Lectures of the Microbiology Laboratory

second stage

Lectures:

1- General introduction of Microbiology
2- Scope of Microbiology
3-Safety and Laboratory Guideline
4- Sterilization
5- Microscopes and the Study of Microbial Structure
6- The Structure and Shape of Bacterial cell
7- Media for Bacterial Growth and Methods of Isolation
8- Transfer Instruments & Methods of Isolation
9- Bacterial stain
10-Techniques of smear preparation
11-Simple stain
12-Negative stain
13-Gram stain
14- Acide fast stain
15- Physical and Chemical agents for the Control of Microbial Growth

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❖ General introduction of Microbiology:-

a specialized area of biology is the study of living organisms are not readily observed without magnification , which is to say they are **microscopic.*** These microscopic organisms are collectively referred to as(**'microorganisms' or 'microbes'**), simple in structure, and usually small in size, that are generally considered to be neither plants nor animals; they include bacteria, algae, fungi, protozoa, and viruses..

❖ **Microorganisms** were first seen and described by the Dutch lens-maker Antonie van Leeuwenhoek (1632–1723).

Branches of Microbiology

1. Medical microbiology 2. Industrial microbiology 3. Food microbiology 4. Soil microbiology 5. Plant microbiology.

- Here we are concerned with medical microbiology. It is studied under following headings:

a. Parasitology :- deals with the study of parasites causing diseases in human being.

b. Mycoloy :- deals with the study of fungi causing diseases in human beings.

c. Immunology :- is concerned with mechanism involved in the development of resistance by body to infectious diseases.

d. Bacteriology :- deals with the study of bacteria.

e. Genetics :- is the study of heredity and variations.

f. Virology :- is the study of viruses.

✓ SO **Medical microbiology** mean (Gr. mikros-small, bios-life, logos-science) is the study of causative agents of infectious disease of human beings and their reactions to such infections. In other words it deals with etiology, pathogenesis, laboratory diagnosis, treatment, epidemiology and control of infection.

❖ Scope of Microbiology :-

*__1.__ Diagnostic, e.g. isolation and identification of causative organism from the pathological lesions. We can also diagnose typhoid fever by doing Widal's test.

*__2.__ Prognosis of disease, e.g. in Widal's test rising titer signifies active disease and ineffective treatment. Falling titer means effective treatment and curing of disease.

*__3.__ Guidance in treatment, e.g. by culturing the organism in pure form and then performing drug sensitivity test we can suggest the effective drug for the treatment of that particular infection.

*__4.__ Source of infection, e.g. in sudden outbreak of infectious disease we can find out the source of infection.

*__5.__ Detection of new pathogens and then development of vaccines.

❖ Safety and Laboratory Guidelines:-

Because microorganisms present varying degrees of risk to laboratory personnel (students, technicians, and faculty), people outside the laboratory, and the environment, microbial cultures must be handled safely, so the **Basic Laboratory Safety** are:-

1. Wear protective clothing (i.e., a lab coat) in the laboratory when handling microbes. Remove the coat prior to leaving the lab (Figure I).
2. Do not wear sandals or open-toed shoes in the laboratory.
3. Wear eye protection whenever you are heating chemicals, even if you wear glasses or contacts (Figure I).
4. Turn off your Bunsen burner when it is not in use.
5. Tie back long hair, as it is a potential source of contamination as well as a likely target for fire.
6. If you are feeling ill, go home. A microbiology laboratory is not a safe place if you are ill.
7. If you are pregnant, immune compromised, or are taking immunosuppressant drugs, please see the instructor.
8. wear disposable gloves If it is your lab's practice while handling microorganisms, staining microbes , handling blood products (plasma, serum, antiserum) or whole blood , be sure to remove them each time you leave the laboratory , and wash your hands (Figure I) .
9. Use an antiseptic (e.g., Betadine) on your skin if it is exposed to a spill containing microorganisms.
10. Never pipette by mouth. Always use mechanical pipettors .
11. Dispose of broken glass or any other item in an appropriate "sharps" or broken-glass container (Figure 2).

Figure 1-wearing a protective lab coat, gloves, and goggles

Figure 2-Sharps Container

❖ Student Conduct in Laboratory :-

1. To reduce the risk of infection, do not smoke, eat, drink, or bring food or drinks into the laboratory room—even if lab work is not being done at the time.
2. Do not apply cosmetics or handle contact lenses in the laboratory.
3. Wash your hands *thoroughly* with soap and water before working in the lab, after handling living microbes, and before leaving the laboratory at any time , Also, wash your hands after removing gloves.
4. Do not remove any organisms or chemicals from the laboratory.
5. Work carefully and methodically. Do not hurry through any laboratory procedure.

❖ Sterilization:-

is the removal of all microbes, including endospores, and can be achieved by Physical , chemicals, mechanical, or radiation methods.

- **Physical Methods**

a) <u>Dry heat</u>

1. **Incineration** (fire) used for sterilize the slides , needles , loops, and test tubes).

2. **ovens** used for sterilize oils , powder, petridishes , pipettes , minerals and surgical instruments , under 160-180°c for 1.5 -2h.

b) <u>Moist heat</u>

1. **under pressure is provided by autoclaving** is used (under 121°c for 20 min and pressure 1-1.5) to sterilize culture media , liquids , waste culture media , and all other substances which damaged by dry heat
2. **without pressure by Fractional sterilization or tyndallization** sterilizer is used (under 100°c for 15 min./3 days) to sterilize any substance which damaged over 100°c like sugar solutions.

✓ **Chemical methods**

a) **<u>Disinfection or disinfectants</u>** Chemical germicides are substances designed to reduce the number of pathogens on a surface (floors, tables, sinks, countertops, surgical instruments, etc.) or liquids such as 0.01%, 0.1%, and 1.0% household bleach and 25%, 50%, and 100% Lysol® Brand II Disinfectant

b) **<u>Antiseptics</u>** Chemical Germicides are substances designed to reduce the number of pathogens on or in living tissue such as 0.03%, 0.3%, and 3% hydrogen peroxide (3% is full strength as purchased at the pharmacy) or 10%, 30%, and 50% isopropyl alcohol (70% is full strength as purchased at the pharmacy)

- **Mechanical Methods** such as : **<u>Filtration</u>** This Method is used for sterilization of biological liquids , which are damage with high temperature degrees , such as the solutions of serum , enzymes , vitamins , and antibiotics .

- **Radiation** such as : **gamma ray** , **X-ray, and alpha ray**. The most substances that sterilized by radiation are plastic petridishes , plastic injections , plastic gloves , plastic pipettes , operation rooms , packaging rooms of drugs , yoghurt production .

✓ Critical thanking:-

- Moist heat is more adequacy for killing a living cell, **Why**?

❖ Microscopes and the Study of Microbial Structure

-Microscope is the device for magnifying objects that are too small to be seen with the naked eye

Microscopes are divided into three categories :

1- Microscopes use a beam of electrons as the source of illumination instead of light

- are subdivided into

a) Scanning electron microscope (SEM) Maximum effective magnification SEM = 650,000X
b) Transmission electron microscope (TEM) Maximum effective magnification TEM = 1,000,000X

2- Microscopes with ultraviolet or laser beam as the source of illumination instead of light, Maximum effective magnification = 1,000. to 2,000 X

- are subdivided into

a) Fluorescent Microscope
b) Confocal Microscope

3- **Microscopes with visible light** as the source of illumination Maximum effective magnification = 1,000. to 2,000.

- are subdivided into

a) Bright-field microscope
b) Dark-field microscope
c) Phase-contrast microscope
d) Differential interference contrast microscope

*As Figure 1-2-3 Respectively

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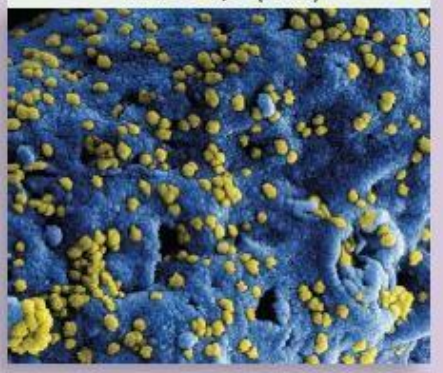
Colorized transmission electron microscope image

Colorized version of the same TEM image. Electron microscopes produce only black and white images, but these are often artificially colored, with the aid of a computer.

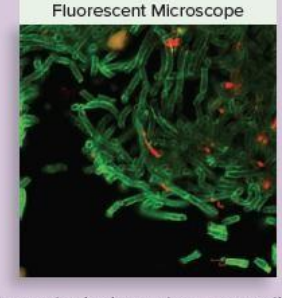
Scanning electron microscope (SEM)



Artificially colorized scanning electron microscope (SEM) image of Middle East respiratory syndrome Coronavirus (MERS CoV) viral particles (colored yellow) on the surface of a eukaryotic cell (colored blue). The electron beam scans over the surface of the cell to produce a three-dimensional image.

2-

Fluorescent Microscope



Cells of Yersinia pestis, the bacterium responsible for plague, viewed at 200X. Ultraviolet light is used to illuminate the specimen. Antibodies labeled with fluorescent dyes emit visible light only if the antibody recognizes and binds to the cell. The specificity of this technique makes it a superior diagnostic tool.

Confocal Microscope

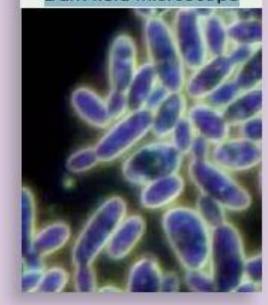
Two views of Paramecium 1,500X, stained by fluorescent dyes, and scanned by a laser beam, form multiple images that are combined into a three-dimensional image. Confocal microscopes may utilize visible or ultraviolet light. Note the fine detail of cilia and organelles.

3-

Bright-field microscope

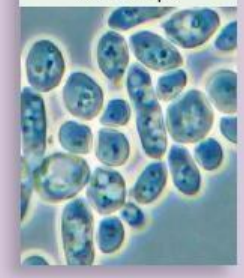
Common multipurpose microscope for live and preserved stained specimens; specimen is dark, field is white; provides fair cellular detail.

Dark-field microscope



Best for observing live, unstained specimens; specimen is bright, field is black; provides outline of specimen with reduced internal cellular detail.

Phase-contrast microscope



Used for live specimens; specimen is contrasted against gray background; excellent for internal cellular detail.

Differential interference contrast microscope

Provides very detailed, highly contrasting, three-dimensional images of live specimens.

- **Bright –field microscope :**

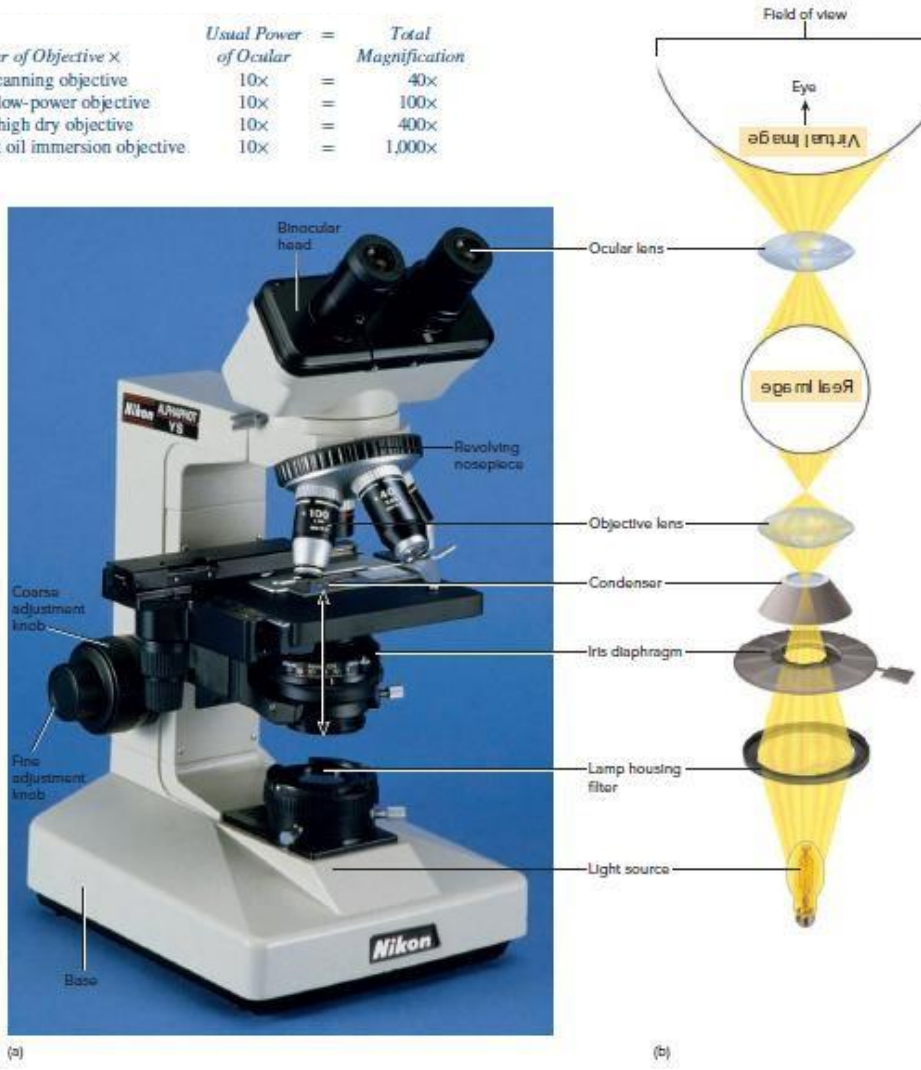
This microscope is used to study the size , shape , and arrangement of the microbial cells , but it provides little information about the internal cell structure . There is two types of this microscope :

* **Simple microscope** : It has a single lens magnifier.

* **Compound microscope** : It employs two or more lenses called ocular and objective lenses.

The magnification of the microscope depends on the type of objective lens used with the ocular lens . So , the total magnification is calculated by multiplying the objective lens by ocular lens.

Power of Objective ×	*Usual Power of Ocular*	=	*Total Magnification*
4× scanning objective	10×	=	40×
10× low-power objective	10×	=	100×
40× high dry objective	10×	=	400×
100× oil immersion objective	10×	=	1,000×



- **Workings of an oil immersion lens :**

To maximize its resolving power, an oil immersion lens (the one with highest magnification) must have a drop of oil placed at its tip. This transmits a continuous cone of light from the condenser to the objective, thereby increasing the amount of light and, consequently, the numerical aperture. Without oil, some of the peripheral light that passes through the specimen is scattered into the air or onto the glass slide; this scattering decreases resolution ,as figure (1)

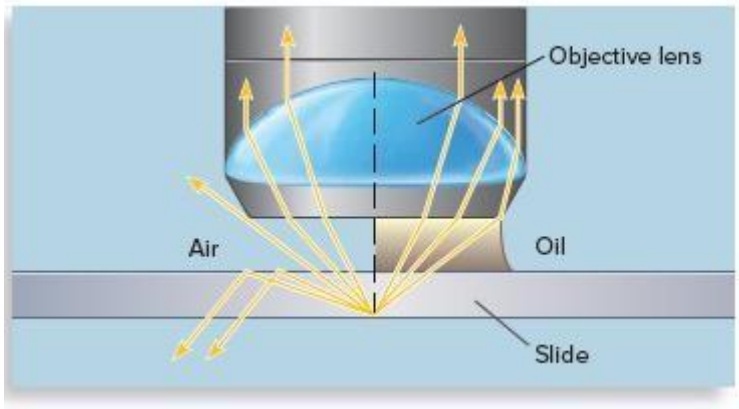


figure (1)

❖ Microscope care

Microscope is a very important tool in microbiology and it must be used carefully and correctly . Follow these guidelines every time you use a microscope :

1- carry the microscope with both hands , one hand beneath the base and the other on the arm . Never slide a microscope a cross a bench surface .

2- Clean the microscope both before and after use . Use only lens paper and lens cleaner .

3-Only use oil when using the 100X oil immersion lens . Do not get oil on the other objective lenses.

4-Do not wrap the cord around the microscope . Instead , fold the cord and place it between the arm and the stage or beneath the stage.

5- Observe the slide with both eyes open , to avoid eyestrain.

6- Always focus with low power first .

7- Keep the stage clean and free of oil .

8- wipe oil off the oil immersion lens before putting your microscope away . Do not touch the lenses with your hands .

9- Clean the ocular lens carefully with lens paper if dust is present .

10- Replace the dust cover before putting the microscope away .

11- Make sure that the specimen is on the top –side of the slide when using the oil immersion lens .

12- Increase the amount of light when using the oil immersion lens .

✓ Citical thinking:-

1- For what purpose each of the following microscope components?
a. Coarse-adjustment knob
b. Fine-adjustment knob
c. Condenser
d. Mechanical stage control

2- How can you calculated the total magnificent for the microscope?

✓ The Structure and Shape of Bacterial cell

- In general the organisms are divided into two groups (As table 1) :-

1- Prokaryotes : Include two kingdoms appear similar in morphology but they have major molecular and biochemical differences :

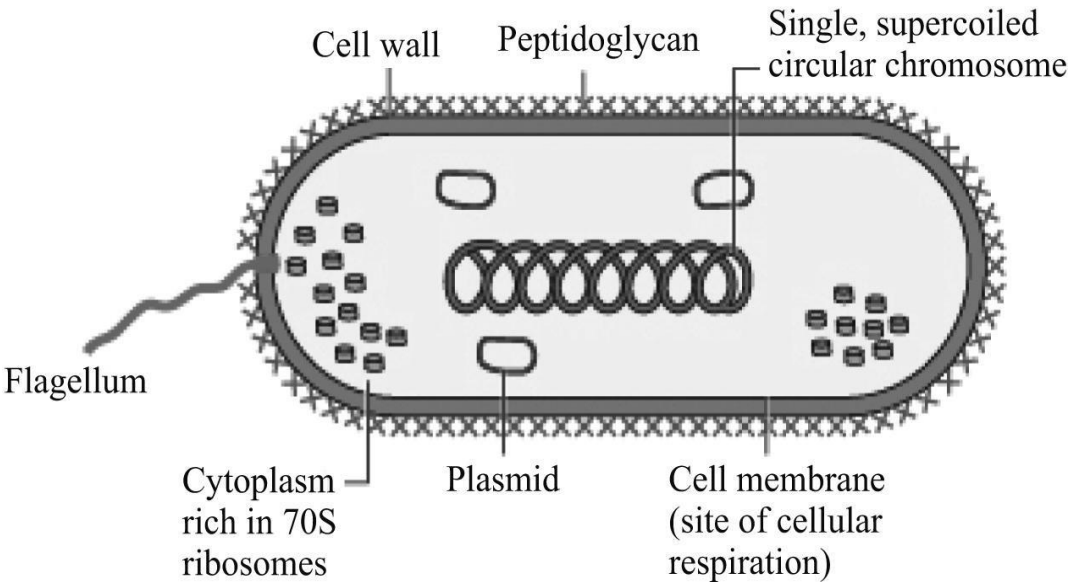
a- Kingdom (1) : Bacteria (Eubacteria) : They are common in the environment and include all bacteria that infect human . They all possess peptidoglycan (murein or cell wall skeleton) which contains a unique sugar and muramic acid, not found elsewhere in nature.

d- Kingdom (2) : Archaea (Archaebacteria) : These organisms do not infect human and they contain a pseudomurein (pseudopeptidoglycan) that is different in structure to eubacterial murein .

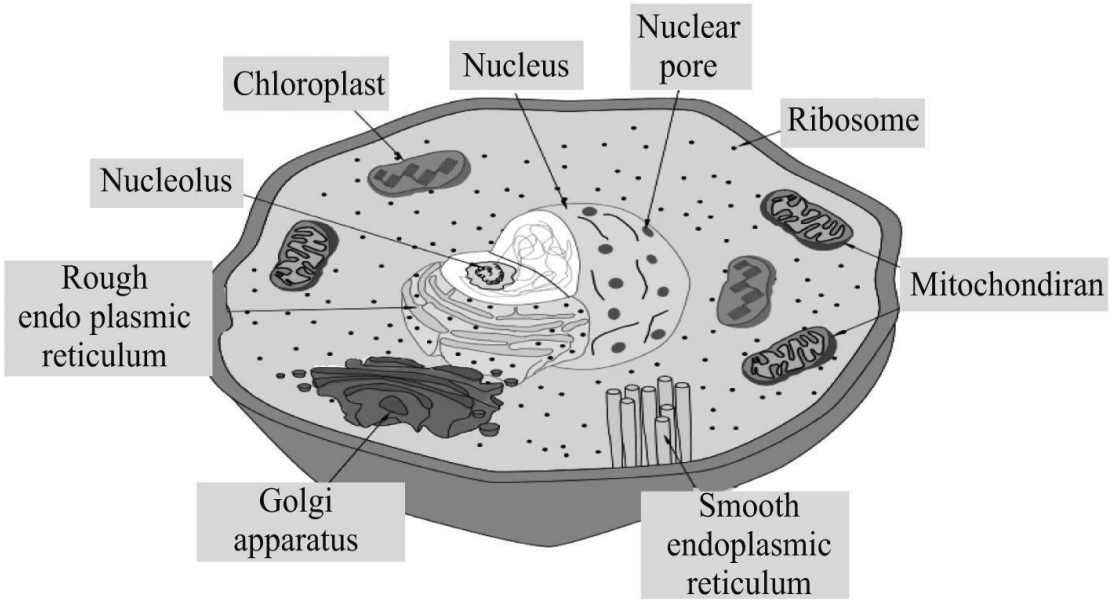
2- Eukaryotes : Include all other forms of life (plants, animals, human, algae, fungi and parasite).

✓ Table (1): Comparison of Prokaryotic and Eukaryotic Cell Organization:-

Characteristic	Prokaryote	Eukaryote
- organelles	Absent	present
- nuclear membrane	Absent	present
- Chromosomal DNA	Circular; complexed with RNA	Linear; complexed with histones and other proteins basic
- Sterols in cytoplasmic membrane	Absent	present
- Ribosomes: site of protein synthesis	Present smaller in size (70S)	Present, larger in size (80S)
- cell division	faster	slower



Prokaryote Cell



Eukaryote Cell

✓ Shapes of Bacteria:-

✓ Microscopic Shapes:-

Bacteria vary in size from 0.4 to 2 μm. They occur in three basic shapes:

- Cocci (spherical)
- Bacilli (rod-shaped)
- Spirochetes (spiral) or (Helical and curved bacteria).

Individual bacteria may form characteristic groupings.

✓ Cocci:- (plural of *coccus*) may occur singly

in pairs (diplococci),

in chains (streptococci),

in four cells grouped together (tetrads)

in eight cells grouped together (sarcinae form)

or in clusters (staphylococci). As figure (1)

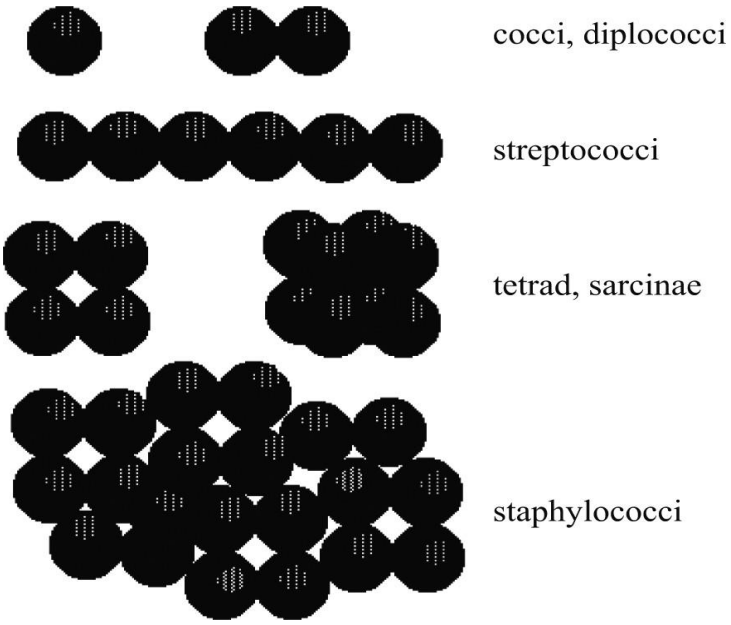
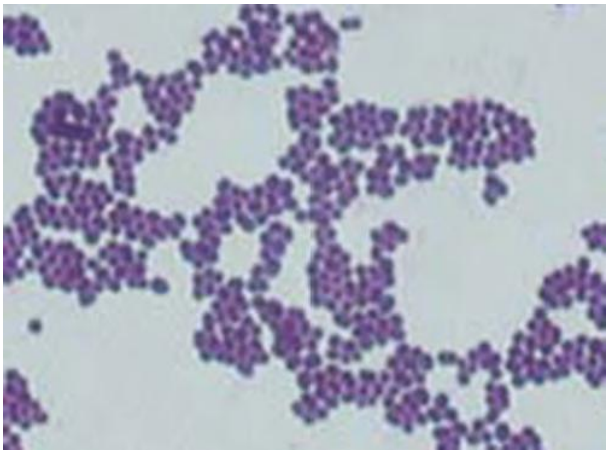


Figure (1):- Arrangement of Cocci.



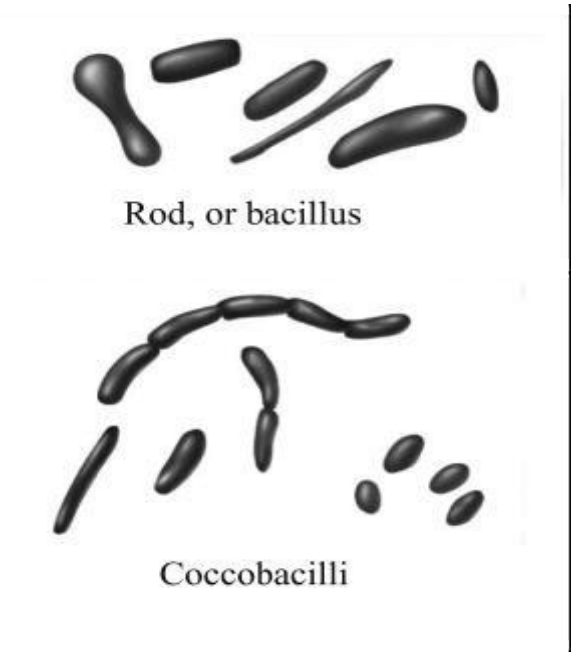
Staphylococcus aureus

- ✓ Bacilli:-

(plural of *bacillus*) Bacilli may occur as single rods, The cells of this group vary in length .

in chains

short coccobacilli to long filamentous rods. As figure (2)



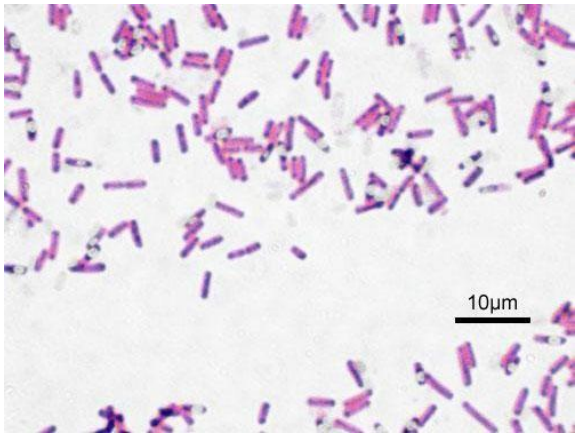
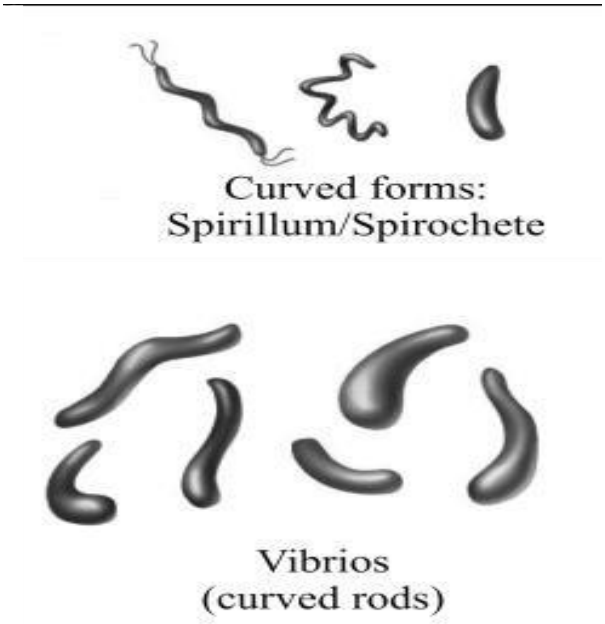


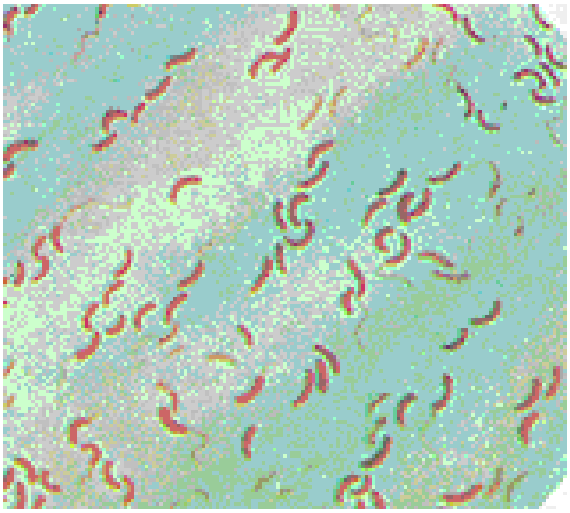
Figure (2):- Arrangement of Bacilli ***Bacillus subtilis***

- ✓ Spirochetes (spiral) or (Helical and curved bacteria):-

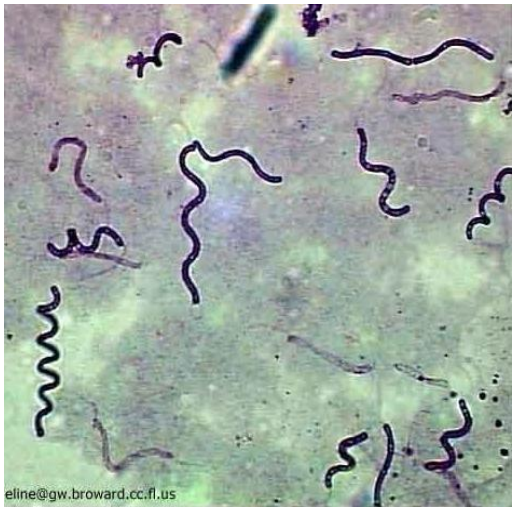
The cells in this group are either helical (spirillum and slender spirochaetes) or curved (comma shape) .As figure(3)



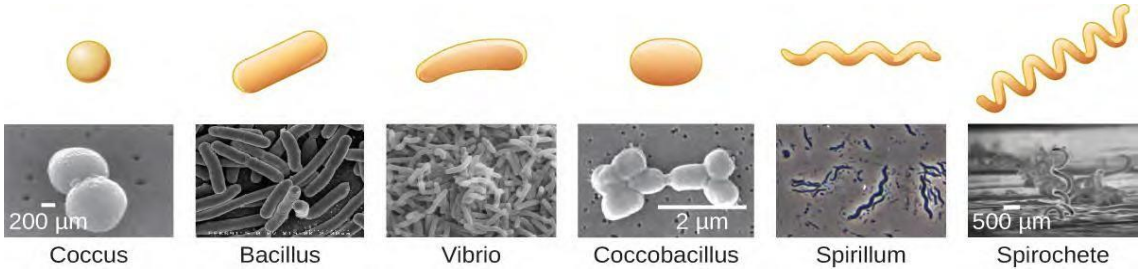
Figure(3):-Arrangement of Spirochetes



Curved bactera*:-Vibrio cholera*



Spirochetes:- ***Treponema pallidum***



❖ Media for Bacterial Growth and Methods of Isolation:-

- ✓ **Culture media** gives artificial environment simulating natural conditions necessary for growth of bacteria. The basic requirement of culture media are:

1. Energy source.
2. Carbon source.
3. Nitrogen source.
4. Salts like sulphates, phosphates, chlorides and carbonates of sodium, potassium, magnesium, ferric, calcium and trace elements like copper, etc.
5. Satisfactory pH 7.2 to 7.6.
6. Adequate oxidation-reduction potential.
7. Growth factor**.**

- ✓ **Media** used for obtaining the growth of bacteria are:

❖ FLUID MEDIA

Bacteria grow very well in fluid media in 3 to 4 hours. Hence, they are used as enriched media before plating on solid media. They are not suitable for the isolation of organism in pure culture. We cannot study colony characters as well Examples of fluid media are nutrient broth, peptone water, etc.

❖ SOLID MEDIA

They are used to study colonies of individual bacteria. They are essential for isolation of organism in pure form. **Agar** it is important constituent of solid media such as blood and nutrient agar ,etc.

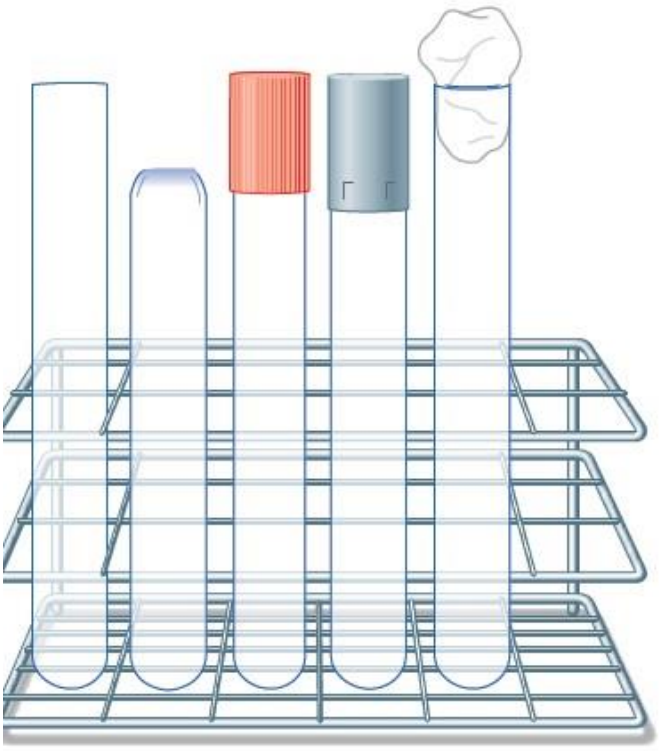
- ✓ **Culture Techniques**

In clinical laboratory indications for culture are:

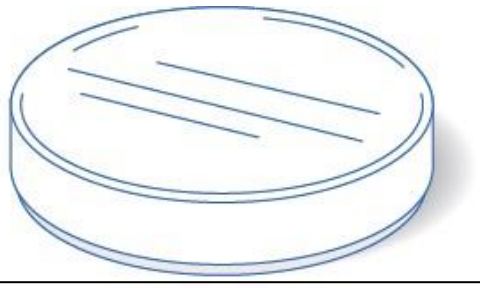
a. Isolation of bacteria in pure culture.
b. To demonstrate their properties.
c. To obtain sufficient pure growth for preparation of antigen and for other tests.
e. To determine sensitivity to antibiotics.

✓ Culture Tubes and Petri Dishes:-

Glass **test tubes** and glass or plastic **Petri dishes** are used to cultivate microorganisms. A suitable nutrient medium in the form of broth or agar may be added to the tubes, while only a solid medium is used in Petri dishes. A sterile environment is maintained in culture tubes by various types of closures. **Figure (1) below** illustrates some of the culture vessels used in the laboratory.



Deep tubes can contain from 15ml to 20ml of broth agar, depending on the size of the tube



Petri dishe i manufactured in various sizes to meet different experimental requiremen

flask can contain 250 ml, 500ml and 1000ml from broth agar, depending on the size of the flask

Figure (1): illustrates some of the culture vessels used in the laboratory.

✓ Transfer Instruments & Methods of Isolation:-

- Microorganisms must be transferred from one vessel to another or from stock cultures to various media by **Wire loops** (Wire loops are made from inert metals such as platinum) to obtain pure culture and maintain for other studies . This transfer is called **subculturing** and must be carried out under aseptic conditions to prevent possible contamination.

-A microbial culture consisting of two or more species is said to be a **mixed culture**, whereas a **pure culture** contains only a single species. Obtaining isolation of individual species from a mixed sample is generally the first step in identifying an organism. A commonly used **isolation technique** is the **streak plate.**

- In the streak plate method of isolation, a bacterial sample (always assumed to be a mixed culture) is streaked over the surface of a plated agar medium by wire loop. During streaking, the cell density decreases, eventually leading to individual cells being deposited separately on the agar surface. Cells that have been sufficiently isolated will grow into **colonies** consisting only of the original cell type.

❖ Application:-

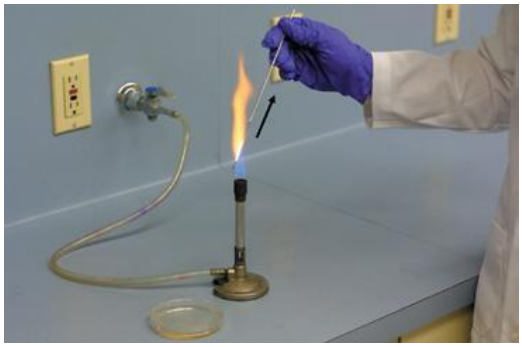
Per Student

✓ inoculating loop

✓ Bunsen burner

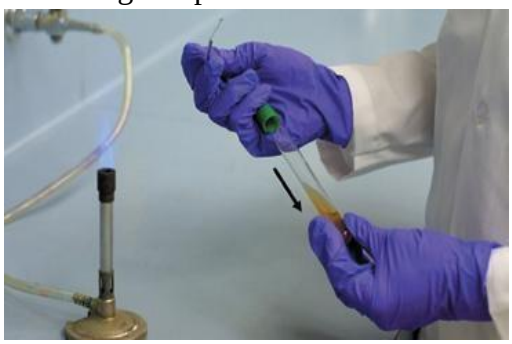
- ✓ sterile fluid media such as Nutrient Broth tubes
- ✓ sterile solid media such as Nutrient and manitol Agar media.
- ✓ marking pens

❖ procedure:-

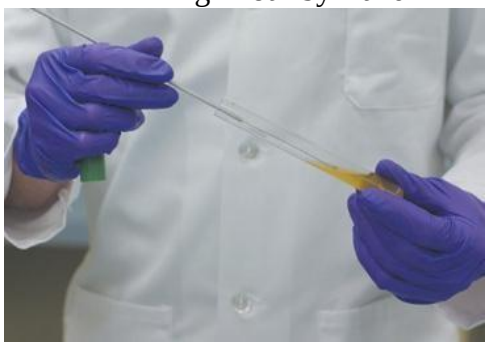


1-Flaming Loop

2- Mixing Broth by Hand

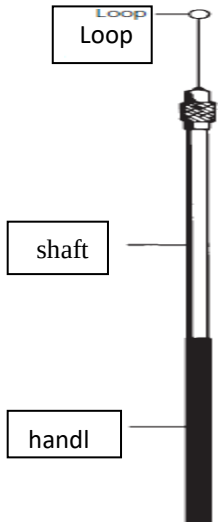


3- Removing the Tube Cap

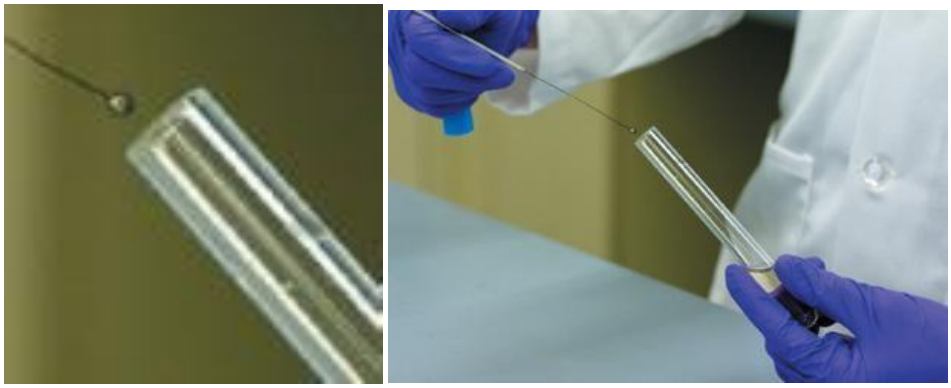


4- Holding the Tube at an Angle

5- 1 Remove the loop, Flaming the Tube and replace the lid.



Wire loop



Note:-Removing the Loop from Broth notice the film of broth in the loop (see inset). Be careful not to catch the loop on the lip of the tube when removing it.

7- Obtain the sample of mixed culture from broth media with a sterile loop on to solid media and Beginning the Streak Pattern. such as figure below.

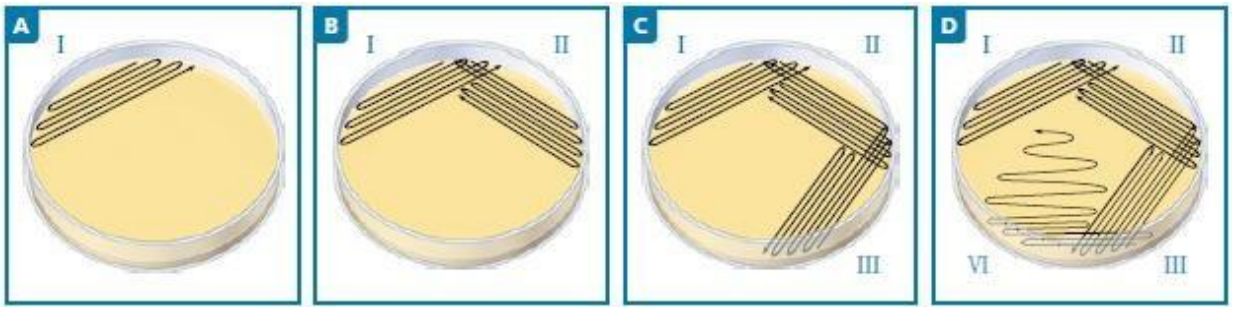


Figure illustrate streak pattern.

A- **Beginning the Streak Pattern:** Label the plate's base. Then, streak the mixed culture back and forth in one quadrant of the agar plate. Do not cut the agar with the loop. Flame the loop, then proceed.

B- **Streaking Again:** Rotate the plate nearly 90° and touch the agar in an uninoculated region to cool the loop. Streak again using the same wrist motion. Flame the loop.

C- **Streaking Yet Again:** Rotate the plate nearly 90° and streak again using the same wrist motion. Be sure to cool the loop prior to streaking. Flame again.

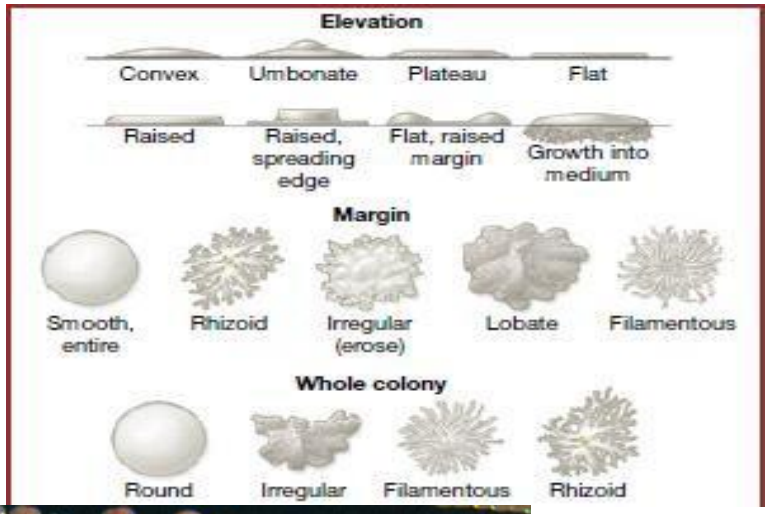
D-**Streaking Into the Center:** After cooling the loop, streak one last time into the center of the plate. Flame the loop. **Then**

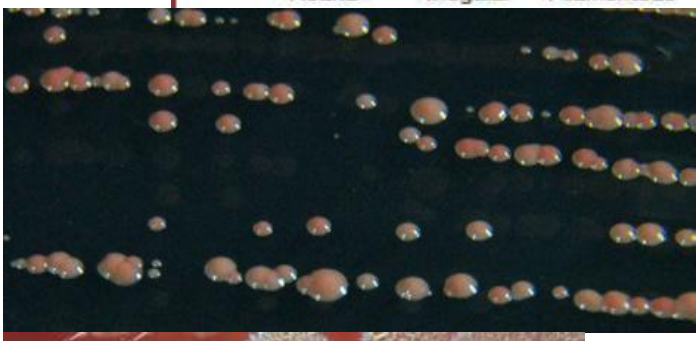
8- Incubate the plate in an inverted position for then assigned time at the appropriate temperature at 37°C at for 24 to 48 hours (incubate temperature depended on type of isolation from soil, water, plants, and animals including humans). after incubation , for isolation and identification of bacteria depended on

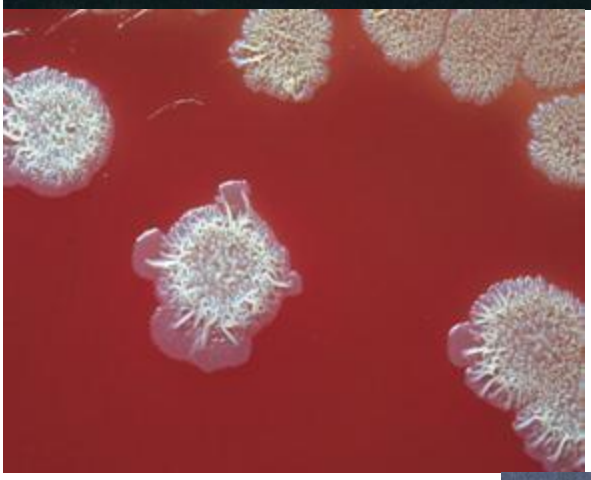
1- examine colony morphology on the solid media plate, this including : colony shape, margin (edge), elevation, texture, and color. such as figure below.

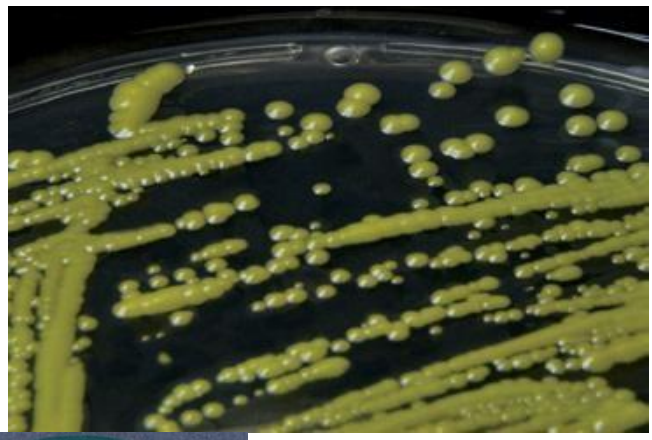
2- Examine shape and arrangement of bacterial cell under microscope after smear preparation and staining, explain in the next lab .

Microbiology Lab ( )..... Lecturer : khulood A.H.....second stage









Critical thinking:-

1- Any method to sterilize Wire loop and any portion in this method (discuses the practical methods)?

2-Petri dishes (after inoculation) are incubated in an inverted position (top down)Why?

❖ Bacterial staining :-

Visualization of microorganisms in the living state is quite difficult, not only because they are minute, but also because they are transparent and practically colorless when suspended in an aqueous medium . To study their properties and to divide microorganisms into specific groups for diagnostic purposes, biological stains and staining procedures in conjunction with light microscopy have become major tools in microbiology.

- Chemical Basis:-

a stain (dye) may be defined as an organic compound containing a benzene ring plus a chromophore and an auxochrome group(**Figure 1**) , The ability of a stain to bind to macromolecular cellular components such as proteins or nucleic acids depends on the electrical charge found on the chromogen portion, as well as on the cellular component to be stained.

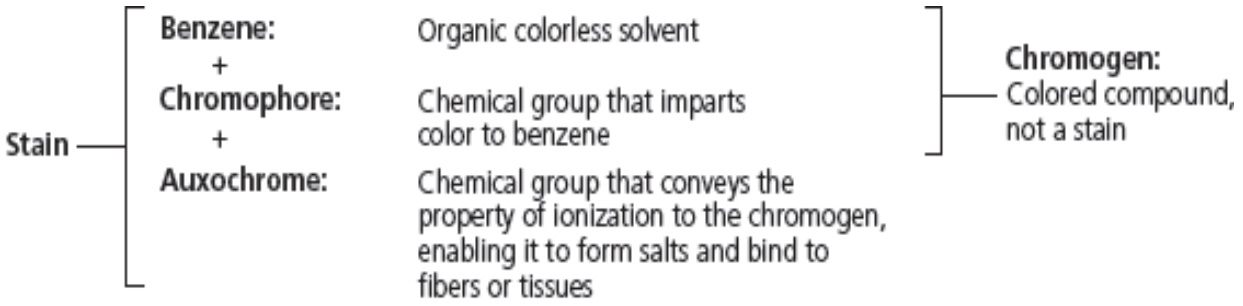


Figure 1 :- Chemical composition of a stain

Bellow, a summary of acidic and basic stains is outlined in Figure 2 .

✓ **Acidic stains** are anionic, which means that, on ionization of the stain, the chromogen portion exhibits a negative charge and therefore has a strong affinity for the positive constituents of the cell. Proteins, positively charged cellular components, will readily bind to and accept the color of the negatively charged, **anionic chromogen** of an **acidic stain**.

- Structurally, like Eosin stain is an example of an acidic stain that produces **Eosinate**$^-$ an anionic chromogen,

Eosin stain:

$$\text{Eosin} \rightleftharpoons \text{Sodium}^+ + \text{Eosinate}^-$$

(Anionic chromogen)

✓ **Basic stains** are cationic, because on ionization the chromogen portion exhibits a positive charge and therefore has a strong affinity for the negative constituents of the cell.

Nucleic acids, negatively charged cellular components, will readily bind to and accept the color of the positively charged, **cationic chromogen** of **a basic stain**.

-Structurally, methylene blue is a basic stain that produces **methylene**$^{+}$ a cationic chromogen

Methylene blue $\rightleftharpoons$ methylene^{+} + Chloride^{-}

cationic chromogen

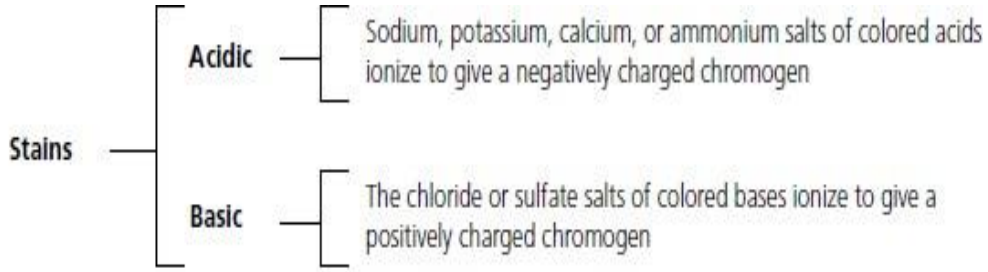


Figure 2:- Acidic and basic stains

▪ **Basic stains** are more commonly used for bacterial staining. The presence of a negative charge on the bacterial surface acts to repel most acidic stains and thus prevent their penetration into the cell.

❖ **Numerous staining techniques are available for :-**

1. visualization
2. differentiation, and
3. separation of bacteria in terms of morphological characteristics

and cellular structures.

A summary of commonly used procedures and their purposes is outlined in **Figure3**

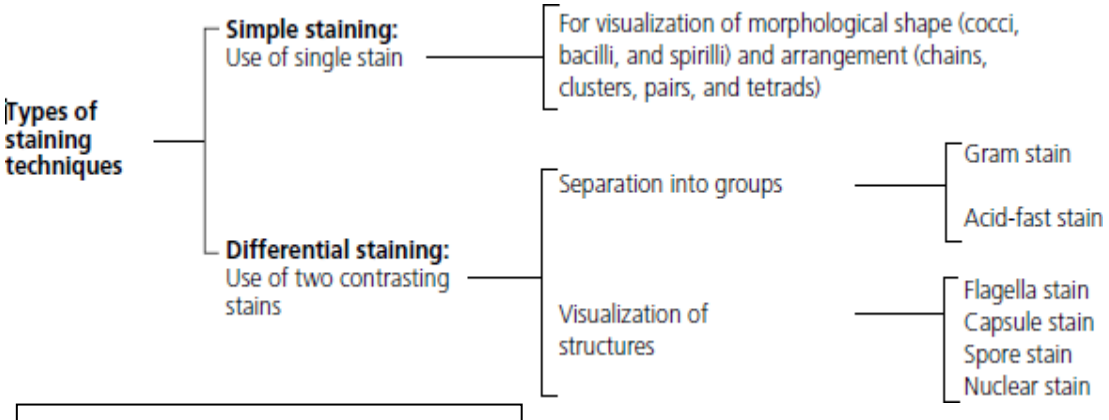


Figure3: **Staining techniques**

❖ Techniques of smear preparation (preparation of bacteial smear):-

▪ Materials

- Cultures

Twenty-four–hour nutrient agar slant culture and a 24-hour nutrient broth culture of any bacteial growth

- Equipment

1. Glass microscope slides
2. Bunsen burner,
3. inoculating loop, and
4. glassware marking pencil.

✓ Smears from a Broth Medium

-Procedure

Label three clean slides with the initials of the organism, and number them 1, 2, and 3. Resuspend the sedimented cells in the broth culture by tapping the culture tube with your finger. The next four steps of this procedure are illustrated

1. With a sterile loop, place one loopful of culture on Slide 1, two loopfuls on Slide 2, and three loopfuls on Slide 3, respectively.

2. With a circular movement of the loop, spread the cell suspension into an area approximately the size of a dime.

3. Allow the slide to air-dry completely. This may be done by placing the slide on a drying tray attached to a microincinerator or by placing the slide on the bench.

4. Heat fix the preparation. Note: Pass the airdried slide in front of the entrance to the microincinerator or pass the slide through the outer portion of the Bunsen flame to prevent overheating, which can distort the morphology through plasmolysis of the cell wall.

-Examine each slide under microscope and record your results in the Lab Report.

✓ Smears from a Solid Medium

-Procedure

Label four clean slides with the initials of the organism. Label Slides 1 and 2 with an L for loop, and Slides 3 and 4 with an N for needle. The next four steps of this procedure are illustrated in

1. Using a loop, place one to two loops of water on each slide.

2. With a sterile loop, touch the entire loop to the culture and emulsify the cells in water on Slide, Then, with a sterile loop, just touch the tip of the loop to the culture and emulsify it in the water on Slide 2. Repeat Steps 1 and 2 using a sterile inoculating needle on Slides 3 and 4.

3. Allow all slides to air-dry completely. This may be done by placing the slide on a drying tray attached to a microincinerator or by placing the slide on the bench.

4. Heat fix the preparation , pass the slide through the outer portion of the Bunsen flame to prevent overheating, which can distort the morphology through plasmolysis of the cell wall.

-Examine each slide under microscope and record your results in the Lab Report.

- **Heat fixing**:- kills the bacteria and the coagulated proteins from the cells will cause cells to stick to the slide. Fixing denatures bacterial enzymes , preventing them from digesting cell parts , which causes the cell to break , a process called autolysis.

✓ Critical thinking:-

1. How does the heaviness of a bacterial smear affect its microscopic analysis?

2. Why should you be careful not to underheat a smear during the heat-fixing process?

3. What is heat fixation? How is it carried out?

❖ **Type of Bacterial Staining:-**

- **1- Simple staining** :-

Principle

In **simple staining**, the bacterial smear is stained with a single reagent, which produces a distinctive contrast between the organism and its background. Basic stains with a positively charged chromogen are preferred because bacterial nucleic acids and certain cell wall components carry a negative charge that strongly attracts and binds to the cationic chromogen.

Clinical Application :-

Simple stains are relatively quick and useful methods of testing for the presence of, determining the shape of, or determining the numbers of bacteria present in a sample. Generally involving a single staining step, simple staining methods are not considered differential or diagnostic and will have limited uses. However, this is a quick procedure for determining whether a clinical sample has the presence of a foreign bacterial pathogen.

The purpose of simple staining is to **elucidate the morphology** and **arrangement of bacterial cells** .

The most commonly used basic stains are

- ✓ methylene blue,
- ✓ crystal violet, and
- ✓ carbol fuchsin.

- **reagents**

Methylene blue, crystal violet, and carbol fuchsin.

- **Equipment**

✓ Bunsen burner, loop, staining tray, microscope, lens paper, and glass slides.

- **Procedure**

Prepare separate bacterial smears of the organisms and following the procedure described in bellow.

Note: All smears must be heat fixed prior to staining.

The following steps are illustrated in **Figure 4a**.

1. Place a slide on the staining tray and flood the smear with one of the indicated stains, using the appropriate exposure time for each: carbol fuchsin, 15 to 30 seconds; crystal violet, 20 to 60 seconds; methylene blue (shown in Figure 4 a), 1 to 2 minutes.

2. *Gently* wash the smear with tap water to remove excess stain. During this step, hold the slide parallel to the stream of water; in this way you can reduce the loss of organisms from the preparation.

3. Using bibulous paper, blot dry, but *do not* wipe the slide.

4. Repeat this procedure with the remaining two organisms, using a different stain for each.

5. Examine all stained slides under oil immersion.

6. In the chart provided in the **Lab Report 1**, complete the following:

a. Draw a representative field for each organism. Refer to page 16 for proper drawing procedure.

b. Describe the morphology of the organisms with reference to their shapes (bacilli, cocci, spirilla) and arrangements (chains, clusters, pairs) as **Figure 4b**.

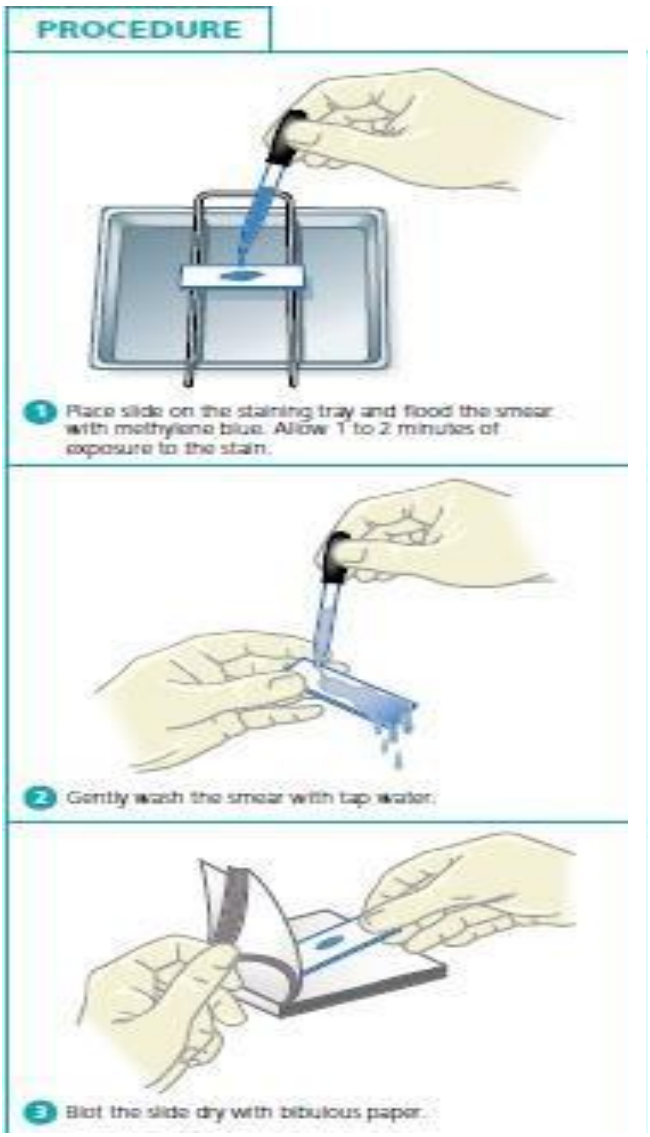
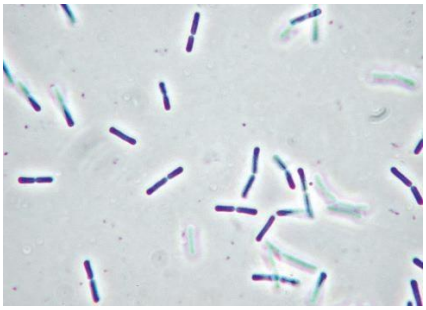
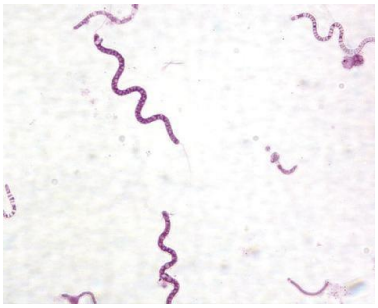


Figure 4a:- Simple staining procedure

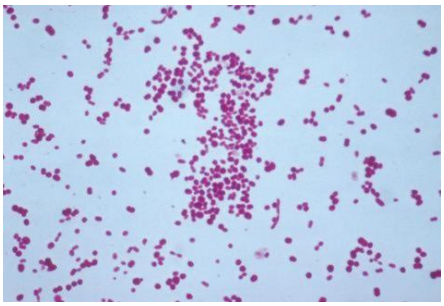
Figure 4 b :-



(a) Bacilli and diplobacilli (rod-shaped) bacteria



(b) Spirilla (spiral-shaped) bacteria



(c) Cocci (spherical-shaped) bacteria: *Staphylococcus*

Name: _____

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1

Lab Report

Observations and Results

	Methylene Blue	Crystal Violet	Carbol Fuchsin
Draw a representative field.			
Organism			
Cell morphology:			
Shape			
Arrangement			
Cell color			

- **Critical thinking :-**

1. Why are basic dyes more effective for bacterial staining than acidic dyes?

- **2-Negative staining:-**

Principle:-

Negative staining requires the use of an acidic stain such as India ink or nigrosin. The acidic stain,with its negatively charged chromogen, will not penetrate the cells because of the negative charge on the surface of bacteria. Therefore, the unstained cells are easily discernible against the colored background.

The practical application of negative staining is twofold:-

-First, since heat fixation is not required and the cells are not subjected to the distorting effects of chemicals and heat, their natural size and shape can be seen.

-Second, it is possible to observe bacteria that are difficult to stain, such as some spirilla. Because heat fixation is not done during the staining process, keep in mind that the organisms are not killed and *slides should be handled with care.* **Figure 5b** shows a negative stain of bacilli.

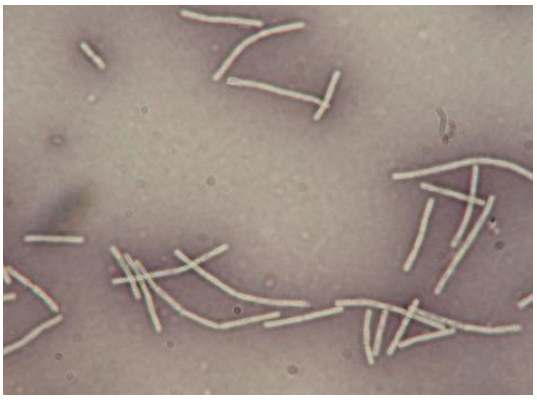


Figure 5b: negative staining: Bacilli (1000×)

Clinical Application :-

Detecting Encapsulated invaders

The principle application of negative staining is to determine if an organism possesses a capsule (agelatinous outer layer that makes the microorganism more virulent), although it can also be used to demonstrate spore formation. The technique is frequently used in the identification of fungi such as *Cryptococcus neoformans*, an important infectious agent found in bird dropping that is linked to meningeal and lung infections in humans.

Materials

- **Cultures**

Twenty-four–hour agar slant cultures of *Micrococcus luteu* or *Bacillus cereus*, and other alternate bacterial cultures.

- **reagent**

Nigrosin. or indian ink

- **Equipment**

Bunsen burner, loop, staining tray, glass slides, lens paper, and microscope.

- **Procedure**

Steps 1–4 are illustrated in **Figure 5a**.

1. Place a small drop of nigrosin close to one end of a clean slide.

2. Using aseptic technique, place a loopful of inoculum from the bacteial culture in the drop of nigrosin and mix.

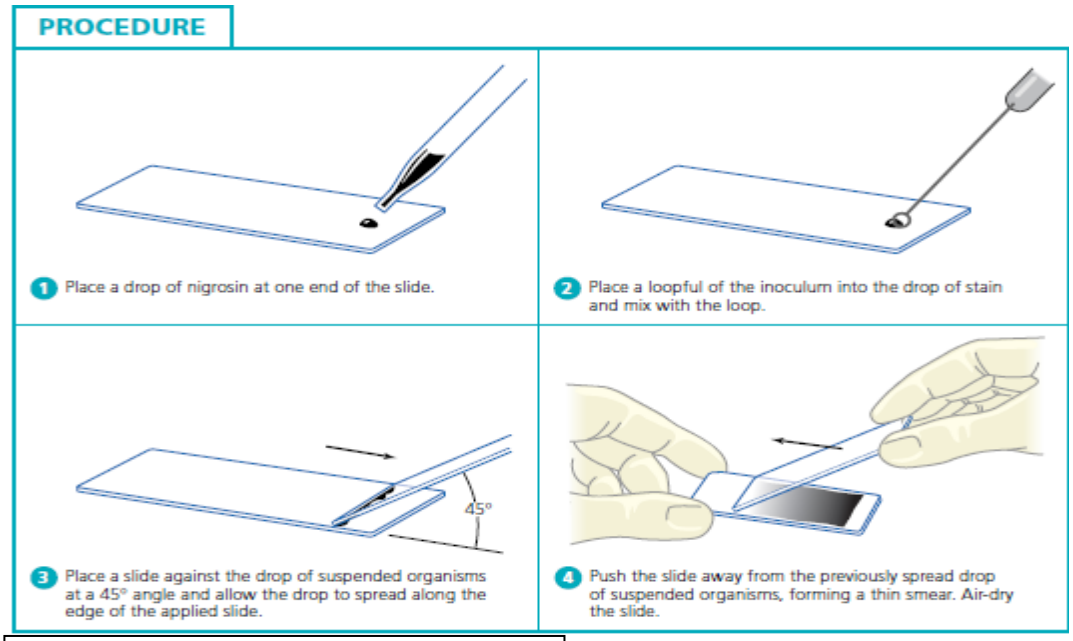
3. Place a slide against the drop of suspended organisms at a 45° angle and allow the drop to spread along the edge of the applied slide.

4. Push the slide away from the drop of suspended organisms to form a thin smear. Air-dry.

Note: Do not heat fix the slide.

5. Repeat Steps 1–4 for slide preparations of the remaining cultures.

6. Examine the slides under oil immersion.



. **Figure 5a:** negative staining procedure

✓ Critical thinking:-

1. Why can't methylene blue be used in place of nigrosin for negative staining? Explain.

2. What is the principle application of negative staining?

3. Why must slides be carefully handled during the negative staining process?

3- Gram stain (Differential staining)

Principle:

Differential staining requires the use of at least **four chemical reagents (discus bellow)** that are applied sequentially to a heat-fixed smear.

-The first reagent is called the **primary stain (Crystal Violet (hucker's))** This violet stain is used first and stains all cells purple. Its function is to impart its color to all cells.

-The second stain is a **mordant(Gram's iodine)**This reagent serves not only as a killing agent but also as a mordant, a substance that increases the cells' affinity for a stain) used to intensify the color of the primary stain. In order to establish a color contrast.

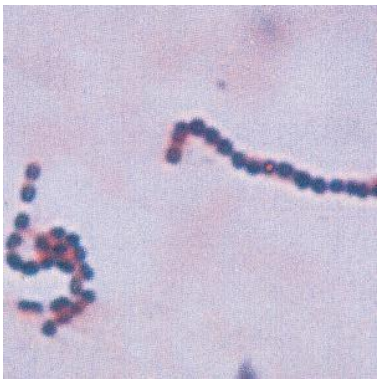
-The third reagent used is the **decolorizing agent** (Ethyl Alcohol, 95%) This reagent serves a dual function as a protein-dehydrating agent and as a lipid solvent. Its action is determined by two factors, the concentration of lipids and the thickness of the peptidoglycan layer in bacterial cell walls.

-The final reagent, the **counterstain** (**Safranin**) This is the final reagent, used to stain pink those cells that have been previously decolorized. Since only gram-negative cells undergo decolorization, they may now absorb the counterstain.

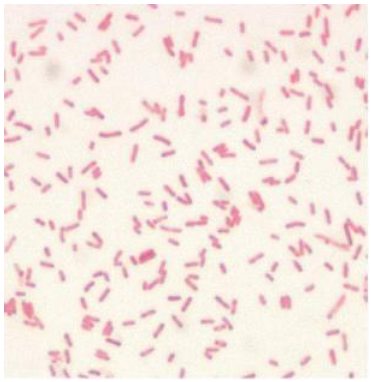
- The most important differential stain used in bacteriology is the **Gram stain**, named after Dr. Hans Christian Gram. **It divides bacterial cells into two major groups, gram positive and gram negative**, which makes it an essential tool for classification and differentiation of microorganisms. **Figure 6B** shows gram-positive and gram-negative cells

- The Gram stain reaction is based on the difference in the **chemical composition of bacterial cell walls**. Gram-positive cells have a thick peptidoglycan layer, whereas the peptidoglycan layer in gram-negative cells is much thinner and surrounded by outer lipid containing layers.

Figure 6B: Gram-stained cells



(a) Gram-positive stain of streptococci



(b) Gram-negative stain of *E. coli*

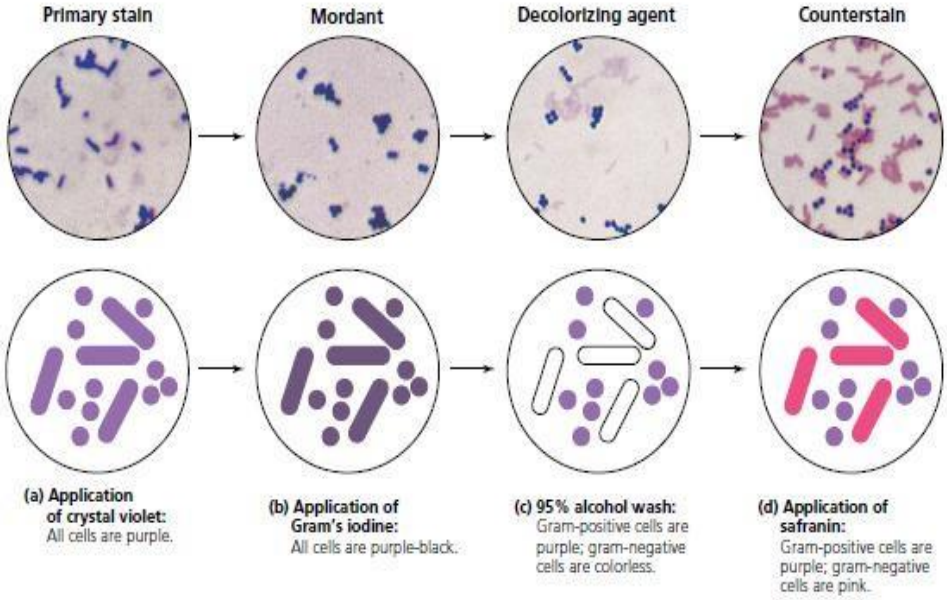


Figure 6C:- Microscopic observation of cells following steps in the Gram staining procedure

- **Materials:-**
- **Cultures:**

Twenty-four–hour nutrient agar slant cultures of *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus.*

- **reagents:**

Crystal violet, Gram's iodine, 95% ethyl alcohol,and safranin.

- **Equipment:**

Bunsen burner, inoculating loop, staining tray, glass slides, lens paper, and microscope.

- **Procedure:**

-The following steps are shown in **Figure 6a:**

1. Smear Preparation and allow smears to air-dry and then heat fix in the usual manner

2. *Gently* flood smears with crystal violet and let stand for 1 minute.

3. *Gently* wash with tap water.
4. *Gently* flood smears with the Gram's iodine mordant and let stand for 1 minute.
5. *Gently* wash with tap water.
6. Decolorize with 95% ethyl alcohol. *Note: Do not over-decolorize.* Add reagent drop by drop until the alcohol runs almost clear, showing only a blue tinge.
7. *Gently* wash with tap water.
8. Counterstain with safranin for 45 seconds.
9. *Gently* wash with tap water.
10. Blot dry with bibulous paper and examine under oil immersion.
11. As you observe each slide under oil immersion, complete the chart provided in the Lab Report.

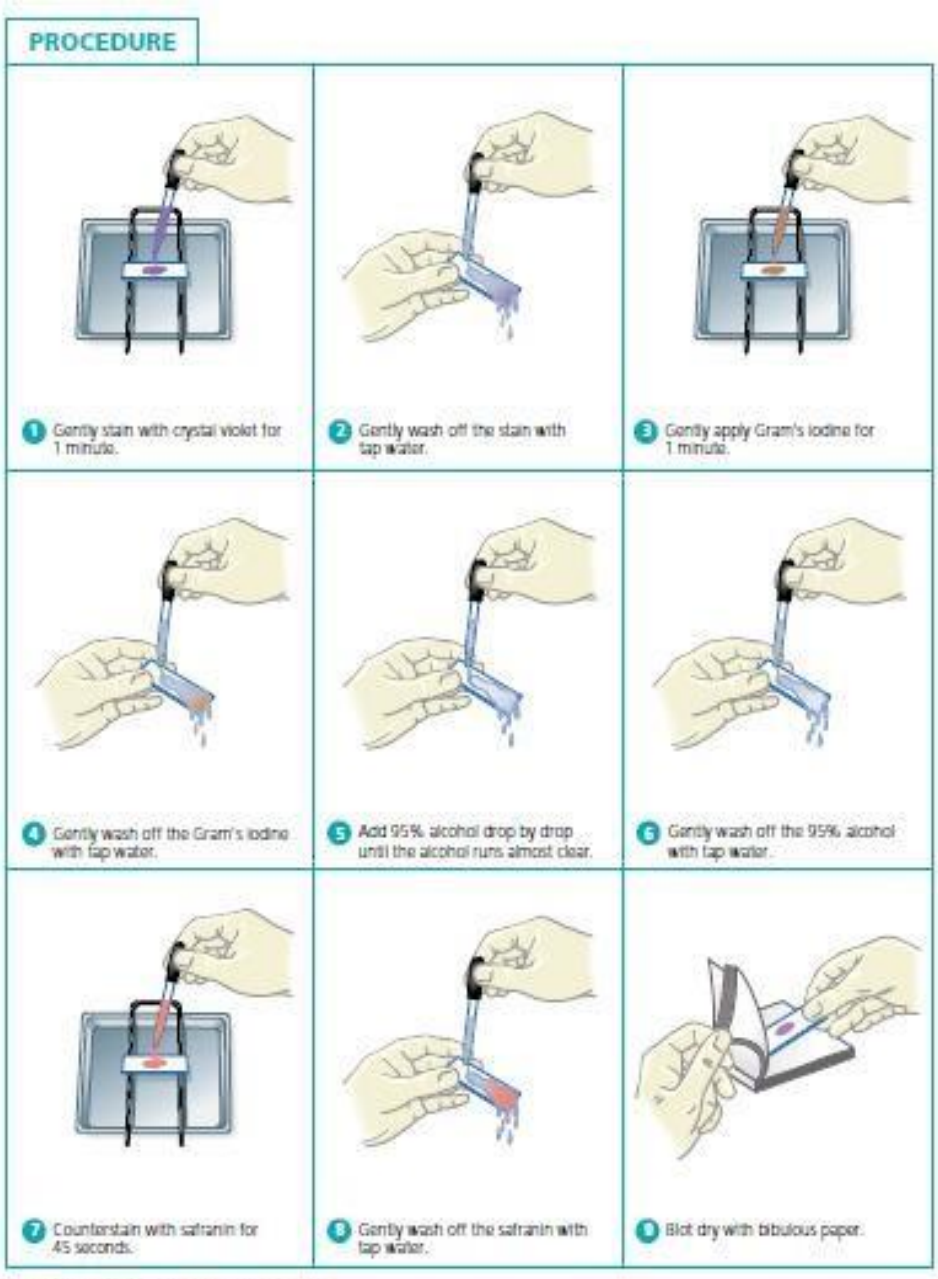


Figure 6a: Gram staining procedure

2

Name:_____

Date:_____ Section:_____

Lab Report

Observations and Results

	E. coli	*B. cereus*	*S. aureus*	*Mixture*
Draw a representative field.				
Cell morphology: Shape Arrangement Cell color Gram reaction				

✓ **Critical thining:-**

1. Explain why only gram-negative cells undergo decolorization during the Gram staining procedure.

2. Cite the purpose of each of the following reagents in a differential staining procedure.

-a. Primary stain:

-b. Mordant:

-c. Decolorizing agent:

-d. Counterstain:

4. What might happen if the Gram staining procedure is performed on a culture incubated for a little over a day?

4- Acid fast stain:-

- **Principle:-**

While the majority of bacterial organisms are stainable by either simple or Gram staining procedures, a few genera, particularly the members of the genus *Mycobacterium,* are visualized more clearly by the **acid-fast** method. Since *M. tuberculosis* and *M. leprae* represent bacteria that are pathogenic to humans, the stain is of diagnostic value in identifying these organisms. The characteristic difference between mycobacteria and other microorganisms is the presence of a thick, waxy (lipoidal) wall that makes penetration by stains extremely difficult.

Mycobacteria tend to clump together, and it is difficult to identify individual cells in stained preparations if this clumping effect occurs. **The acid-fast stain uses three different reagents.**

-Primary Stain

Carbol Fuchsin Unlike cells that are easily stained by ordinary aqueous stains, most species of mycobacteria are not stainable with common dyes such as methylene blue and crystal violet. Carbol fuchsin, a dark red stain in 5% phenol that is soluble in the lipoidal materials that constitute most of the mycobacterial cell wall, does penetrate these bacteria and is retained. Penetration is further enhanced by the application of heat, which drives the carbol fuchsin through the lipoidal wall and into the cytoplasm. This application of heat is used in the **Ziehl-Neelsen method**. The **Kinyoun method**, a modification of the Ziehl-Neelsen method, circumvents the use of heat by addition of a wetting agent (Tergitol®) to this stain, which reduces surface tension between the cell wall of the mycobacteria and the stain. Following application of the primary stain, all cells will appear red.

- Decolorizing Agent

Acid-Alcohol (3% hCl + 95% Ethanol) Prior to decolorization, the smear is cooled, which allows the waxy cell substances to harden. On application of acid-alcohol, acid-fast cells will be resistant to decolorization since the primary stain is more soluble in the cellular waxes than in the decolorizing agent. In this event, the primary stain is retained

and the mycobacteria will stay red. This is not the case with non–acid-fast organisms, which lack cellular waxes. The primary stain is more easily removed during decolorization, leaving these cells colorless or unstained.

- Counterstain

Methylene Blue This is used as the final reagent to stain previously decolorized cells. As only non–acid-fast cells undergo decolorization, they may now absorb the counterstain and take on its blue color, while acid-fast cells retain the red of the primary stain.

✓ **At the bench:-**

- **Materials**
  - **Cultures:-**

Seventy-two– to 96-hour Trypticase™ soy broth culture of *Mycobacterium smegmatis* and 18- to 24-hour culture of *Staphylococcus aureus* BSL -2 .

- **reagents:**

Carbol fuchsin, acid-alcohol, and methylene blue.

- **Equipment:**

Bunsen burner, hot plate, 250-ml beaker, inoculating loop, glass slides, lens paper, staining tray, and microscope.

Procedure:-

Steps 1–7 are pictured in **Figure 7a**

1-Smear Preparation and allow smears to air-dry and then heat fix in the usual manner.

2. a. Flood smears with carbol fuchsin and place over a beaker of water on a warm hot plate, allowing the preparation to steam for 5 minutes. *Note: Do not allow stain to evaporate; replenish stain as needed. Also, prevent stain from boiling by adjusting the hot-plate temperature.*

b. For a heatless method, flood the smear with carbol fuchsin containing Tergitol® for 5 to 10 minutes.

2. Wash with tap water. Heated slides must be cooled prior to washing.

3. Decolorize with acid-alcohol, adding the reagent drop by drop until the alcohol runs almost clear with a slight red tinge.

4. Wash with tap water.

5. Counterstain with methylene blue for 2 minutes.

6. Wash smear with tap water.

7. Blot dry with bibulous paper and examine under oil immersion.

8. In the chart provided in the Lab Report, complete the following:

a. Draw a representative microscopic field for each preparation.

b. Describe the cells according to their shapes and arrangements.

c. Describe the color of the stained cells.

d. Classify the organisms as to reaction: acidfast or non–acid-fast.
Refer to **Figure 7b** for a photograph of an acid-fast stain.

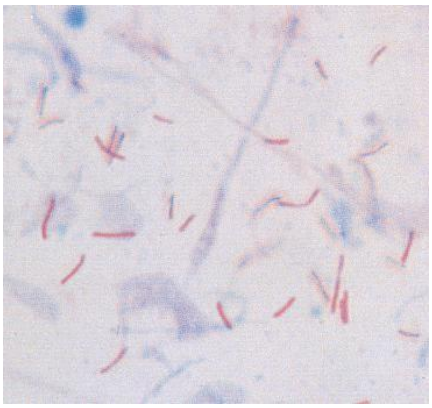


Figure 7b:- Acid-fast stain of mycobacteria.

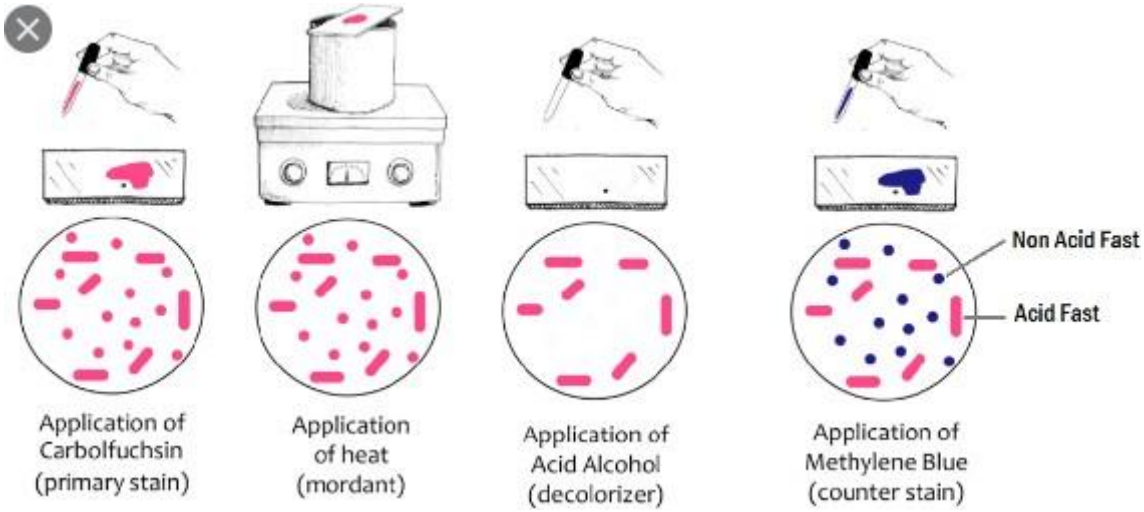


Figure 7a: acid fast stain procedure

3

Name: _____

Date: _____ Section: _____

Lab Report

Observations and Results

	M. smegmatis	*S. aureus*	Mixture
Draw a representative field.			
Cell morphology: Shape Arrangement Cell color Acid-fast reaction			

✓ Critical thinking:-

1. Why must heat or a surface-active agent be used with application of the primary stain during acid-fast staining?

2. Explain the importance of using methylene blue as the counterstain in the acid-fast staining method

3.Why is the application of heat or a surface-active agent not required during the application of the counterstain in acid-fast staining?

❖ Physical and Chemical agents for the Control of Microbial Growth:-

Physical Methods for Control of Microbial Growth:

The modes of action of the different physical agents of control vary, although they all produce damaging effects to one or more essential cellular structures or molecules in order to cause cell death or inhibition of growth such as **heat (**Explain beforehand in lab. 2). Sites of damage that can result in malfunction are :-

1. Cell-wall injury: Failure to synthesize a missing segment of the cell wall results in an unprotected protoplast.

2. Cell-membrane damage: This may be the result of lysis of the membrane, which will cause immediate cell death

3. Alteration of the colloidal state of cytoplasm:

Certain agents cause denaturing of cytoplasmic proteins. Denaturing processes are responsible for enzyme inactivation and cellular death

4. Inactivation of cellular enzymes

5. Interference with the structure and function of the DNA molecule

Chemical Methods for Control of Microbial Growth such as :-

1. Antiseptics

2. Disinfectants

Explain beforehand in lab. 2

3. Chemotherapeutic agents:

Chemotherapeutic agents are chemical substances used in the treatment of infectious diseases (that destroy or inhibit the growth of microorganisms in living tissues). Their mode of action is to interfere with microbial metabolism, thereby producing a **bacteriostatic** or **bactericidal** effect on the microorganisms,without producing a like effect in host cells.Chemotherapeutic agents act on a number of cellular targets.

Their mechanisms of action include

1-inhibition of cell-wall synthesis

2- inhibition of protein synthesis

3- inhibition of nucleic acid synthesis

4-disruption of the cell membrane, and

5- inhibition of folic acid synthesis.

These drugs can be separated into two categories:

1. Antibiotics are synthesized and secreted by some true bacteria, actinomycetes, and fungi that destroy or inhibit the growth of other microorganisms. Today, some antibiotics are laboratory synthesized or modified; however, their origins are living cells.

2. Synthetic drugs are synthesized in the laboratory. To determine a therapeutic drug of choice, it is important to determine its mode of action, possible adverse side effects in the host, and the scope of its antimicrobial activity. The specific mechanism of action varies among different drugs, and the short-term or long-term use of many drugs can produce systemic side effects in the host. These vary in severity from mild and temporary upsets to permanent tissue damage (**Table 2**).

TABLE 2 Prototypic Antibiotics

ANTIBIOTIC	MODE OF ACTION	POSSIBLE SIDE EFFECTS
Penicillin	Prevents transpeptidation of the *N*-acetylmuramic acids, producing a weakened peptidoglycan structure	Penicillin resistance; sensitivity (allergic reaction)
Streptomycin	Has an affinity for bacterial ribosomes, causing misreading of codons on mRNA, thereby interfering with protein synthesis	May produce damage to auditory nerve, causing deafness
Chloramphenicol	Has an affinity for bacterial ribosomes, preventing peptide bond formation between amino acids during protein synthesis	May cause aplastic anemia, which is fatal because of destruction of RBC-forming and WBC-forming tissues
Tetracyclines	Have an affinity for bacterial ribosomes; prevent hydrogen bonding between the anticodon on the tRNA–amino acid complex and the codon on mRNA during protein synthesis	Permanent discoloration of teeth in young children
Bacitracin	Inhibits cell-wall synthesis	Nephrotoxic if taken internally; used for topical application only
Polymyxin	Destruction of cell membrane	Toxic if taken internally; used for topical application only
Rifampin	Inhibits RNA synthesis	Appearance of orange-red urine, feces, saliva, sweat, and tears
Quinolone	Inhibits DNA synthesis	Affects the development of cartilage

✓ **Principle:-**

A standardized diffusion procedure with filterpaper discs on agar, known as the **Kirby-Bauer method**, is frequently used to determine the drug susceptibility of microorganisms isolated from infectious processes.

✓ **Media**

Per designated student group: seven Mueller- Hinton agar plates.

✓ **Antimicrobial-Sensitivity Discs**

Penicillin G, 10 μg; streptomycin, 10 μg; tetracycline, 30 μg; chloramphenicol, 30 μg; gentamicin, 10 μg; vancomycin, 30 μg; and sulfanilamide, 300 μg.

✓ **Equipment**

Sensi-Disc™ dispensers or forceps, microincinerator or Bunsen burner, sterile cotton swabs, glassware marking pencil, 70% ethyl alcohol, and millimeter ruler.

✓ Procedure:-

1. Place agar plates right side up in an incubator heated to 37°C for 10 to 20 minutes with the covers adjusted so that the plates are slightly opened, allowing the plates to warm up and the surface to dry.

2. Label the bottom of each of the agar plates with the name of the test organism to be inoculated.

3. Using aseptic technique, inoculate all agar plates with their respective test organisms as follows:

a. Dip a sterile cotton swab into a well-mixed saline test culture and remove excess inoculum by pressing the saturated swab against the inner wall of the culture tube.

b. Using the swab, streak the entire agar surface horizontally, vertically, and around the outer edge of the plate to ensure a heavy growth over the entire surface.

4. Allow all culture plates to dry for about 5 minutes.

5. Using the Sensi-Disc dispenser, apply the antibiotic discs by placing the dispenser over the agar surface and pressing the plunger, depositing the discs simultaneously onto the agar surface (**Figure 8**, Step 1a). Or, if dispensers are not available, distribute the individual discs at equal distances with forceps dipped in alcohol and flamed (**Figure 8**, Step 1b).

6. Gently press each disc down with the wooden end of a cotton swab or with sterile forceps to ensure that the discs adhere to the surface of the agar (**Figure 8**, Step 2). *Note: Do not press the discs into the agar.*

7. Incubate all plate cultures in an inverted position for 24 to 48 hours at 37°C.

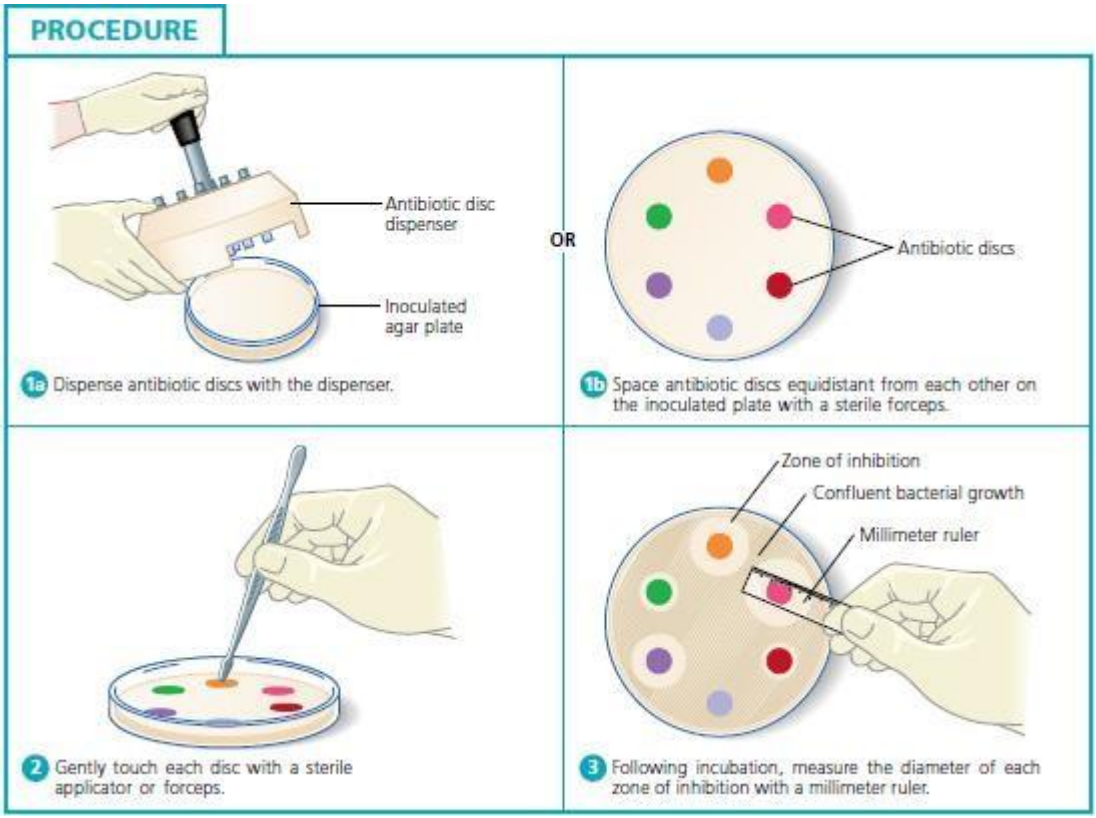


Figure 8:- Kirby-Bauer antibiotic sensitivity procedure

TABLE 3 Zone Diameter Interpretive Standards for Organisms Other Than *Haemophilus* and *Neisseria gonorrhoeae*

		ZONE DIAMETER, NEAREST WHOLE MM		
ANTIMICROBIAL AGENT	DISC CONTENT	RESISTANT	INTERMEDIATE	SUSCEPTIBLE
Ampicillin				
when testing gram-negative bacteria	10 µg	≤13	14–16	≥17
when testing gram-positive bacteria	10 µg	≤28	—	≥29
Carbenicillin				
when testing *Pseudomonas*	100 µg	≤13	14–16	≥17
when testing other gram-negative organisms	100 µg	≤19	20–22	≥23
Cefoxitin	30 µg	≤14	15–17	≥18
Cephalothin	30 µg	≤14	16–17	≥18
Chloramphenicol	30 µg	≤12	13–17	≥18
Clindamycin	2 µg	≤14	15–20	≥21

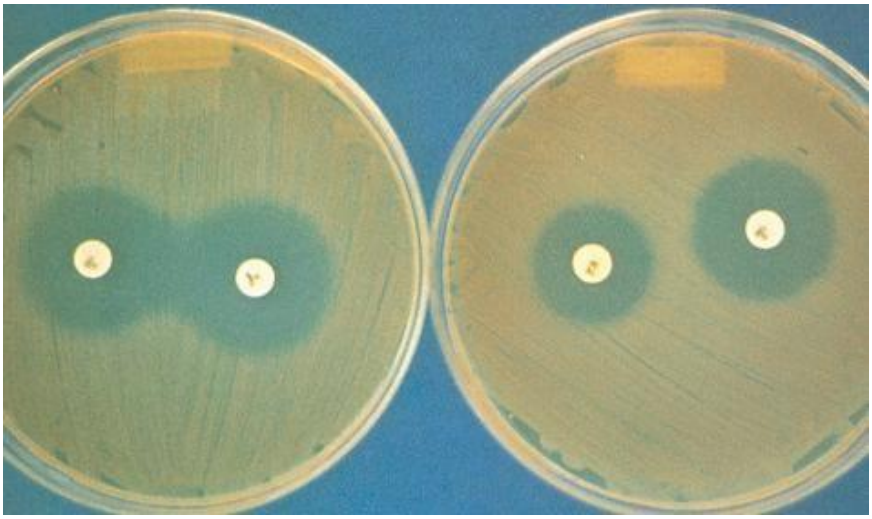


Figure 8: Kirby-Bauer antibiotic sensitivity test.

- Reference:-

1- Cappuccino J.G.; and Welsh C. (2018). Microbiology a laboratory manual, Pearson Education Limited.

2- Mahon C.R.;and Lehman D.C.(2015) .Textbook of Diagnostic Microbiology. SAUNDERS ELSEVER.

3- Satish G.M.(2010).Medical Microbiology (Including Parasitology). Jitendar P Vij.

Medical Parasitology:

- **Medical parasitology** is the study of the parasites which cause disease in man. Here, as a matter of fact we study host/parasite relationship, geographical distribution, habitat, morphology, lifecycle, mode of infection, disease manifestations, host response, laboratory diagnosis, treatment.
- **parasite** a living organism which gets nourishment from another living organism (Host) where it lives is called parasite, may be:

❖ Ectoparasite (living on the surface of other organisms lice, ticks, mites, etc.)

❖ Endoparasites (lives inside the body of other organism, e.g. *Entamoeba histolytica, Ascaris lumbricoides*, etc.)

❖ Obligate parasite (who must spend some part of their life cycle in or on host, e.g. plasmodium).

❖ Facultative parasite (may be free living but can obtain the nutrition from hosts too).

- **Host** An organism which harbors the parasite. Host may be of following types:

❖ Definitive host (when it harbors parasite in adult form or where parasite utilizes sexual method of reproduction).

❖ Intermediate host (harbor's larval stages of parasite).

❖ Natural host (which is naturally infected with certain species of parasite).

❖ Accidental host (which is by and large under normal circumstances not infected with parasite).

- The parasites divided into two subkingdom are **Protozoa** and **Helminths.**

✓ Protozoa:-

Protozoa are:-

1- unicellular

2-consist of tow membrane bound nucleus and cytoplasm

protozoa are subdivided into four groups :-

1-Amoebae

2-Flagellates

3- Sporozoa , and

4- Ciliates

✓ Helminthes:-

Helminthes are :-
1- multicellular
2- Elongated , bilaterally symmetrical covered with thick cuticle and vary in length.

Helminthes are subdivided into four groups :-

1-Trematodes

2-Cestodes

3- Nematodes

.....

- **Subkingdom**: protozoa

Genus: *Entamoeba*

Species: *Entamoeba histolytica*

Cause disease (amebiasis)

▪ Geographical Distribution

Entamoeba histolytica has been found in all populations throughout the world where search has been conducted. Predominantly infecting humans and other Mammals such as dogs and cats

▪ Habitats

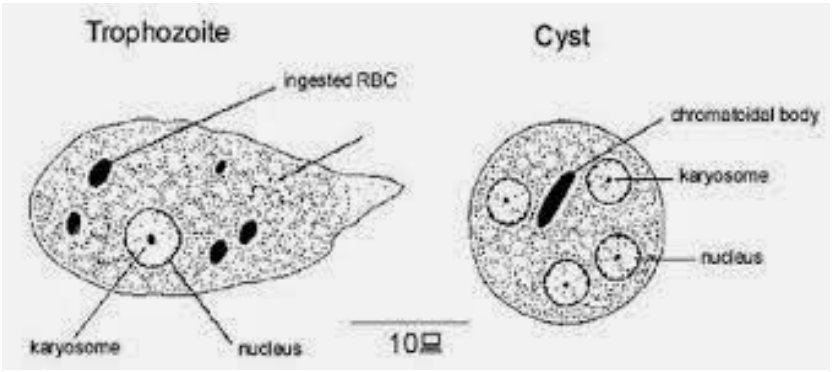
Trophozoites of *Entamoeba histolytica* live in the mucous and submucous layers of large intestine.

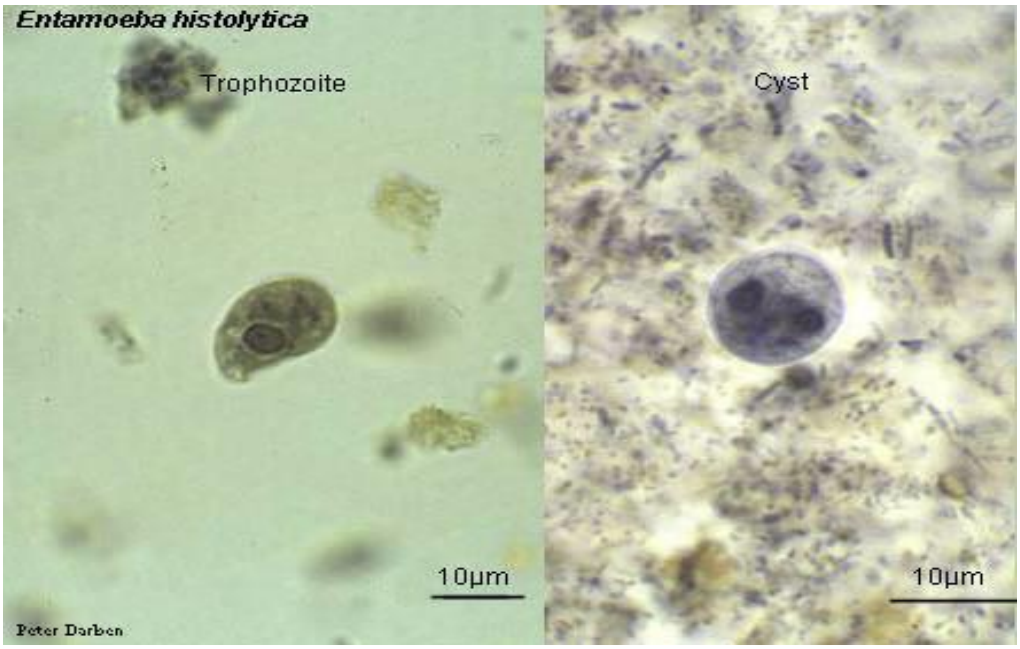
- **Morphology**

Three stages are encountered:-

(a) active ameba trophozoite

(b) inactive cyst and





—**Transmision**: Transmitted by fecal-oral , swallowing cysts in contaminated water or food.

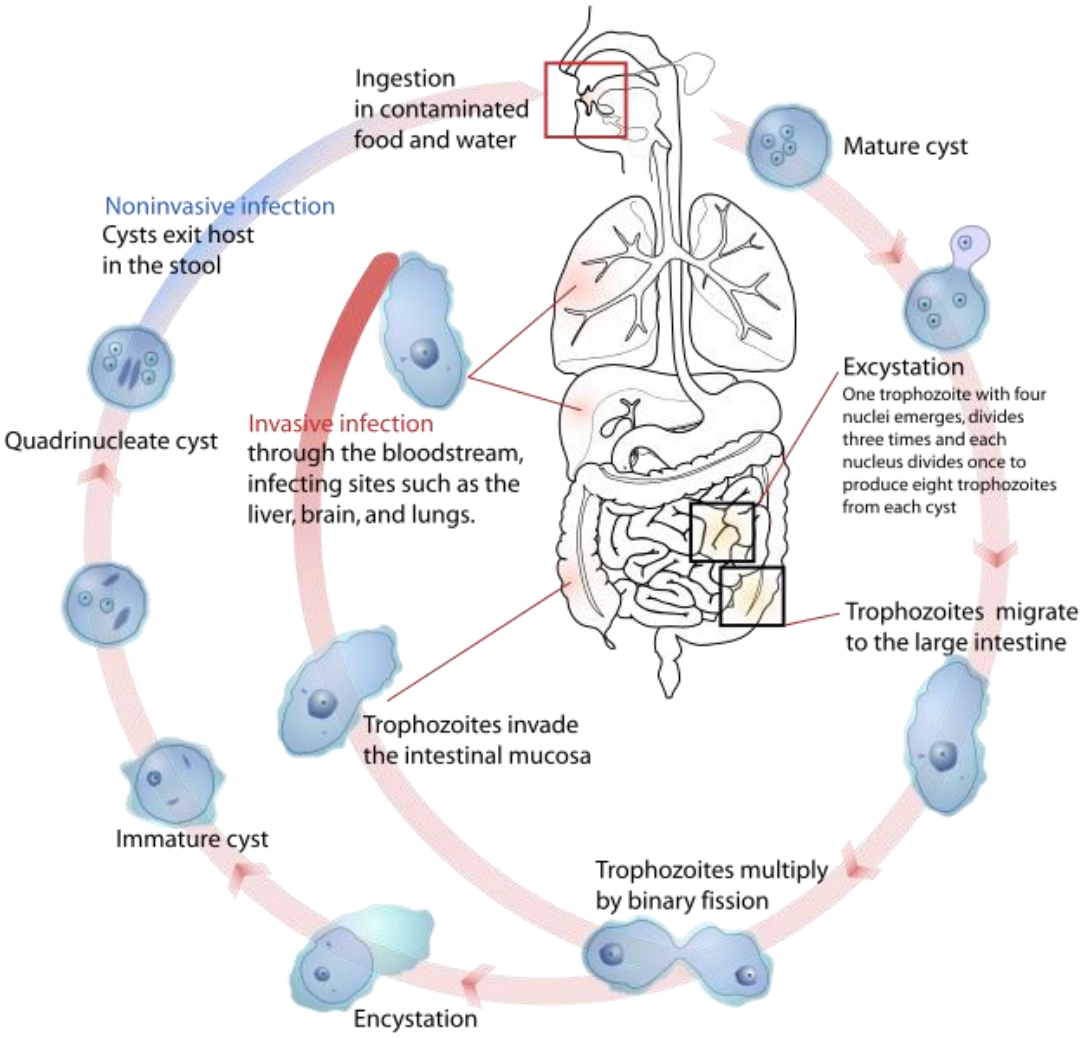
- **Pathology**

• Man is the reservoir of infection. Infections occur by cysts.

• *Entamoeba histolytica* produces dysentery with frequent passing of stools mixed with mucus and blood.

- **Life cycle of *Entamoeba histolytica***

The active (trophozoite) stage exists only in the host and in fresh loose feces; cysts survive outside the host in water, in soils, and on foods, especially under moist conditions on the latter. The cysts are readily killed by heat and by freezing temperatures, and survive for only a few months outside of the host. When cysts are swallowed they cause infections by excysting (releasing the trophozoite stage) in the digestive tract. is pathogenic; infection can be asymptomatic or can lead to amoebic dysentery or amoebic liver abscess.



- **Laboratory Diagnosis**

• Macroscopic examination of stool (dark red stool mixed with blood and mucus).

• Microscopic examination of stool for demonstration of trophozoite or cyst of *Entamoeba histolytica,*

• Proctosigmoidoscopy, scraping and biopsy samples collected under direct vision by endoscopy

• Culture techniques can be done

• Serological techniques

• DNA examination techniques

.....

Subkingdom: protozoa

Genus: ***Giardia***

Species: ***Giardia lamblia***

Cause disease (Giardiasis)

- **Geographical Distribution:-**

It occurs all over the world. It is prevalent in 2to 25 percent population.

- **Habitat:-**

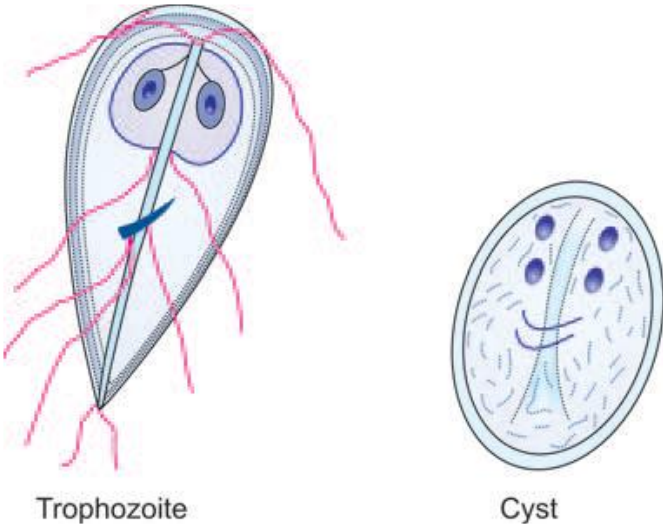
is an aerobes **flagellated protozoan** parasite that colonizes and reproduces in the Duodenum and upper part of small intestine.

- **Morphology:-**

It is found in the following two forms:-

1-**Trophozoite:** It resembles longitudinally-cut pears, The dorsal surfaceis convex and ventral surface is concave.There are a pair of axostyles, two nuclei and 4 pairs of flagellae. It multiplies by binary fission.

2-**Cyst:** Trophozoites are transformed into cysts under unfavorable conditions. The cyst is Oval, Contains 4 nuclei ,They are passed in stools.



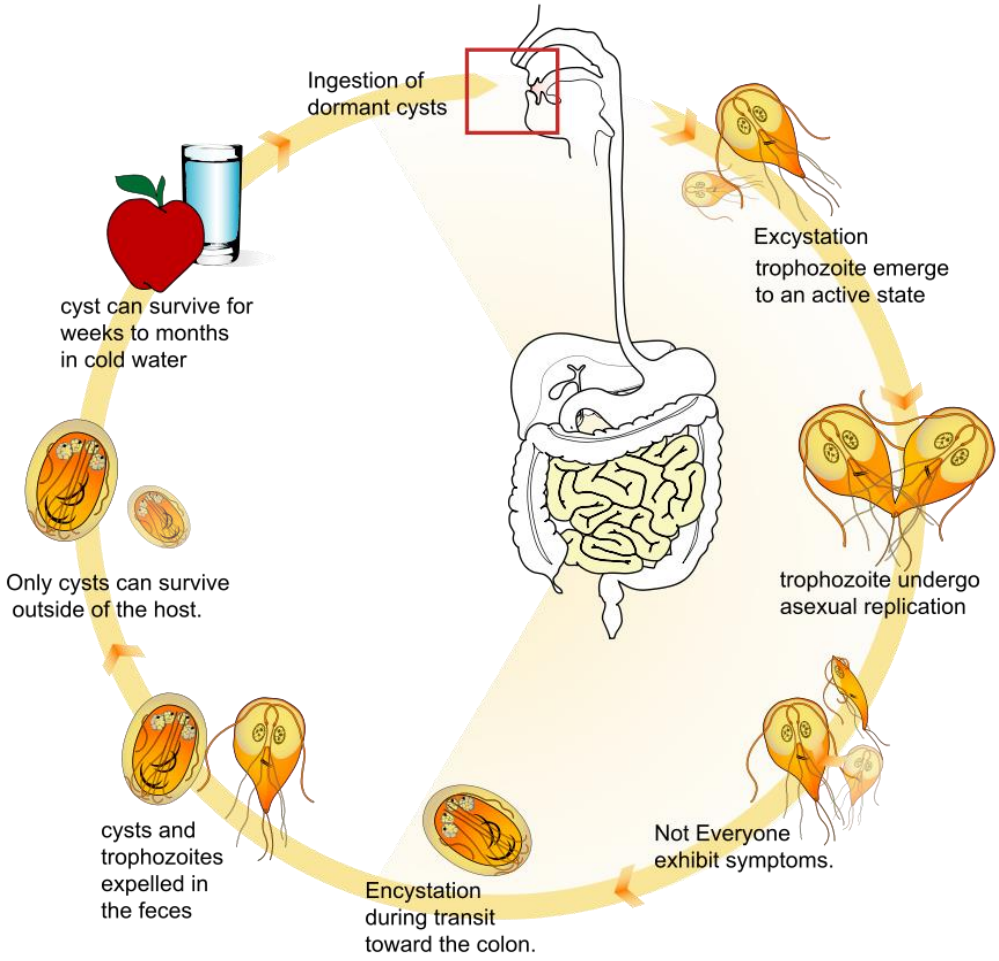
Giardia lamblia (cyst and trophozoit)

Transmission

Giardia infection can occur through ingestion of dormant microbial cysts in contaminated water, food, or by the fecal-oral route.

Life cycle of *Giardia lamblia*

Cyst is the infective form. Cysts are ingested through water and edibles. Acidic pH of stomach initiates excystation which is completed in duodenum thus releasing trophozoites (2 trophozoites from each cyst). The trophozoites establish themselves in the intestinal villi and start multiplying by binary fission. It can also localize itself in biliary tract.



- **Laboratory Diagnosis**

• Demonstration of cysts in the stool microscopically.

• Demonstration of trophozoites in duodenalaspirate.

• Intestinal biopsy.

• Immunological techniques like ELISA

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- **Subkingdom**: **protozoa**

Genus: ***Leishmania***

Species: ***Leishmani donovani***

Leishmani tropica

Leishmani brasiliensis

Cause disease (Leishmaniasis)

Leishmania: is a disease caused by protozoan flagellates parasites and spread by the bite of certain types of sandflies. The disease can present in three main ways as:

- ***Leishmani donovani* :**

Habitat :

The natural habitat of *Leishmania donovani* in man is reticuloendothelial system especially spleen, liver, bone marrow, intestinal mucosa, and also in the macrophages of intestinal wall. causes visceral leishmaniasis (kala azar).

Geographical Distribution

Visceral leishmaniasis is widely distributed,It is endemic in many places in America, Africa, China, South Europe, Europe and India.

- ***Leishmani tropica*:**

Geographical Distribution

Central and Western India. The infection does not coexist with kala-azar.

Habitat :

Amastigote in reticuloendothelial cells of skin (clasmatocyte). Promastigote form in sandfly causes cautaneous leishmaniasis (oriental sore,Baghdad boil).

- ***Leishmani brasiliensis*:**

Geographical Distribution

Central and South America.

Habitat :

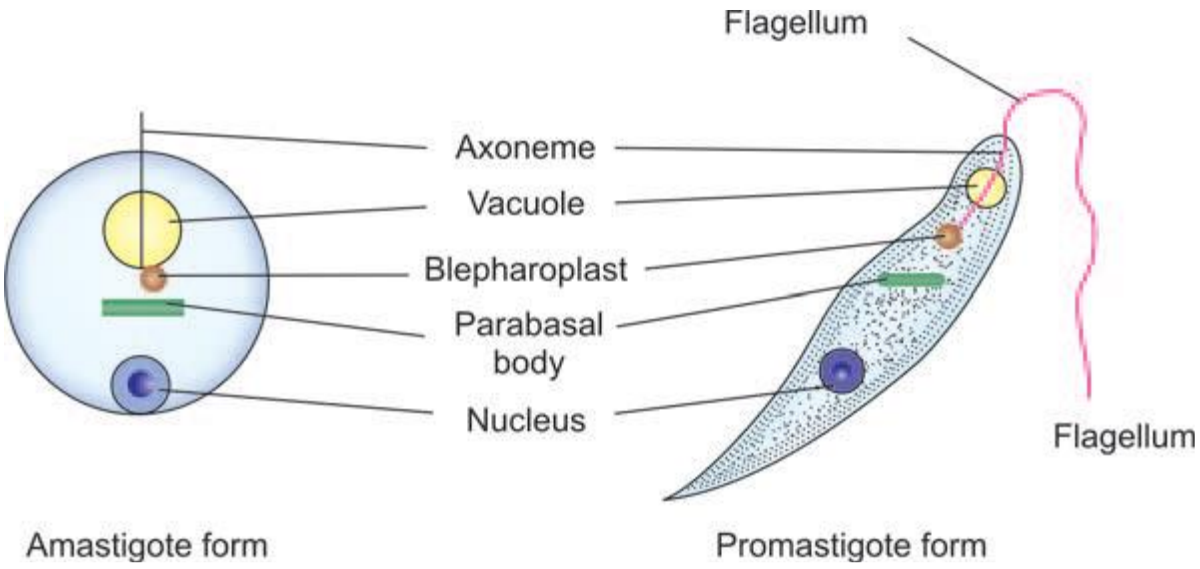
Amastigote form occurs in the macrophages of skin and mucous membrane of the nose and buccal cavity. causes mucocutaneous leishmaniasis.

Morphology

Leishmania exists in two forms:

(a)- amastigote form also called aflagellar form , and

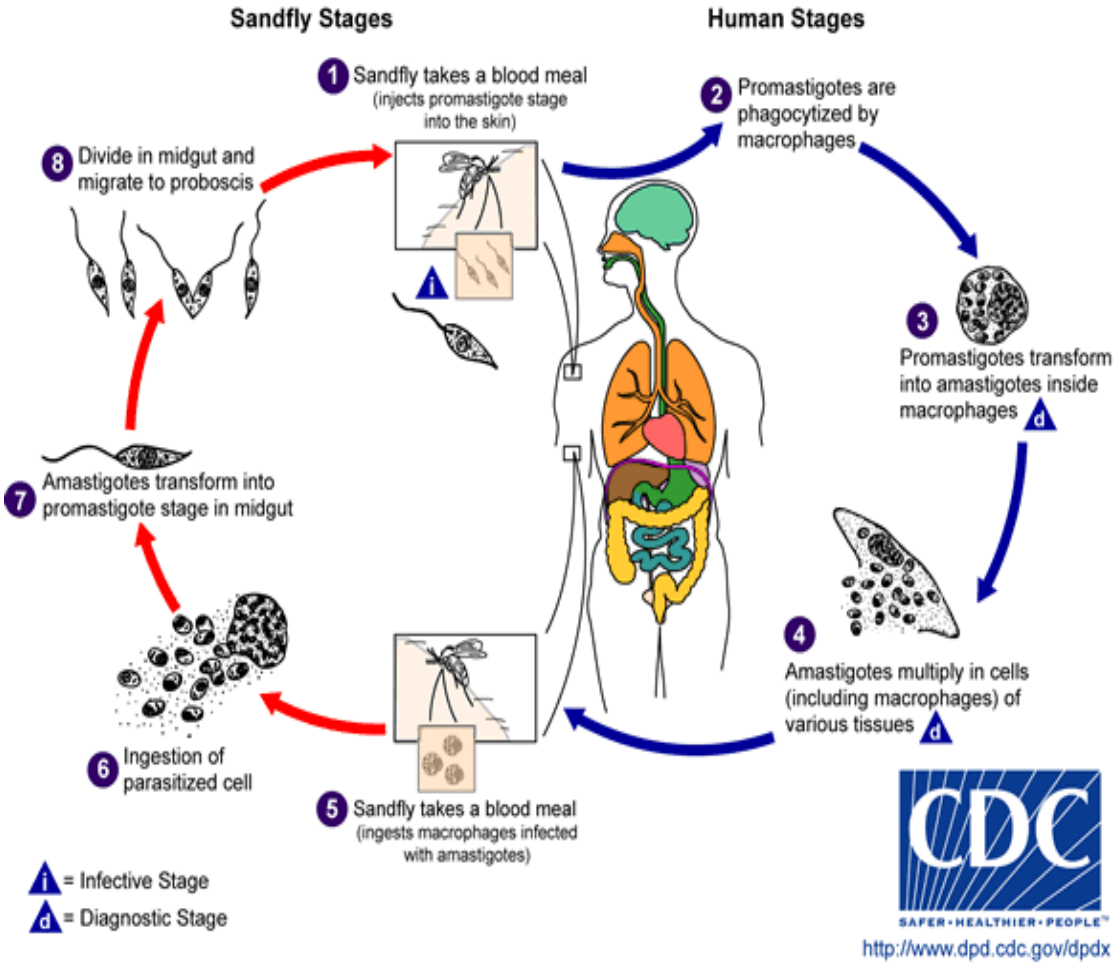
(b)- Promastigote form also called flagellar form .



❖ Life cycle:

The female sandfly after sucking leishmania along with blood of the patient,female sandfly is small hairy fly (1.5 to 3.5 mm). Its usual biting time is at dusk or night. *Leishmania spp.* undergoes development inside the body of female sandfly. The promastigote forms after multiplication ascend to pharynx and reach the proboscis. It takes 9 days to complete the cycle in sandfly. Ultimately the buccal cavity of sandfly is blocked by promastigote form. For taking second meal the sandfly has to release the promastigote form from its mouth into the bite wound caused by its proboscis. The promastigotes thus enter the circulation are mainly destroyed by vertebrate host (man). Still some promastigotes take shelter inside cells of reticuloendothelial system where promastigote form is transformed into amastigote one. They undergo multiplication

there at a slow rate. When the infected cells of reticuloendothelial system rupture, the free amastigote forms attack other cells. Sometimes they may be phagocytosed.



Laboratory Diagnosis

- Direct smear, culture and serological techniques
- Biopsy or aspirate from these specimens is smeared on clean glass slide fixed with methyl alcohol and stained with Giemsa stain.
- Immunological tests include tests to detect antigen ,e.g. ELISA

- **Subkingdom**: **protozoa**

Genus: ***Trichomonas***

Species: ***Trichomonas vaginalis***

Cause disease (Trichomoniasis).

-Is an anaerobic , **flagellated protozoan.**

Geographical Distribution

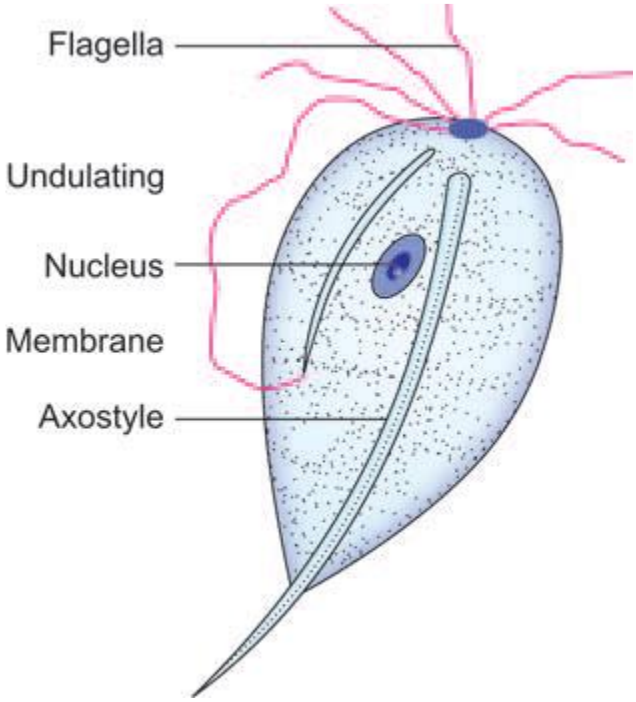
world wide distribution

Habitat

In female it is found mainly in vagina and in male it is in urethia.

Morphology

It is found only in trophozoitic form which bears



Mode of Transmission

It is primarily a venereal disease in which transmission can also be from person-toperson contact. However, newborns may get infected during birth. Fomities also form another way of transmission of infection.

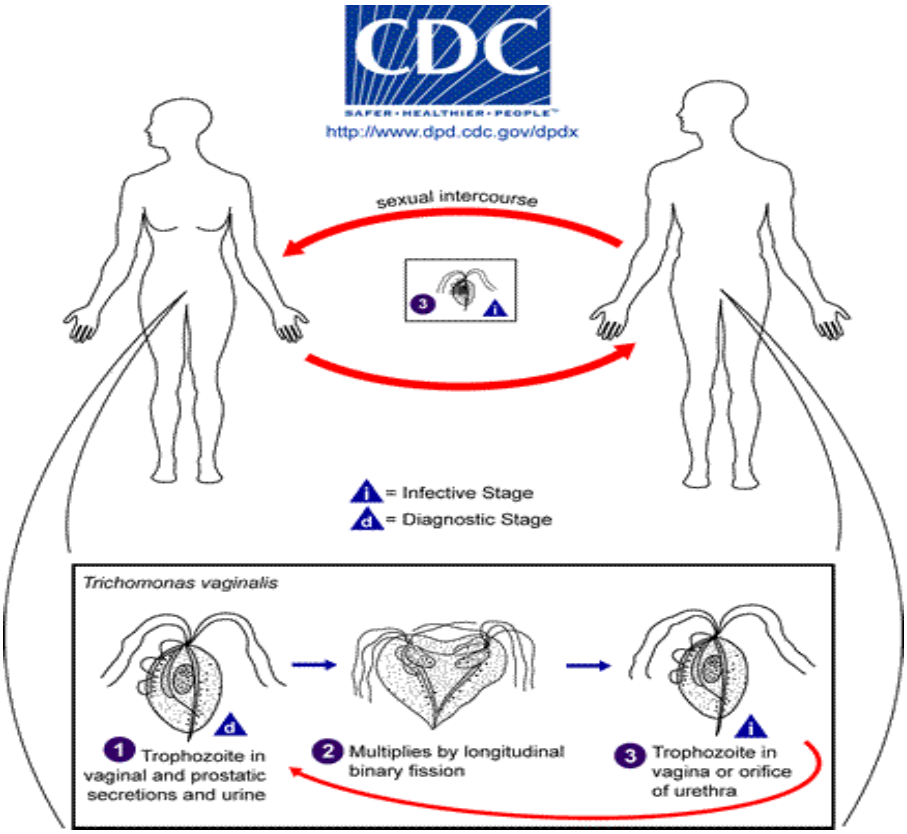
Incubation Time

It varies from 4 to 30 days.

Laboratory Diagnosis

- In female patient, *Trichomonas vaginalis* may be demonstrated in sedimented urine, vaginal secretion
- In male patient *Trichomonas vaginalis* may be found in the centrifuged urine and prostatic secretions
  - *Culture:* It is quite sensitive technique.

- **Life cycle**



- **Subkingdom**: **protozoa**

Genus: *Plasmodium* (Malarial parasite)

Species*: Plasmodium falciparum*

Plasmodium vivax

***Plasmodium ovale* and**

***Plasmodium malariae*.**

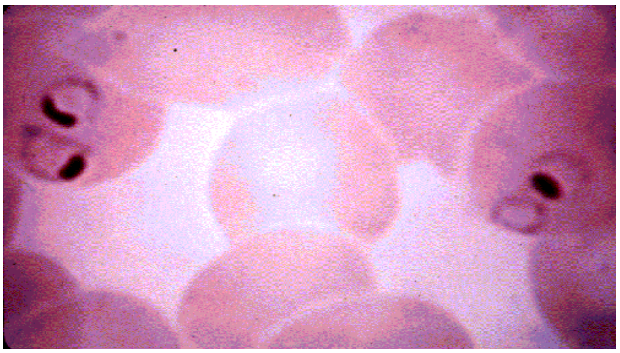
GEOGRAPHICAL DISTRIBUTION

It is sporozoa parasite, occurs in all countries in the tropics and subtropics.

Transmission: The infection is initiated when sporozoites are injected with the saliva of a feeding mosquito

Habitat :

It is found in parenchymal cells of liver, erythrocytes and other organs.



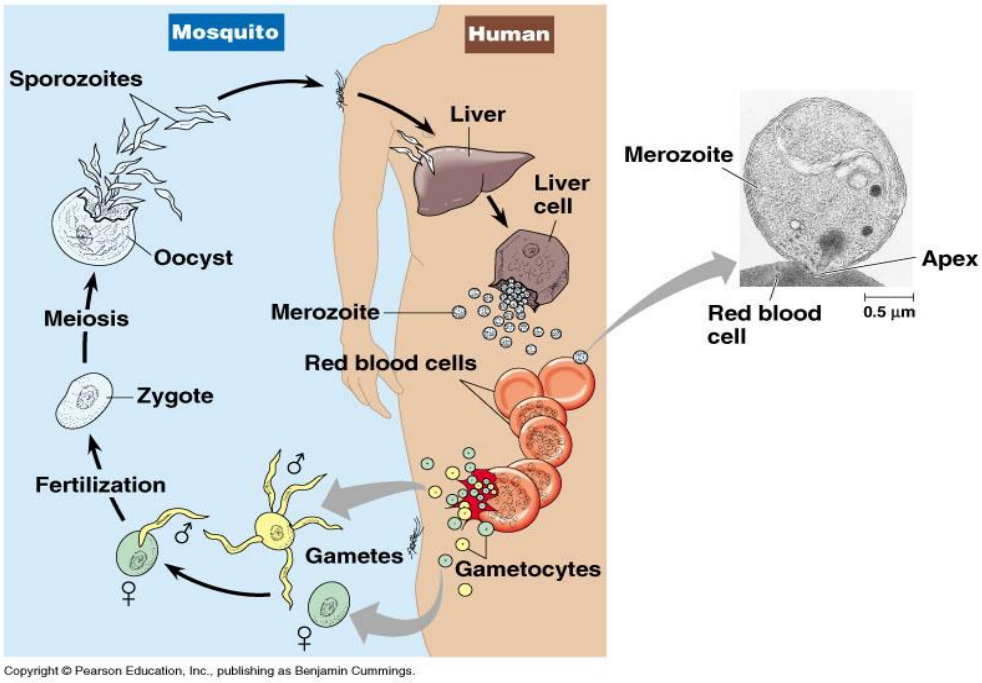
Life cycle: All species complete life cycle in man and female anopheles mosquito. The life cycle of *Plasmodium* involves several distinct stages in

- **the insect** and
- **vertebrate hosts.**

In infected mosquitoes, parasites in the salivary gland are called sporozoites. When the mosquito bites a **vertebrate host**, sporozoites are injected into the host

with the saliva. From there, the sporozoites enter the bloodstream and are transported to the liver, where they invade and replicate within hepatocytes. At this point, some species of *Plasmodium* can form a long-lived dormant stage called a hypnozoite which can remain in the liver for many years. The parasites that emerge from infected hepatocytes are called merozoites, and these return to the blood to infect red blood cells. Within the red blood cells, the merozoites grow first to a ring-shaped form and then to a larger form called a trophozoite. Trophozoites then mature to schizonts which divide several times to produce new merozoites. The infected red blood cell eventually bursts, allowing the new merozoites to travel within the bloodstream to infect new red blood cells. Most merozoites continue this replicative cycle, however some merozoites upon infecting red blood cells differentiate into male or female sexual forms called gametocytes. These gametocytes circulate in the blood until they are taken up when a mosquito feeds on the infected vertebrate host, taking up blood which includes the gametocytes.

In the mosquito, the gametocytes move along with the blood meal to the mosquito's midgut. Here the gametocytes develop into male and female gametes which fertilize each other, forming a zygote. Zygotes then develop into a motile form called an ookinete, which penetrates the wall of the midgut. Upon traversing the midgut wall, the ookinete embeds into the gut's exterior membrane and develops into an oocyst. Oocysts divide many times to produce large numbers of small elongated sporozoites. These sporozoites migrate to the salivary glands of the mosquito where they can be injected into the blood of the next host the mosquito bites, repeating the cycle.



Laboratory Diagnosis

• Peripheral blood film for parasites (thick and thin smear) is studied microscopically after staining

• Serological techniques like ELISA

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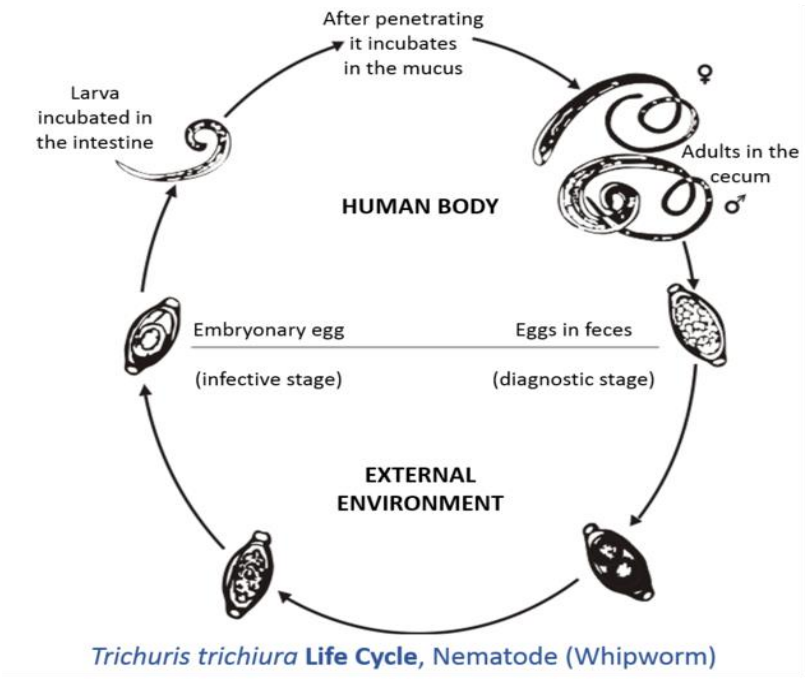
-2-class: NEMATODES

species: *Trichuris trichiura*

- **Geographical Distribution :**Worldwide.
- **Habitat :**Adult worm lives in large intestine of man.
- **Disease:** causes **Trichuriasis**
- **Transmission:** A soil transmitted swallowing infective eggs in contaminated soil, food or water.

- **Life cycle:** **No intermediate host is required**. Eggs are passed in stools of infected patient. A rhabditiform larva develops from egg and infection to healthy person occurs by ingestion of embryonated eggs in food and water. The egg shell is dissolved in the stomach and larvae liberated pass down the cecum which grow into adult worms and embed their anterior parts in the intestinal mucosa. They grow in adult form. The life-cycle is completed in one host, i.e. man.



Trichuris trichiura **Life Cycle**, Nematode (Whipworm)

- **Laboratory Diagnosis :**It is established by detecting characteristic eggs

in stool. Sometimes adult worm may be detected in stools but rarely.

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2-species :*Ascaris lumbricoides*

- **Geographical Distribution :**It is cosmopolitan.
- **Habitat :**Adult worm lives in the lumen of the small intestine (jejunum) of man.

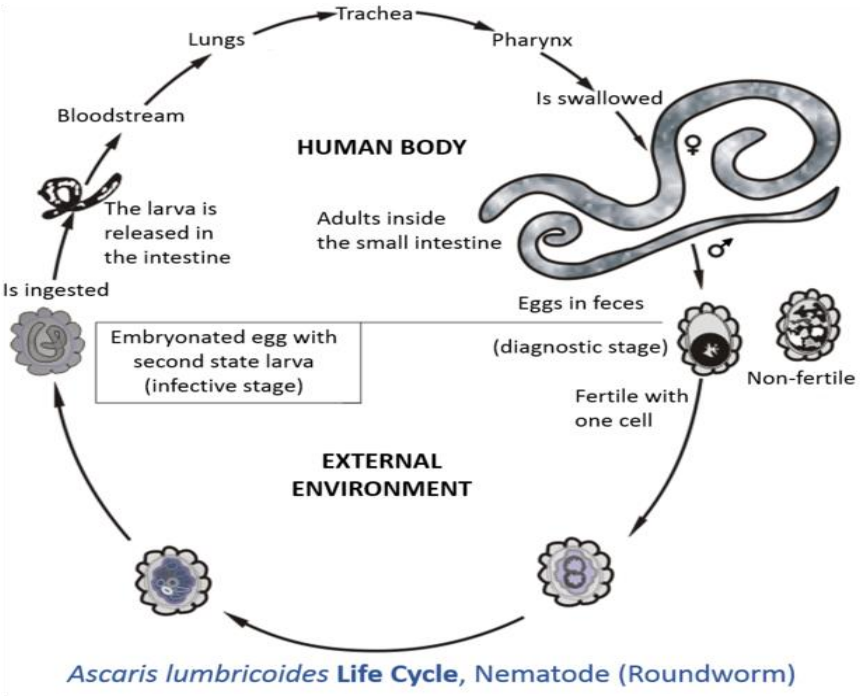
- **Transmission**: swallowing infective eggs in contaminated soil, food or water.
- **Disease: Ascariasis**
- **Life Cycle**

- Infects humans when an ingested fertilised egg becomes a larval worm that penetrates the wall of the duodenum and enters the blood stream. From there, it is carried to the liver and heart, and enters pulmonary circulation to break free in the alveoli, where it grows and molts. In three weeks, the larva passes from the respiratory system to be coughed up, swallowed, and thus returned to the small intestine, where it matures to an adult male or female worm. Fertilization can now occur and the female produces as many as 200,000 eggs per day for a year. These fertilized eggs become infectious after two weeks in soil; they can persist in soil for 10 years or more

- **Laboratory Diagnosis**

❖ Detection of adult worms in stool.

❖ Microscopic detection of eggs in feces or bile obtained by duodenal intubation.



Ascaris lumbricoides **Life Cycle**, Nematode (Roundworm)

3-Specise:*Enterobius vermicularis*

- **Geographical Distribution :**It is cosmopolitan.

- **Habitat :**Adult worm (female resides in cecum and appendix of man).
- **Disease:** cause **Enterobiasis**
- **Transmission:** Do not need to rely on a vector for transmission. infection usually occurs via ingestion of infectious eggs by direct anus-to-mouth transfer by fingers.
- **Life Cycle**

The female worm when fully gravid passes down to migrate several inches outside the anus to deposit eggs. These eggs are transferred by fingers (autoinfection) and by contaminated food or fomites to the mouth and they are swallowed. On reaching the intestine, outer shell is dissolved by digestive enzyme thus liberating the larvae. In the presence of oxygen, larvae become infective.

- **Laboratory Diagnosis**

• Detection of adult worm in the stools.

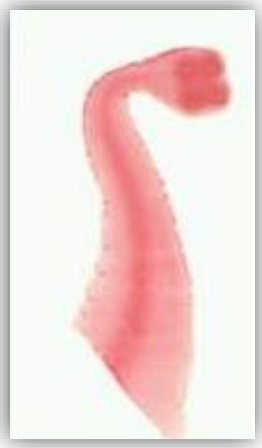
• Demonstration of eggs in stool and finger nails.

3-Class :Cestoda (Tapeworms) :- live in the digestive tracts of vertebrates as adults.

- **Transmission**: Humans are subject to parasitism by several species of tapeworms if they eat undercooked meat such as pork (*Taenia solium*), beef (*T. saginata*), and fish (*Diphyllobothrium* spp.), or if they live in, or eat food prepared in, conditions of poor hygiene (*Hymenolepis* or *Echinococcus* species).
- **Laboratory Diagnosis**

- Demonstration of proglottids or eggs.
- Serodiagnosis is done with the help of tests like indirect hemagglutination, and ELISA.

Taenia solium



Taenia saginata

Echinococcus granulosus