different concentration of Standard Antibiotic solution & Test Antibiotic solutions are made
- Now one ml of each concentration of Test antibiotic and Standard Antibiotic solutions are added into different test tube.
- To each tube 9 ml of Nutrient Medium (containing Micro-organism) is added.



(Separate For Test & Standard)

- All the tubes are placed in the incubator at 37°C For 3-4 hours or 18-24 hrs.
- Now after the incubation measure the growth of bacteria in the form of turbidity Spectrophotometrically.

- Then compare the turbidity of Test Antebiotic in comparison to Standard
- Now make a Standard curve relating Concn of Antebiotic at the X-axis & Turbidity observed on Y-axis



Model Questions & Answers
B. Pharmacy, 3rd Semester
Sub: Pharmaceutical Microbiology, Sub. Code: BP303T
Unit-V

Short Answer Type Questions **(05 marks)**

Q1. Write the evaluation of microbial stability of pharmaceutical formulations.

Ans.

- Microbial stability of a formulation is dependent on effectiveness of its preservative.
- Chemical assay and biological assay may assure effectiveness of preservative but it may lose its activity
- due to presence of other ingredients in the formulation.
- Some formulations do not require preservative because they act as self-preservative.
- Some formulation does not require preservative because it contains antimicrobial agents like antibiotics as ingredient.
- Some formulation may contain high sugar concentration, salt concentration and may act as self-preservative.
- So, the ability of the formulation to protect itself from microbial growth must ascertain to determine microbial stability of the formulation. It is done by preservative efficacy test.
- Basic principle of this test is to inoculate products with different types of specified microorganism with specific quantity. Little amount of inoculated product is removed at a specific interval.
- Then viable count of this withdrawn sample is determined. United States Pharmacopoeia, European Pharmacopoeia etc. recommends this type of tests.
- The concentration of the test organism should be 10^5-10^6 cells per ml or gm.
- Total microbial count is performed in 0 hr., 6 hrs., 24 hrs., 48 hrs. 7 days, 14 days and 28 days. British
- pharmacopeia recommends test even after 28 days.
- Different bacterial species are used for this purpose. They are *Staphylococcus aureus*,
- *Pseudomonas aeruginosa* and E. coli.
- Different fungus species are also used. They are *Candida albicans*, *Aspergillus niger* etc.
- This test allows to add designated micro-organisms if required. After withdrawal of sample and before viable count, sample is mixed with chemicals which can deactivate preservative because presence of even very minute quantity of preservative may hamper microbial viable count.

Q1. Define and classify Preservatives with suitable examples. Write the ideal characteristics of preservatives.

Ans.

The main function of antimicrobial preservative is to prevent the growth of unwanted microorganisms in pharmaceutical preparations. Preservatives are widely employed in pharmaceutical, cosmetics and nutraceuticals preparations for better stability.

Antimicrobial preservatives can be classified into four major groups

1. Acidic preservatives - Exp. Benzoic acid, Acetic acid, Citric acid
2. Neutral preservatives- Exp. Parabens, Phenoxyethanol, Potassium sorbate.
3. Mercurial preservatives- Exp. Thiomersal, Phenyl mercuric acetate and Phenyl mercuric nitrate.
4. Quaternary ammonium salts- Exp. Benzalkonium chloride, Cetyltrimethyl ammonium bromide.

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Preservatives used in preparation of pharmaceutical formulations:
Tablets – Methyl paraben (0.1%)
Liquids- Bronopol (0.02%)
Methyl paraben (0.1%)
Chlorocresol (0.1%)
Injections- Benzyl alcohol (1-2%)
Thiomersal (0.01%)
Eye drops- Benzalkonium chloride (0.01%)
Phenyl mercuric nitrate (0.002%)
Semisolids- Cetyltrimethyl ammonium bromide (0.05-0.1%)

Ideal characteristics of preservatives:

- Preservatives should be able to kill the microbial contaminants rapidly not to be irritant or toxic to the patient.
- It should be stable and effective throughout the life of the medicine.
- Combination of two or more preservatives are used to extend the range and spectrum of preservation.

Long Answer Type Questions **(10 marks)**

Q1. What is cell culture and mention its importance? Explain in details about the general procedure for cell culture.

Ans.

Cell culture or tissue culture is the process by which cells are grown under controlled conditions, generally outside of their natural environment. After cells of interest have been isolated from living tissue, they can subsequently be maintained under carefully controlled conditions. They need to be kept at body temperature (37 °C) in an incubator.

Types:
Cell culture is classified into three types on the basis of origin, chromosomal characters and the no. of generations through which they can be maintained.

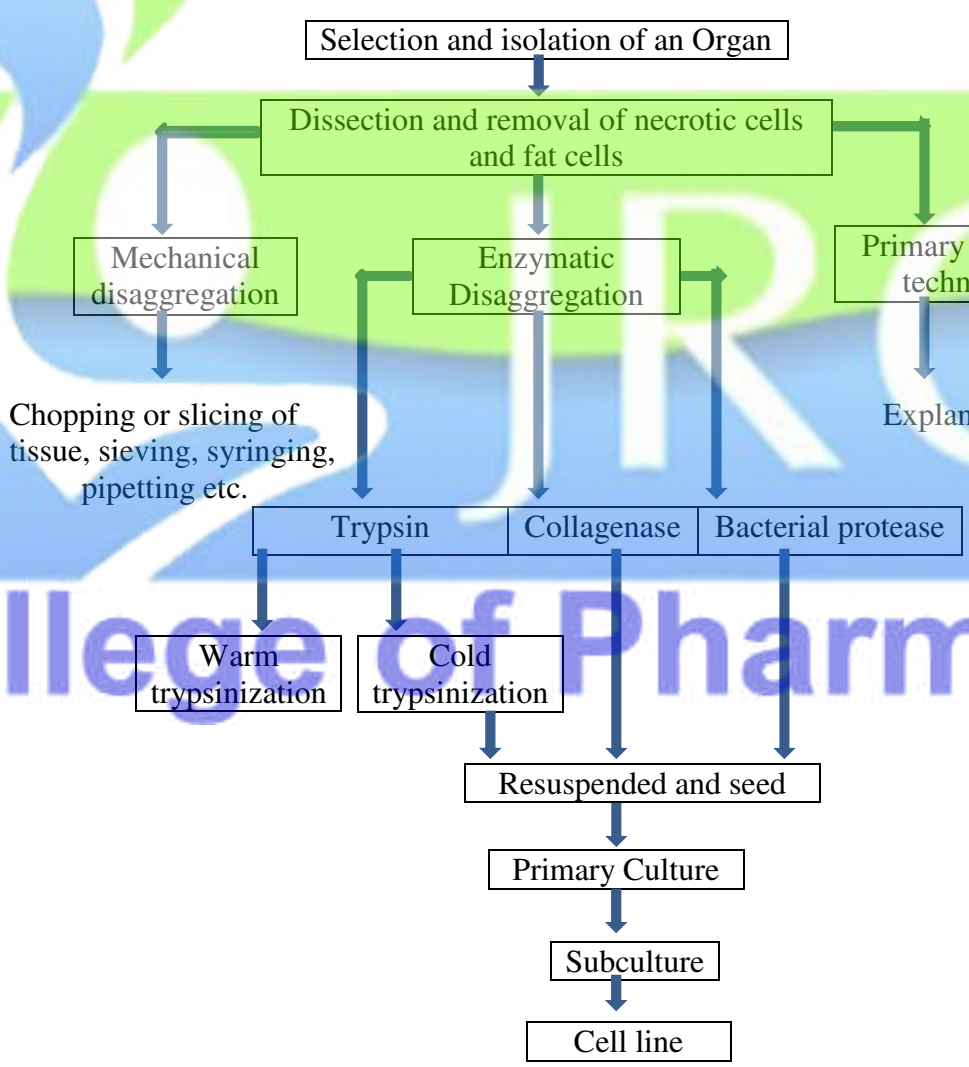
- Primary cell culture
- Diploid cell strain
- Continuous cell line

History:
The 19th-century English physiologist Sydney Ringer developed salt solutions containing the chlorides of sodium, potassium, calcium and magnesium suitable for maintaining the beating of an isolated animal heart outside the body.
In 1907 the zoologist Ross Granville Harrison demonstrated the growth of frog embryonic cells that would give rise to nerve cells in a medium of clotted lymph while working at Johns Hopkins Medical School and Yale University.

Requirements:

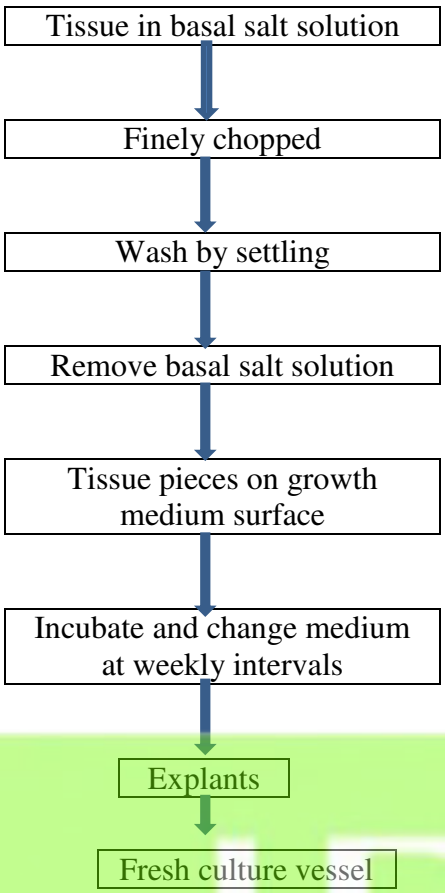
Components	**Functions**
Carbon source (glucose/glutamine)	Source of energy
Amino acid	Building blocks of protein
Vitamins	Promote cell survival and growth
Balanced salt solution	An isotonic mixture of ions to maintain optimum osmotic pressure within the cells and provide essential metal ions to act as cofactors for enzymatic reactions, cell adhesion etc.
Phenol red dye	pH indicator. The color of phenol red changes from orange/red at pH 7–7.4 to yellow at acidic (lower) pH and purple at basic (higher) pH.
Bicarbonate /HEPES buffer	It is used to maintain a balanced pH in the media.
Temperature	Should be at 37^0C.

General Procedure of cell culture:



Mr. Biswajit Biswal

Primary explant technique



Importance of cell culture:

- Provide model system for basic cell biology and biochemistry.
- Toxicity testing.
- Research in cancer biology.
- Virology
- Cell based manufacturing.
- Genetic engineered protein
- Vaccine production
- Genetic counselling
- Replacement tissues or organ
- Gene therapy
- Drug screening and developments