Unit-1

Pharmaceutical Microbiology

Unit I 10 Hours

- Introduction, history of microbiology, its branches, scope and its importance.
- Introduction to Prokaryotes and Eukaryotes
- Study of ultra-structure and morphological classification of bacteria, nutritional requirements, raw materials used for culture media and physical parameters for growth, growth curve, isolation and preservation methods for pure cultures, cultivation of anaerobes, quantitative measurement of bacterial growth (total & viable count).
- Study of different types of phase constrast microscopy, dark field microscopy and electron microscopy.

Introduction of Microbiology:

- Microbiology is the scientific study of microorganisms, which are living organisms that are too small to be seen with the naked eye.
- This includes bacteria, archaea, viruses, fungi, prions, protozoa, and algae. Microorganisms are found in all environments on Earth, including the air, water, soil, and inside all living things.
- The term of microbiology is derived from 3 word-

Micro: Small

Bio: Living

Logy: Study

These micro-organism may be unicellular or multicellular and micro-organism like- spherical, cylindrical, and rod shaped and comma shaped.

Some microorganism is responsible for different-2 disease in human body.

History of Microbiology:

- 1st time **'Aristotle'** gave the concept of living and non-living organism.
- In 13th century **'Roger Bacon'** gave the term disease and it is caused by micro-organism.
- Fracastorius in 1546 gave the concept of communicable disease which affect the healthy person when they comes in contact with infected person.
- In 1st concept of living micro-organism was given by the **Antonie-Van-Leeuwenhoek** in 1675, so he is known as **Father of Microbiology**.
- He gave the term animal clues for protozoa and bacteria.
- In 1729 **Spallanzani** prepare the 1st culture media in which bacteria and virous can be grow.
- Scientist John Tyndall say that the micro-organism are kills at high temperature this is called Tyndall effect.
- The term microbiology was given by **Lewis Pasture** and he is also known as **father of microbiology.**
- **Louis pasteur** gave the term aerobic and Aerobic bacteria.
- **Louis pasteur** explain that when milk is heated at 62.8 for 30 minutes then all microbes are killing and milk becomes pure this process is known as pasteurisation.
- Lord joseph lister gave the concept of use of antiseptic before surgery.
- He is also known as father of antiseptic surgery.
- **Alexender flaming discover** the 1st antibiotic penicillin from fungus penicillium notatum which was isolated from tobacco leaf.

Branches of Microbiology:

The major branches of microbiology include:

- **Bacteriology:** The study of bacteria
- **Virology:** The study of viruses
- **Mycology:** The study of fungi
- **Parasitology:** The study of parasites
- **Immunology:** The study of the immune system
- **Environmental microbiology:** The study of microorganisms in the environment
- **Food microbiology:** The study of microorganisms in food
- **Industrial microbiology:** The use of microorganisms in industry

Scope and its Importance:

1. Production of Antibiotic:

Antibiotic are capable of inhibiting growth of micro-organism.

Antibiotic are mainly 2 type-

- Bacteriostatic [Inhibit the growth of microorganism]
- Bactericidal [Kill the bacteria]

2. Production of enzyme vaccine and alcohol:

- List of micro-organism which are helping in the production of enzyme, vaccine, and alcohol.

3. Use the production of dairy product:

- Dairy product such as manufactured bacterial activity.
  - Cheese- Lactobacillus lactis
  - Yogurt – lactobacillus bulgaricus.

4. Industrial Microbiology:

- Using microorganism to make product such as antibiotics, vaccines, steroids, alcohols and other solvents, vitamins, amino acids, enzymes etc.

5. Genetic Engineering:

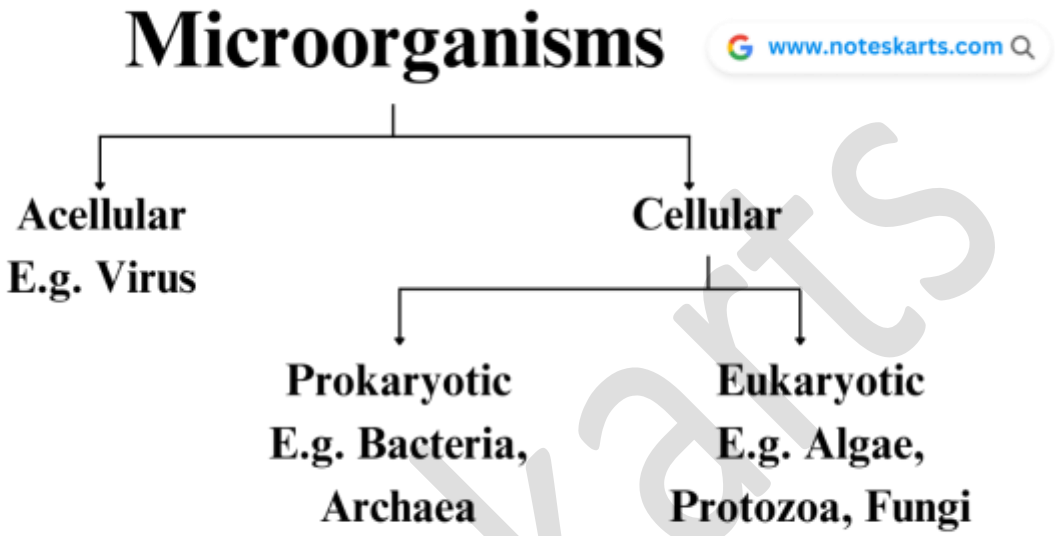
- Engineered microorganisms used to make hormones, antibiotic, vaccines and other products.

6. Cosmetic and perfumes:

- Some species use for making perfume and soap like romatina.

Classification of Microbiology:

On the basis of cellular level microbiology can be classified as following-



Acellular:

- In this type of micro-organism single poorly developed cell is present in which cell membrane and nucleus is absent.

Eg: virus

Cellular:

- This is the complete cell structure in which the cell membrane and nucleus is developed it further can be divided into 2 types-

a. **Prokaryatic Cell:** Cell membrane is developed but cell organellis and nucleus is absent.
Eg. Bacteria, Archaea.

b. **Eukaryotic cell:** These cells are well developed and they have well developed nucleus and cell arganelis.
Eg. Fungi, Protozoa, Alagae,

Archaea:

- Archaea are similar to bacteria, but they are more closely related to eukaryotes.
- They are found in extreme environments, such as hot springs and salt lakes.

Fungi:

- Fungi are eukaryotic organisms that are neither plants nor animals.
- They play an important role in the decomposition of organic matter and the recycling of nutrients.

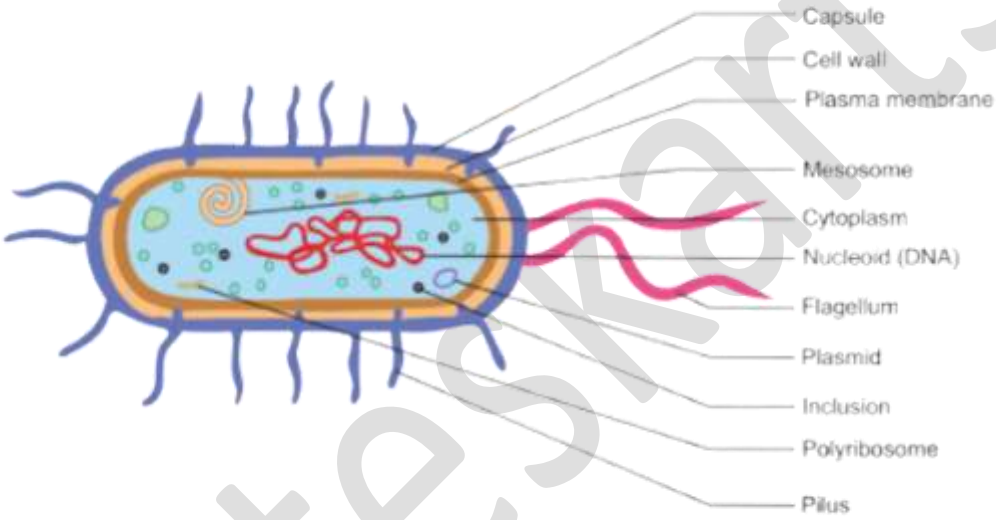
Protozoa:

- Protozoa are single-celled eukaryotic organisms that can move.
- They can be found in a variety of habitats, including water, soil, and the bodies of animals.

Bacteria:

"Bacteria are the unicellular organisms belonging to the prokaryotic group, where the organisms lack several organelles and a true nucleus."

Structure of Bacteria:



Capsule:

- It is the outer most thick and slippery structure and it is rigid and flexible.
- The composition of capsule is about 98% water and 2% glycoprotein.
- This glycoprotein is different type in different bacteria like homopolysaccharide, Hemipoly saccharide and hetropolysaccaride.

On the basis of thickness capsule is of 2 types:

Macro capsule- thickness more than 0.2µ

Micro capsule- Thickness less than 0.2 µ

Cell Wall:

- It is also thick structure made up with peptidoglycan byers.
- We can identify bacteria as gram(+) or Gram (-) on the basis of cell wall.
- Gram(+): 20-80nm thickness of peptidoglycan

- Gram(-) 7-8nm thickness of peptidoglycan.
- It provide protection shape and identification to the cell bacteria.

Cell Membranes:

- Cell membrane is are thin layer in prokaryotic cell or bacteria it is composed of 20-30% phospholipid and 60-70% protein.

Flagella:

- It is long thick hair like structure.
- Which is surrounded by sheath.
- Its diameter is 20nm and length is 15-20 μm.

Cytoplasm:

- The cytoplasm is the jelly-like substance inside the cell. It contains the cell's organelles and other components.

Mesosome:

- Mesosome are present in the cell membrane of bacterial cell which helps in cellular respiration.

Ribosome:

- Ribosomes are the protein factories of the cell. They are responsible for making proteins.
- In bacteria 70S type of ribosome is present which helps in protein synthesise.

Pilus:

- It is small thin 8-10 hair like structure which helps in the attachment of bacteria with other bacteria and transfer in genetic material.

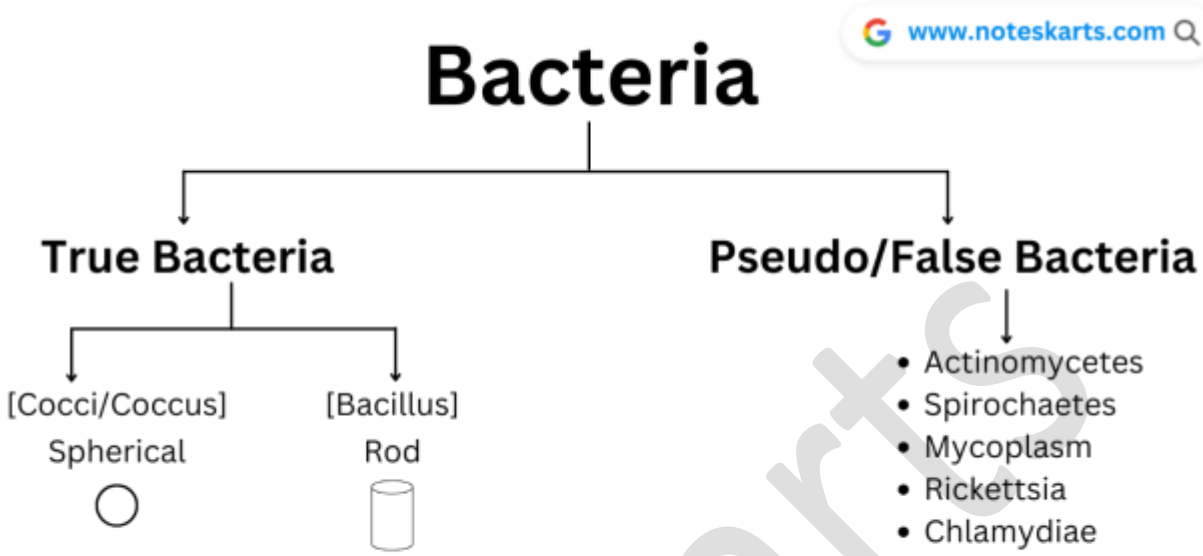
Nucleoid:

- The less developed nucleus without nucleoplasm and nuclear membrane is called nucleoid.
- In the nucleoid of bacteria about 60% DNA 30% RNA and 10% protein is present.

Morphological Classification of bacteria:

According to external appearance:

- On the basic of structure, shape, size and appearance bacteria can be classified into 2 categories



True bacteria:

They are the group of bacteria which shows the real characteristics of bacteria.

It is 2 types-

1. Coccus Bacteria: It is spherical in shape-

a. Monococcus: In which cocci is in single form.
b. Diplococcus: In which cocci is in pair
c. Tetracoccus: In which cocci is in group of four.
d. Streptococcus: cocci is in chain form
e. Staphyllococcus:

2. Bacillus:

a. Monobacillus
b. Diplobacillus
c. Tetradbacillus
d. Strepto bacillus
e. Sarcino bacillus
f. Staphyllo bacillus

False/ Pseudo Bacteria:

These are those bacteria which are actually a bacteria but their shape is different from bacteria.

i. **Acetinomycetes:**
- They are rigid bacteria like a fungus.
- They are filoments in shape and branching is present.
- They are gram +ve in nature.
- They are mostly present in soil.

Eg. Mycobacteriaceae.

ii. **Spirochates-**
- They are long chain structure non branched and consist of double membrane.
- They are chemohetrophic in nature and gram negative bacteria.
- They length of this bacteria is between 3 to 500 μm.

Eg. Leptospirainterrogans.

iii. **Mycoplasma:**
- They are smallest bacteria do not have rigid cell membrane.
- They looks like virus and pathogenic in nature.

Eg. Mycoplasma-pneumoniae, Mycoplasma-genitalium

iv. **Rickettsia:**
- They are non mortile gram-ve
- These are look like as filaments in which no branched and no chain present.
- Its diameter is 0.1-04 μm.

v. **Chlamydiae:**
- It is oval shape.
- Peptidoglycan protein are present in it.
- It is mostly responsible for the disease in human eye.

Culture media:

- Culture media are mediums that provide essential nutrients and minerals to support the growth of microorganisms in the laboratory.
- Microorganisms have varying nature, characteristics, habitat, and even nutritional requirements, thus it is impossible to culture them with one type of culture media. However, there are also microorganisms that can't grow on a culture media at all in any condition – these are called obligate parasites.
- Culturing microorganisms is essential for diagnosing infectious diseases, obtaining antigens, developing serological assays for vaccines, genetic studies, and identification of microbial species.

a. Types of culture media based on consistency/ physical state

- **Solid medium**
- **Semi-solid medium**
- **Liquid medium**

b. Types of culture media based on chemical composition/application

There are seven routine laboratory media.

- Basal media
- Enriched media
- Selective media
- Enrichment media
- Indicator media or differential media
- Transport media
- Storage media

c. Types of culture media based on oxygen requirement

- Aerobic media
- Anaerobic media

d. Types of Special purpose culture media

- Assay media
- Minimal media
- Fermrntation media

a. Types of culture media based on consistency/ physical state

1. **Solid Medium:**
   - It is for the isolation of bacteria as a pure culture on a solid medium.
   - Robert Koch realized the use of solid media.
   - Agar is used to hardening the media at 1.5- 2.0% concentration. Solid media allows the growth of bacteria as colonies by streaking on the medium. It solidified at 37 degrees Celsius.
   - Agar is an un-branched polysaccharide extracted from red algae species like Gelidium. Colonies identification is done on this medium.

   Ex: Blood agar, Chocolate agar.

2. **Semi-solid media:**
   This media shows the motility of bacteria and the cultivation of microaerophilic bacteria. This media has agar at a concentration of 0.5% or less. It has a jelly consistency.

Eg: Amies media

3. **Liquid Media:**
   - This media shows the growth of a large number of bacteria.
   - It is called Broth that allows bacteria to grow uniformly with turbidity. The growth occurs at 37ºC in an incubator for 24hrs.
   - Liquid media don't have the addition of agar; it is for fermentation studies.

   Eg: Nutrient broth

b. Types of culture media based on chemical composition/application

1. Basal media:

- This media is simple as it enhances the growth of many microorganisms. It's a routinely used medium in the lab, having Carbon and Nitrogen.
- This media allows the growth; of non- fastidious bacteria without any enrichment source; used for sub-culturing.
- It's a non-selective medium.
- ***Staphylococcus*** **and** ***Enterobacteriaceae*** grow in this media.

  Ex: Nutrient Agar

2. Enriched media

- This media requires the addition of other substances like blood, egg, or serum.
- An enriched media allows the growth of devised microorganisms but inhibits other and fastidious microbes grow as they require nutrients like vitamins and growth-promoting substances.

  Eg: Blood Agar

3. Selective media

- This culture media is specific for any bacteria and only 1 type of bacteria can grow in this medium.

  Eg: Mannitol Agar

4. Enrichment media

- It is a liquid medium, which also permits the growth of desired bacteria at a low density.
- The media provides an environment and conditions as selective media and inhibits unwanted bacteria from growing.
- It is for the isolation of the soil and fecal microorganisms.

  Eg: Selenite F-broth

5. Indicator or differential media

- This media shows visible changes due to the presence of an indicator.
- It differentiates bacteria based on colony color growing on the same plate; biochemical characteristics show organism's growth with chemical indicators like neutral red, phenol red, methylene blue.

  Eg: Mannitol salt agar (mannitol fermentation shows yellow color colonies)

6. Transport media

- The media transport specimens after collection to control the overgrowth of organisms.
- For the cultivation, this media act as temporary storage.
- It also maintains the viability of pathogens in the specimen and prevents them from drying.
  Eg: Stuart's transport medium

7. Storage Media

- It maintains the longevity of bacterial culture.
  Examples are- cooked meat broth, NA egg saline.

Types of culture media based on oxygen requirement

Microorganisms have different requirements for growth depending on oxygen requirements.

1. Aerobic media

- In this media, it is easy to cultivate microbes, on solid media, the growth occurs by keeping the culture in the incubator.
- It shows the growth; of non-fastidious microorganisms.
  Examples of aerobic media are- liquid media, solid media
    - Peptone water- 1%peptone + 0.5% Nacl +100ml water.
    - Nutrient agar- nutrient broth +2% agar.

2. Anaerobic media

- The media cultivates anaerobic bacteria at low oxygen, reducing oxidation-reduction potential. Anaerobic media contains extra nutrients like vitamin K, hemin, and oxygen that get reduced by a physical or chemical process.
- The addition of glucose (1%), thioglycollate(0.1%), ascorbic acid (0.1%), cysteine (0.05%), or iron fillings added to cause the medium to reduce.
- The medium is boiled in a water bath to force out dissolved oxygen and packed with sterile paraffin.

Examples of Anaerobic media

- **RCM (Robertson cooked meat) isolation for *Clostridium* sp.**

Types of special purpose culture media

1. Assay media

- The media assay vitamins, amino acids, and antibiotics. Example- Antibiotic sensitivity test the media used is Muller-Hinton agar has 1.7% agar for better diffusion of antibiotics.
- It also contains starch, which absorbs toxins released by bacteria.
- In this media plate Zone of inhibition is seen around antibiotics.

2. Minimal media

- Minimal media is a defined medium with different compositions depending on microorganisms cultured. It contains a carbon source like sugar/succinate and inorganic salts like magnesium,

nitrogen, sulfur, phosphorus.

- Carbon is a source of energy; magnesium and ammonium salts are the sources of ions for metabolism stimulation. Phosphate is a buffering agent.
- The growth comparison of microbe culture and mutant forms- Minimal media and supplementary-minimal media- allow the differentiation of wild-type and mutant cells.

Use- The selection of recombinants, for the growth of wild-type microorganisms.

3. Fermentation media

- The media is for optimum microorganisms. Fermentation media produce high yields of the product; media provide energy and nutrients for growth, and medium gives the substrate for the synthesis of products in the fermentation.
- Fermentation media contains major and minor components-
  Major components – Carbon and nitrogen for energy.
  Minor components- This contains inorganic salts, growth factors, vitamins, buffer, anti-foaming agents, dissolved oxygen, gases, growth inhibitors, enzymes.
- The nutrients in fermentation media depend on the organism and type of fermentation process.

4. Resuscitation culture media

- The resuscitation method is for the stressed bacterial recovery; this is a specialized medium that allows the growth of microbes that have lost the ability to produce because of the environmental harness.
- The culture provides nutrients and recovers their metabolism.

For example- Tryptic Soy Agar.

Application of culture media

- To culture microbes.
- To identify the cause of infection.
- To identify characteristics of microorganisms.
- To isolate pure culture.
- To store the culture stock.
- To observe biochemical reactions.
- To test microbial contamination in any sample.

Nutrition Requirement for Bacterial Culture medium

For the preparation of culture medium for bacteria following requirements are necessary.

- Major Macronutrients
- Major micronutrients
- Carbon Energy source
- Growth factor
- Vitamin

Major Macronutrients:

These dements are required in larger amount.

- **Carbon:** The main source is organic compounds and CO_2. It is the main component of cellular materials.
- **Hydrogen:** The main source is organic compounds. It is the main component of cell water.
- **Oxygen:** The main source is organic compounds, CO_2, and O_2. It is the main constituent of cell material and cell water. It is an electron acceptor in aerobic respiration.
- **Nitrogen:** The main source is organic compounds, NH_3, NO_3, N_2, etc. It is the main constituent of amino acids, nucleic acids nucleotides, and coenzymes.
- **Sulfur:** The main source is organic sulfur compounds. It is the main constituent of some amino acids like cysteine, methionine, glutathione, and several coenzymes.
- **Phosphorus:** The main source is inorganic phosphates. It is the main component of nucleic acids, nucleotides, phospholipids, etc.
- **Potassium:** The main source is potassium salt. It is the main component of cellular inorganic cation and cofactor for certain enzymes.
- **Magnesium:** The main source is magnesium salt. It is the main component of inorganic cellular cation, a cofactor for certain enzymatic reactions.
- **Iron:** The main source is iron salt. It is the main component of cytochromes and certain non-heme iron-proteins and a cofactor for some enzymatic reactions.
- **Calcium:** The main source is calcium salt. It is the main component of inorganic cellular cation, a cofactor for certain enzymes, and a component of endospores.
- **Manganese:** The main source is manganese salt. It is the main component of inorganic cellular cation, a cofactor for certain enzymes.

Major Micronutrients:

This elements are required in small amount.

- **Manganese (Mn)-** Assists in carbohydrate, amino acid and cholesterol metabolism.
- **Zinc-** Necessary for normal growth immune function and wound healing.
- **Copper-** Required for connective tissue formation as well as normal brain and nervous system function.
- **Chloride-** often found in combination with sodium helps maintain fluid balance and is used to make digestive juices.

Carbon energy source:

- Carbon is the main source of energy in the form of Co2 source.
- The photosynthetic bacteria is required Co2 and sunlight, chemosynthetic bacteria requires co2 and chemicals as a source of energy like ammonia, nitrate etc.

Growth factor:

- All the bacteria require small amount of organic compound for growth because they are essential as growth factors.

- Eg. Purine and Pyrimidine- this is essential for DNA synthesis

Vitamins:

- Vitamins act as co-enzymes for the growth of purine pyrimidine, vitamins, protein and harmones in the body of bacteria.
- There are different enzymes required for bacterial growth.

Factors affecting bacterial growth:

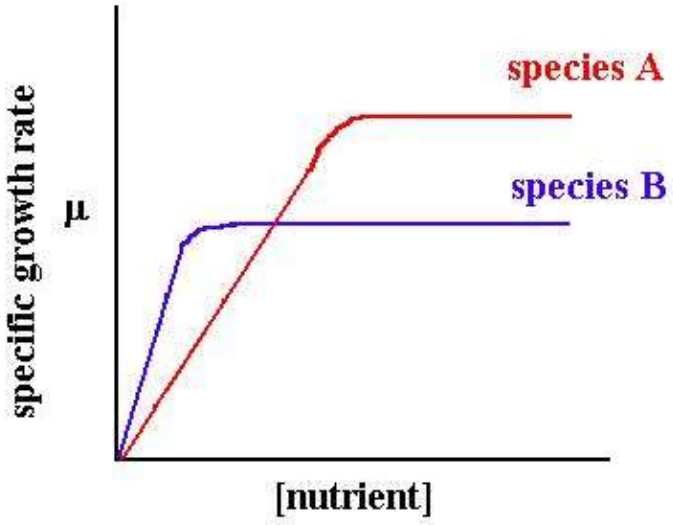
- Growth of bacteria is affected by many factors such as nutrition concentration and other environmental factors.

Some of the important factors affecting bacterial growth are:

- Nutrition concentration
- Temperature
- Gaseous concentration
- pH
- Ions and salt concentration
- Available water

Nutritional concentration:

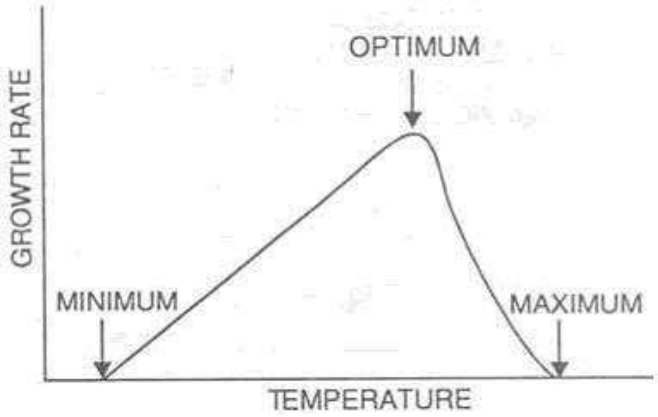
- If culture media is rich in growth promoting substance, growth of bacteria occurs faster. Decrease in nutrient concentration decreases the growth rate.
- Different bacteria have different nutritional requirement.



- With increase in concentration nutrition, growth rate of bacteria increases up to certain level and then growth rate remains constant irrespective of nutrition addition.

2. Temperature:

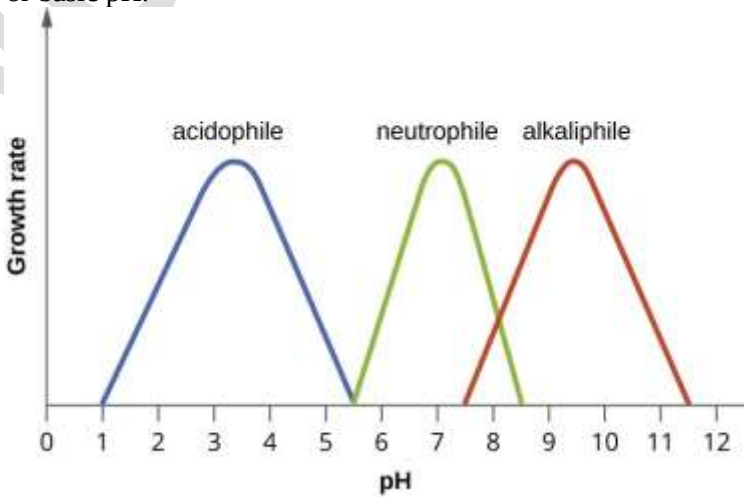
- Temperature affects the growth of bacteria by various ways.
- The lowest temperature that allows the growth is called minimum temperature and the highest temperature that allows growth is called maximum temperature.
- There is no growth below minimum and above maximum temperature.
- Below minimum temperature cell membrane solidifies and become stiff to transport nutrients in to the cell, hence no growth occurs.
- Above maximum temperature, cellular proteins and enzymes denatures, so the bacterial growth ceases.



- When temperature is increases continuously from its minimum, growth rate of bacteria increases because the rate of metabolic reaction increases with increase in temperature.
- At certain temperature the growth rate become maximum, this temperature is known as optimal temperature.
- On further increasing the temperature above optimal, growth rate decreases abruptly and completely ceases with reaching maximum temperature.

3. pH:

- pH affects the ionic properties of bacterial cell so it affects the growth of bacteria.
- Most of the bacteria grow at neutral pH (60.5-7.5). However there are certain bacteria that grow best at acidic or basic pH.



4. Ions and salt:

- All bacteria requires metal ions such as K+, Ca ++, Mg++, Fe++, Zn++, Cu++,Mn++ etc to synthesize enzymes and proteins.
- Most bacteria do not require NaCl in media however they can tolerate very low concentration of salt.
- There is some halophilic bacteria such as *Archeobacteria* that require high concentration of salt in media.

5. Gaseous requirement:

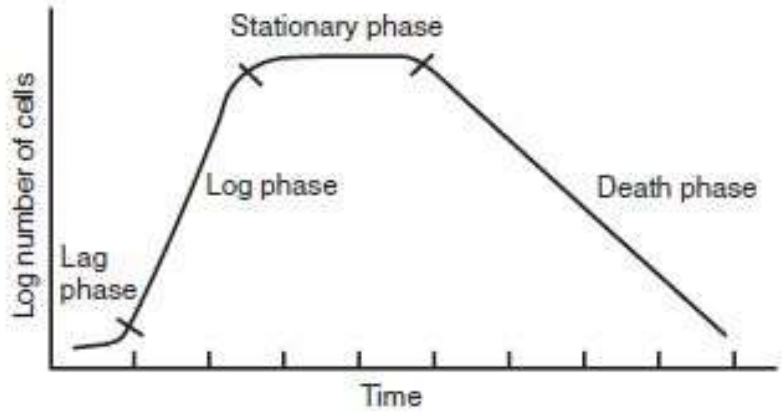
- Oxygen and carbon-dioxide are important gases that affects the growth of bacteria.
- Oxygen is required for aerobic respiration and obligate aerobic bacteria must require O2 for growth. Eg. *Mycobacterium, Bacillus*
- For obligate anaerobes Oxygen is harmful or sometime lethal. However facultative anaerobes can tolerate low concentration of O2.
- Carbon-dioxide is needed for capnophilic bacteria. Such as *Campylobacter, Helicobacter pylori*

6. Available water:

- Water is the most essential factor for bacterial growth.
- Available water in the culture media determines the rate of metabolic and physiological activities of bacteria.
- Sugar, salts and other substances are dissolved in water and are made available for bacteria.

Growth Curve of bacteria:

- The growth curve is hyperbolic due to exponential bacterial growth pattern.



The growth curve has following phases

- Lag phase
- Log phase or exponential phase
- Stationary phase
- Death phase or decline phase

Lag Phase:

- When culture medium is prepare then upto 2 to 3 hours there is no growth of bacteria only sterile nutrition medium is present.
- In log phase there is no growth of bacteria.

Log Phase:

- After the qermination of bacteria they reproduce in fast rate and the no. of bacteria is increase.
- In log phase the concentration of nutrition medium is more than the bacteria so the growth of bacteria is maximum in this phase this is also called exponential phase.

Stationary phase:

- The growth rate slow as a result of nutrient depletion and accumulation of toxic product.
- This phase is reached as the bacteria begin to exhaust the resources that are available to them.
- This phase is a constant value as the rate of bacterial growth is equal to the rate of bacterial death.

Death Phase (Decline Phase):

- In this phase no of bacteria is increase but nutrition medium is very less. So due to lack of nutrients bacteria started to death in Decline Phase

Isolation of Pure Culture:

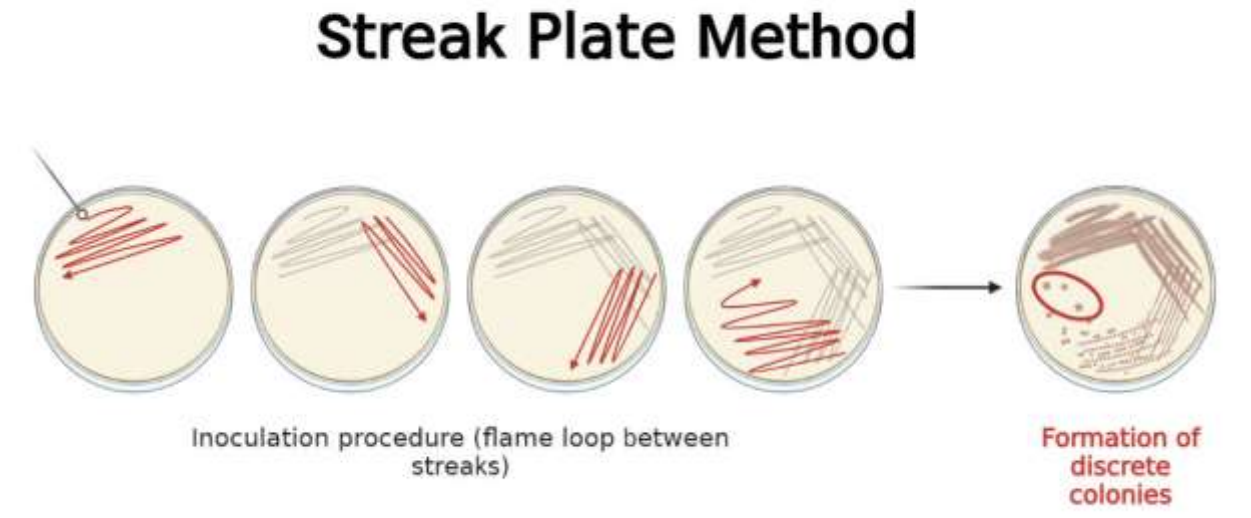
A pure culture is a culture of microorganisms that contains only one type of organism. It is important to isolate pure cultures in order to study the individual properties of each organism.

There are 4 main methods for isolating pure cultures:

- Streak plate method
- Pour plate method
- Spread plate method
- Serial dilution method
- Special Method

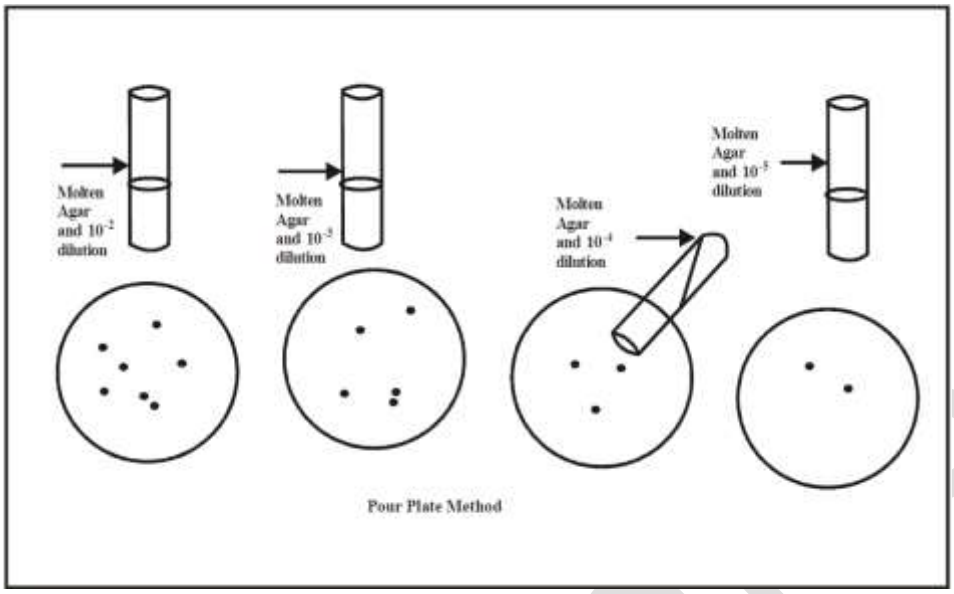
Streak plate method:

- In this technique,the bacteria is isolated from (Mixed culture media) by applying streak on the surface of culture media with the help of inoculation loop.
- Firstly inoculation loop is heated for sterilization and then streak on the surface of mixed culture then it streak on new sterile culture media.



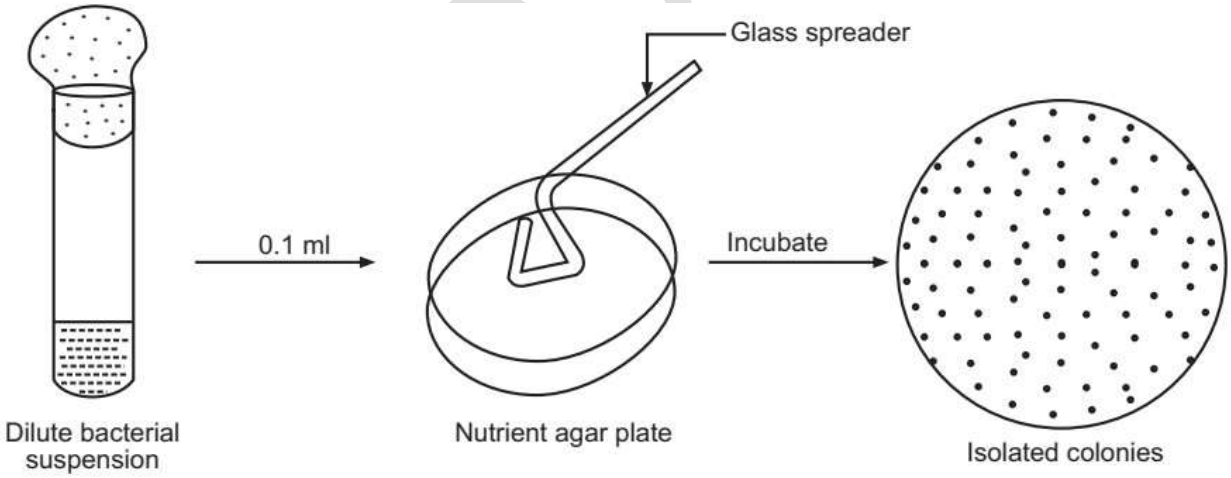
Pour Plate method:

- In this method Dilute agar solution (Agar mix with some water) is mixed in the mixed culture media, and dilute the mixed culture media.
- Now this diluted culture medium is pare into different petri-dish.



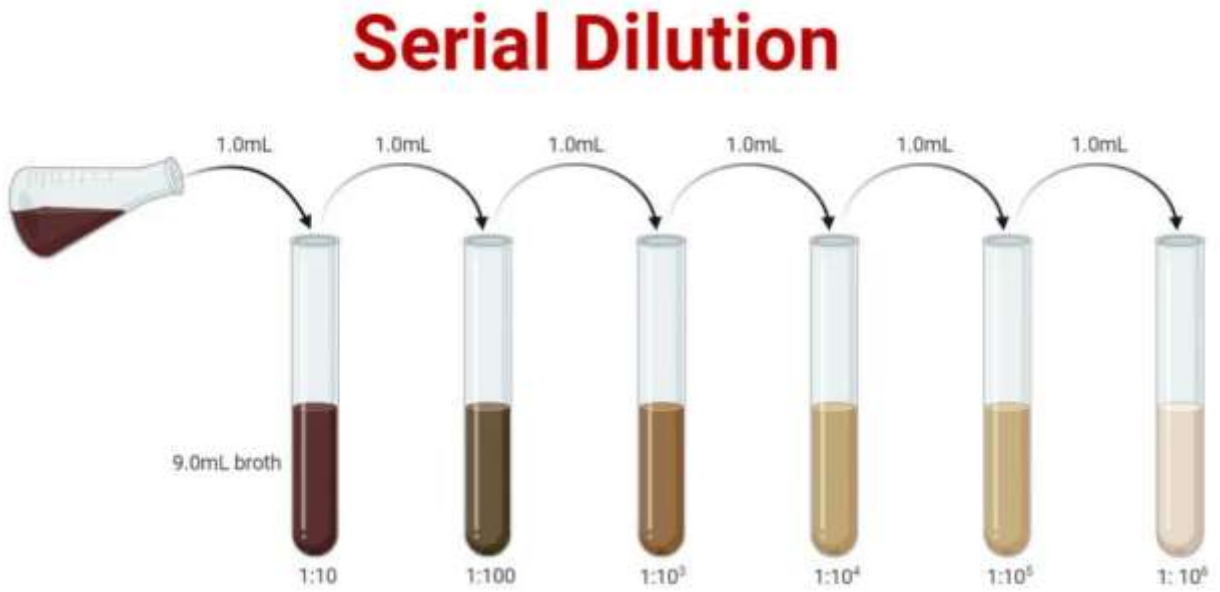
Spread plate method-

- In this method the culture medium is diluted with sterile saline solution.
- Now take 1ml of culture medium and placed on the surface of new sterile culture medium.
- Spread this drop with glass spreader in all over the surface and allow to grow the bacteria.



Serial dilution method:

- In serial dilution method the 1ml of culture medium is added into 9ml sterile solution.
- Now take 1ml again from this 10 ml culture medium solution and add into another 9ml solution.
- Repeat this procedure in the serial as well as no of test tube increase and the concentration of bacteria is decrease and the dilution is increase.
- This method is used for actinomycetes bacteria.



Preservation method for pure culture:

- Preservation is a process to maintain pure culture for long period in viable condition.
- Once in any culture medium bacteria is grow then it placed for long time and there is lot of chance to be contaminated.
- The preservation of pure culture can be achieved by following method-
  - Subculturing
  - Storage in sterile condition
  - Saline suspension
  - Refrigeration
  - Paraffine suspension
  - Lypophillization

Subculturing:

- In this method the culture medium solution is regulary changed.
- In fresh culture medium the chances of contamination is less.

Storage in sterile condition

- In this method calcium carbonate solution is added and the container is closed with cotton plug and placed under incubator.
-

Saline suspension:

- Bacteria cannot grow in saline solution so 1% Nacl solution is added into culture medium so the growth of bacterial will stop.

Refrigeration:

- In this method the culture medium is placed under refrigerator add 0 to 4°C for 2 to 3 days so the microbes will kill.

Paraffin method:

- In this method the paraffine oil is added in culture medium it form a thin layer over the surface of culture medium so the aerobic bacteria cannot grow.

Lypophilization:

- In this method culture put in very low then reduced the pressure. So microbial cells are dehydrated and their metabolic activity are stopped.
- After it seated and stored in the dark at 4°C in refrigerators.
- Also called Freeze dried pure cultures.

Quantitative measurement of bacterial growth:

Bacteria are growing in culture medium after placing in incubator.

The growth of bacteria is calculated in 2 terms-

1. Total count
2. Viable count

Total count means total no of living and non-living bacteria and viable count means total no of living bacteria only.

The quantitative measurement of no and growth of bacteria is determined by following method:

1. Total Count:

- In this measurement count all bacteria (either it lives on deid)
- It is also called direct method because in which we directly count the no of colonies of bacteria by using microscopes.

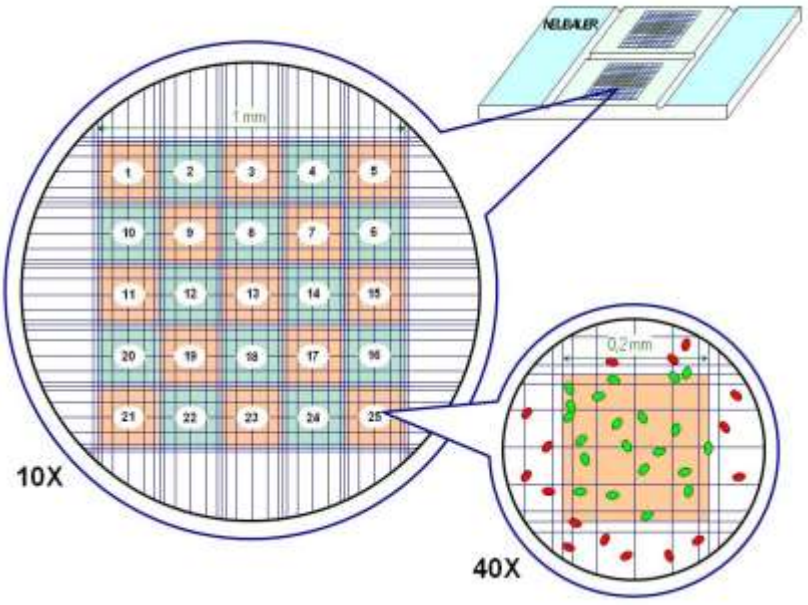
Here we count by two methods following:

- Counter chamber method
- Electron counter method

Counter chamber method:

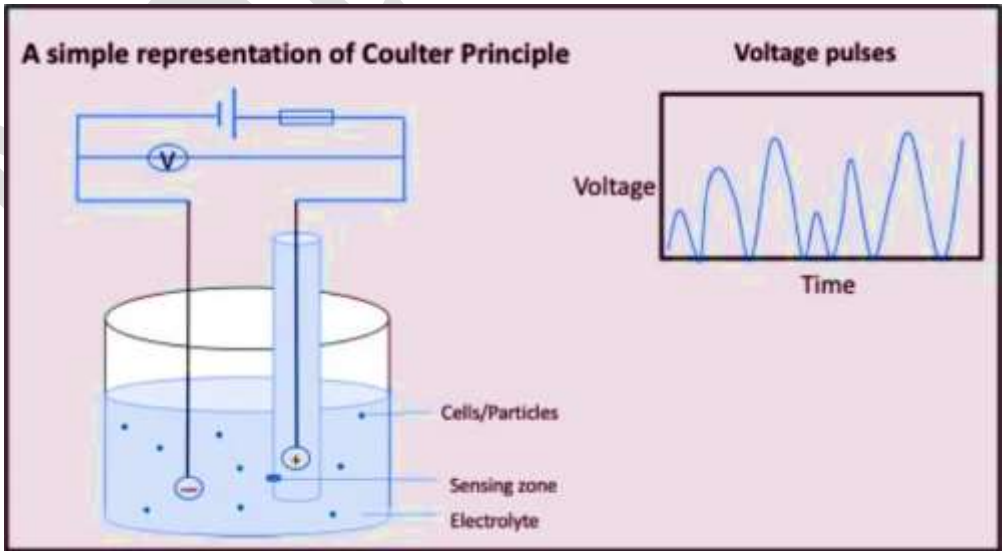
- This is the simple and less time consuming method and this requires calonie counting chamber and hemocytometer.
- In hemocytometer different scales are made and each chamber 62 to 70 bacteria are present.
- The petri dish containing culture medium is placed inside the counting chamber and hemocytometer is fixed.

- The total no of colonies of bacteria can be count and no of bacteria is calculated.



Electron counter method:

- This method is also known as flow cytometry method.
- In this method the culture medium containing bacteria is pass through the dectrodes by a small orifice.
- When bacteria is pass between the electrodes then the voltage is qenerated.
- By counting peakes the total no of bacteria is calculated.
- This method is also known as coulter counter method.



Viable Count (Indirect Method)

- In this method the optical density of light is calculated and co-relate with no of bacteria.
- This culture media is added in clear solution and if bacteria are present then solution becomes turbot.
- The turbidity is measured by passing the light from the medium.
- More turbidity means more bacteria present.

Study of different types of phase constrast microscopy, dark field microscopy and electron microscopy

Microscope:

A microscope is a scientific instrument that magnifies small objects that are not visible to the naked eye. Microscopes use lenses to bend light toward the eye, making an object appear larger than it actually is.

Phase Contrast Microscopy:

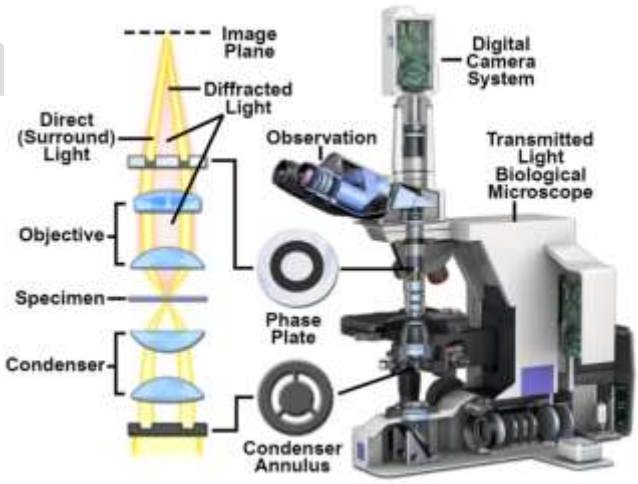
- It is a type of light microscopy that converts phase shifts in light passing through a transparent specimen to brightness changes in the image.
- Phase shifts themselves are invisible, but become visible when shown as brightness variations.

Principle:

- Phase contrast microscopy is based on the principle that light passing through a transparent object will undergo a phase shift, depending on the refractive index of the object.
- The phase shift is caused by the different speeds at which light travels through different materials.

Working:

- A phase contrast microscope uses a special phase plate to interfere with the light passing through the specimen.
- The phase plate consists of a transparent disk with a dark ring in the center. The dark ring retards the phase of the light passing through it by a quarter of a wavelength.
- When light passes through the specimen, it is refracted and its phase is shifted slightly. The refracted light then passes through the phase plate.
- The light that passes through the dark ring will be retarded by a quarter of a wavelength, while the light that passes through the rest of the phase plate will not be retarded.
- The two beams of light then interfere with each other. If the two beams are in phase, they will constructively interfere and produce a bright image.
- If the two beams are out of phase, they will destructively interfere and produce a dark image.



Advantages:

- It allows unstained living cells to be visualized in high contrast.
- It has a high resolution, allowing small details to be seen.
- It is a relatively simple technique to use.

Disadvantage:

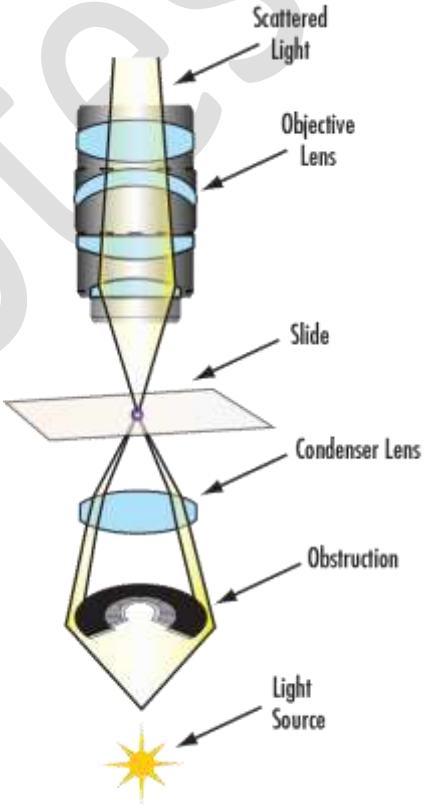
- It can be expensive to purchase a phase contrast microscope.
- The phase plate is delicate and must be carefully aligned.

Dark Field Microscopy:

- The microscope which forms a bright image against a dark background is called field microscopy.

Principle:

- The effect produced by the dark field technique is that of a dark background against which object are brilliantly illuminated.
- The condenser has a stop plate which blocks the light over the centre of the field where specimen lies
- Used to see unstained samples
- Stop plate is placed under condenser lens due to which the light scattered by object on slide reached to eyes.



Advantages:

- Ideal for viewing object that are unstained, transparent and absorb light or no light.
- Simple and effective.

Electron Microscopy:

Principle of Electron Microscopy:

- Electron microscopy is a type of microscopy that uses a beam of electrons to create an image of the specimen.
- The wavelength of electrons is much shorter than the wavelength of light, which allows for much higher magnifications and resolutions.

Working:

- The basic principle of operation of an electron microscope is similar to that of a light microscope. A beam of electrons is directed at the specimen, and the interaction of the electrons with the specimen produces an image.
- The image can then be magnified and viewed on a screen or photographed.

Types:

There are two main types of electron microscopes:

- **Transmission electron microscopy**
- **Scanning electron microscopy**

Transmission electron microscopy (TEM):

- In TEM, a beam of electrons is transmitted through the specimen.
- The electrons that are transmitted through the specimen are then collected and focused to form an image.
- TEM is used to study the internal structure of thin specimens, such as cells and viruses.

Scanning Electron Microscopy (SEM)

- In SEM, a beam of electrons is scanned across the surface of the specimen.
- The electrons that interact with the surface of the specimen produce secondary electrons, which are then collected and focused to form an image.
- SEM is used to study the surface morphology of specimens.

Advantages:

- Very high magnification and resolution
- Can be used to study a wide range of specimens, including biological, geological, and materials science samples
- Can be used to obtain three-dimensional images of specimens

Disadvantages:

- Expensive to purchase and maintain
- Requires specialized training to operate

- Specimens must be prepared in a vacuum environment
- Live specimens cannot be studied

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