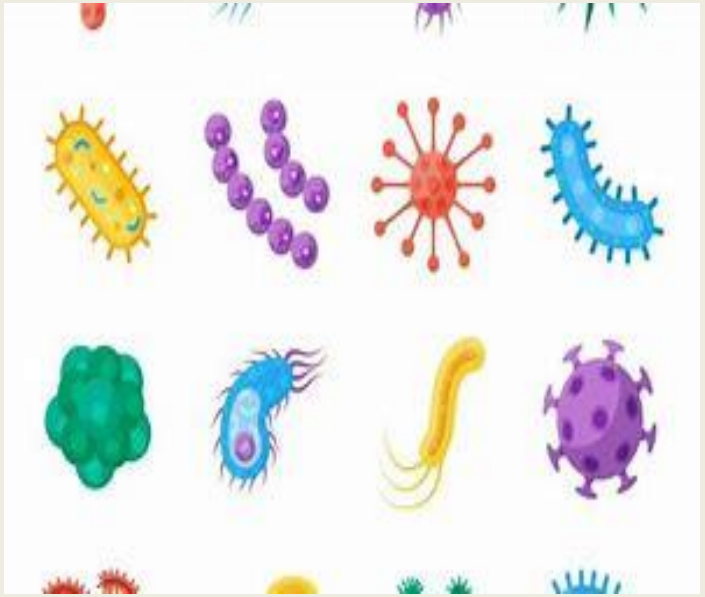
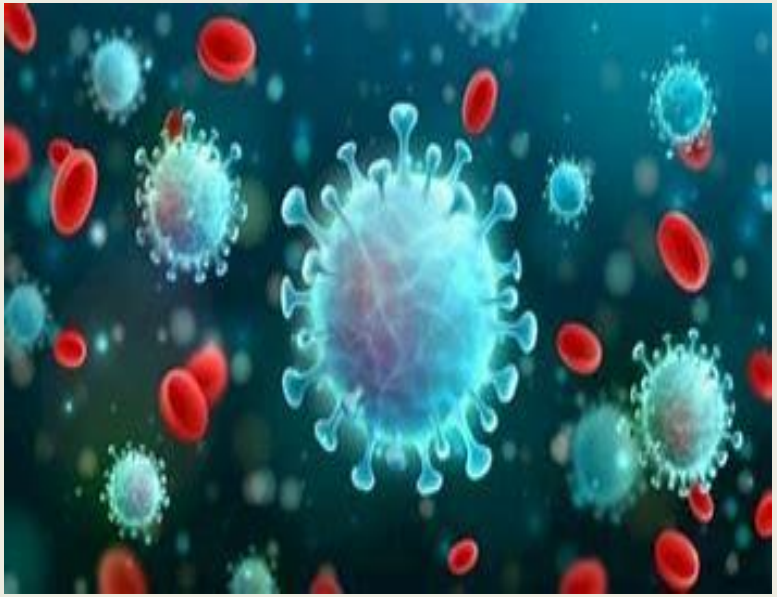
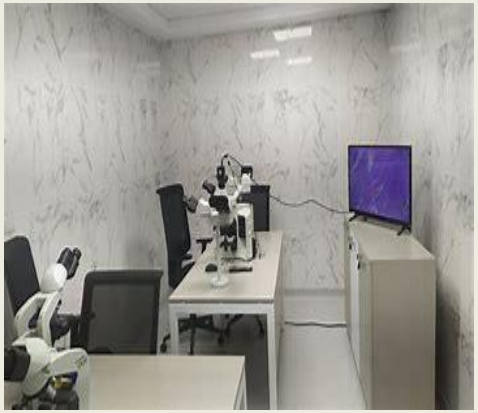


Microbiology
Lab 1

MSc: Zainab S.H



Orientation to The Microbiology Laboratory



- Microbiology is the science that study of microorganisms (MO.) and including bacteriology, mycology, virology, and parasitology.
- Microbiology lab. is a place to grow and study tiny organisms, called microorganisms, and these Microbes can include bacteria, fungus, virus, and parasite.

Rules of conduct and general safety

- 1. Wear protective safety glasses, gloves and laboratory coat when processing specimens.
- 2. Long hair should be bound back neatly away from shoulders.
- 3. Keep fingers, pencils, bacteriological loops, etc. out of your mouth.
- 4. Do not lick labels with tongue (use tap water).
- 5. Do not wander about the laboratory: uncontrolled activities cause:- Accidents - Distract others - Promote contamination.

Rules of conduct and general safety

- 6. Do not place contaminated pipettes on the bench top.
- 7. Do not discard contaminated culture, glass ware, pipettes, tubes or slides in wastepaper basket or garbage can.
- 8. Do not eat, drink, smoke, apply cosmetics or manipulate contact lenses in work area.
- 9. Decontaminate work surface at least once a day and after any spill of potentially infectious material.

Rules of conduct and general safety

- 10. If you have cuts or abrasions on the skin of your hands, cover them with adhesive dressing.
- 11. If you use any sharp instruments, dispose of them in a "sharps" container for decontamination.
- 12. Remove gloves and wash your hands after completing any task involving the handling of Pathological specimens

The common modes of infection

- Self- inoculation with a syringe needle, spilling and splattering of cultures and infective fluids, pipetting by mouth, injuries through broken glass and through .food and water.

Steps to face mishaps with contaminated materials

1- Skin cut or pricks

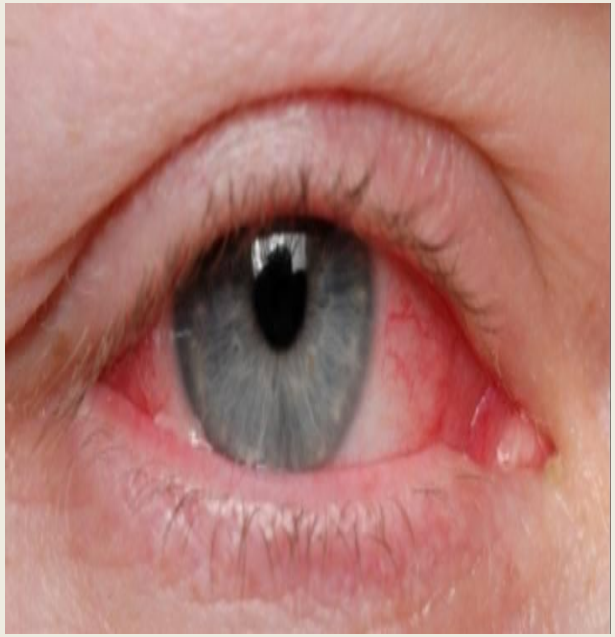
- wash the area with soap and water then apply tincture of iodine. Keep the wound .covered

Steps to face mishaps with contaminated materials

2- Mouth contamination

- do not mouth pipette. In case any accidental intake of contaminated material, spit out and rinse the mouth .repeatedly with water

Steps to face mishaps with contaminated materials



3- Eye contamination

- Wash with running water

Steps to face mishaps with contaminated materials

5- Spilling on floor

- pour disinfectant on the spilled water wait for 10 minutes and then wipe with a disposable cloth or paper. Discard the .latter after autoclaving .

Microscope

- Microscope is an instruments designed to produce magnified visual or photographic images of objects too small to be seen with the naked eye

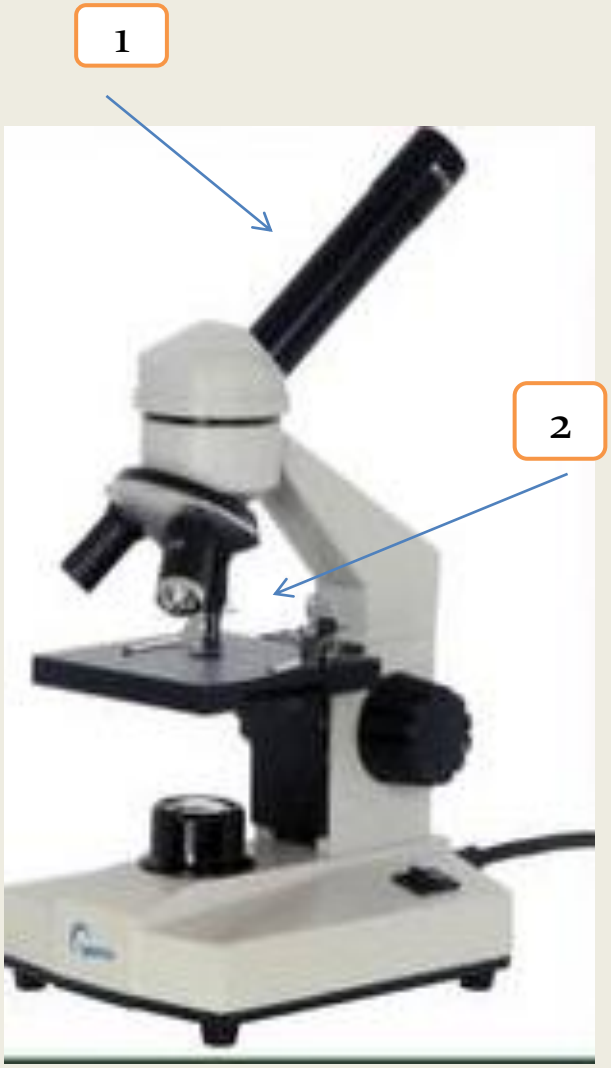
Types of microscope

- Depending on the number of eyepiece, the microscopes were classified to:
- 1- Monocular microscope
- 2- Binocular microscope

Types of microscope

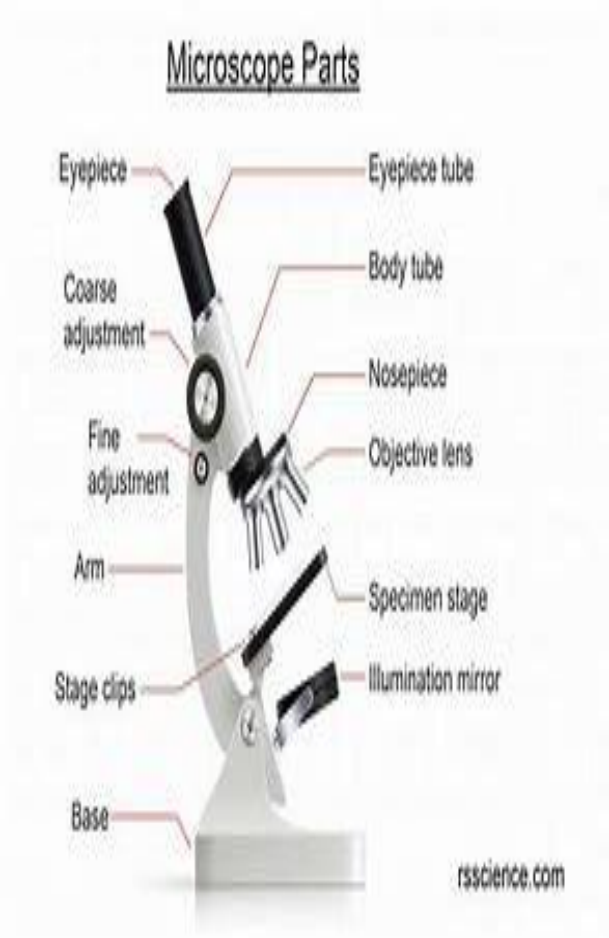
- Depending on the source, the microscopes were classified to:
- 1-Light microscope
- 2- electron microscope
- Other major types of microscopes are the fluorescence microscope the electron microscope (both the transmission electron microscope and the scanning electron microscope) .

Part of microscope



- 1- Ocular :The lens that one looks into for see the specimen. The eyepiece usually contains a 10X power lens.
- 2- Objective lenses: One of the most important parts of a compound microscope, as they are the lenses closest to the specimen. A standard microscope has three, four, or five objective lenses in power 4X, 10X, 20X, 40X and 100X.

Part of microscope



- 3- Body tube (Head): The body tube hold the eye piece and connects it to the objective lenses.
- 4- Arm: The arm connects the body tube to the base of the microscope, it helps carry the microscope. One can hold the arm with on hand and put another hand under the base of the microscope.
- 5- Base: The base supports the microscope and it's where illuminator is located.

Part of microscope

- 6-Stage: The flat platform where the slide is placed.
- 7-Stage clips: Metal clips that hold the slide in place and provide stability to the slides.
- 8- Stage height adjustment (Stage Control): These knobs move the stage left and right or up and down.
- 9- Aperture: The hole in the middle of the stage that allows light from the illuminator to reach the specimen.

Part of microscope

- 11-Coarse adjustment: Brings the specimen into general focus.
- 12- Fine adjustment: Fine tunes the focus and increases the detail of the specimen.
- 13-On/off switch: This switch on the base of the microscope turns the illuminator off and on.

Part of microscope

- 15- Iris diaphragm: Adjusts the amount of light that reaches the specimen.
- 16- Condenser: Gathers and focuses light from the illuminator onto the specimen being viewed.

How to use a microscope

- Place the slide on the stage
- Use stage clips to secure slide
- Adjust nosepiece to lowest setting
- (Lowest = shortest objective)
- Look into eyepiece
- Use coarse focus knob

Rules of using a microscope

- Always carry with 2 hands
- Only use lens paper for cleaning
- Do not force knobs
- Always store covered
- Be careful of the cords

Thank you for listening

Questions??

Sterilization and Disinfection Microbiology : lab 2

Sterilization and Disinfection

- Sterilization: means any process, either chemical or physical methods, which kills or removes all forms of life of pathogenic microorganism including vegetative cells, bacterial spores or viruses.
- Disinfection: any process that destroy pathogenic microbes by disinfectants to reducing the number of them to point where they no longer cause disease.

Advantages of sterilization

- 1- Prevent transmission of diseases
- 2-Prevent contamination and growth of undesirable bacteria
- 3- Prevent spoilage of material by microorganisms.

The processes of sterilization primarily use moist or dry heat and those of disinfection are often restricted to the use of chemicals. There are three methods :

Methods of sterilization

- There are three methods: Physical, Chemical, & Mechanical methods.
- Physical methods
- A- Heat:
- 1-Dry heat:
- a- Red heat: is sterilizing the tools (loope , needle, and forceps).
- b- Flaming is the sterilizing of upper pit of the glasses (test tubes, flasks, and the surface of slides) on the Bunsen light flame with sloping way.

Methods of sterilization

- 2. Dry hot air: by using apparatus oven (at 160-180°C for 1.5-2 hr) is sterilizing the glass (Petri dish, pipettes, bottles, test tubes) filter papers and metal tools .

Methods of sterilization

- 3. Moisture heat: is sterilizing the culture media& clothes by autoclave (1.5 bar for 20 mint).
- Boiling: The temperature employed is 100°C for 15 min. The method is sufficient to kill most of pathogenic organisms but not all or bacterial spores. This method used to sterilize metallic articles and glassware by boiling in water bath.

Methods of sterilization

- Radiation:
- A. Ionizing radiation (X-ray and y-ray):

It is used for sterilization of heat-sensitive items (disposable plastic Petri dishes, plastic tubes, disposable syringes, gloves, sutures.).

- B. Non-ionizing (UV-light and IR-light):

It is used to sterilize any surface in laboratory, air in hospital operating room and preparation of vaccine.

Methods of sterilization

- Filtration(mechanical sterilization):

Bacteria can be removed from liquid materials by passing them through filters that have very small pores (millipore filters with pore size of 0.22 µm). Filtration is used for:

- 1. Sterilizing substances that sensitive to heat (like serum, urine, sugar solutions, etc).
- 2. Preparation of antibiotic solutions.
- 3. Preparation of vaccines

Chemical methods

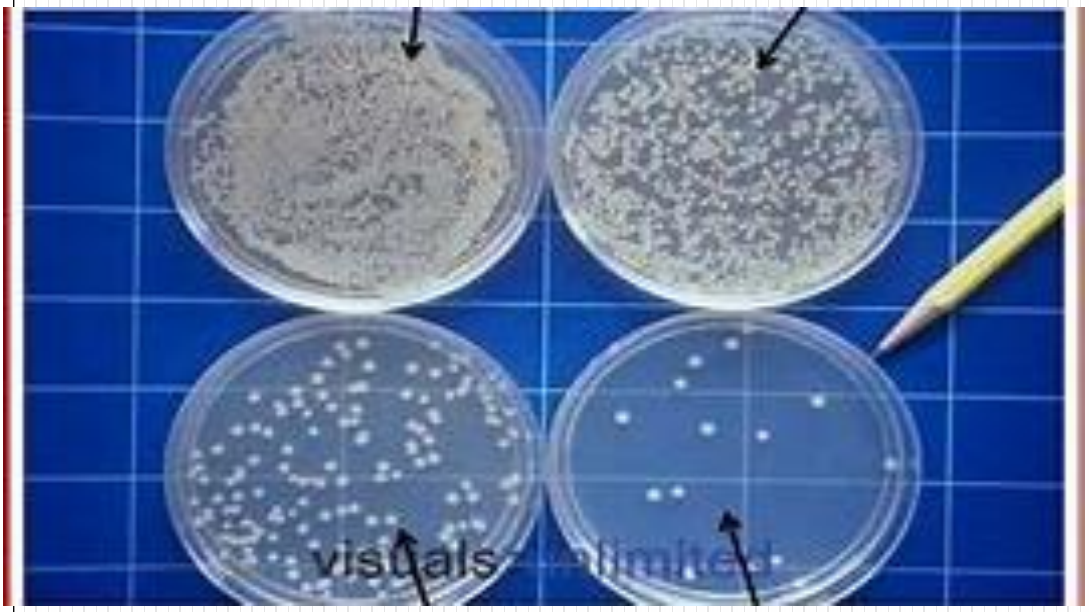
Chemical agent that kills pathogenic and non pathogenic microorganisms but not spores. Disinfection and antiseptic are generally applied to different types of substance, it cannot kill all types of microorganisms but reduce no., to be not effect or produce disease

Chemical methods

- Phenol and Phenolic
- Alcohols
- Halogens
- Heavy metals
- Gaseous agents
- Soap and detergents


Thank you
for listening

Culture Media



Lab 3

Zainab Sahib

culture medium

- The food material or substances required for growing microorganisms in vitro (outside the body) is called culture medium.
- Composition of culture media
- Water
- Energy source
- Carbon source
- Nitrogen source
- Mineral salts
- Special growth factors

Uses of culture medium

1. to identify the cause of infection from the clinical sample, so that proper treatment can be given.

- 2. to study the characteristics or properties of microorganisms.
- 3. to prepare biological products like vaccines, toxoides, antigens…etc.

Types of culture media

- I. Classification based on physical state
- a) solid medium :agar is the most commonly used solidifying agent
- b) semi solid medium : are useful in demonstrating bacterial motility and separating motile from non- motile strains.
- c) liquid medium: Sometimes referred to as "uniformly growing bacteria", e.g. nutrient broth

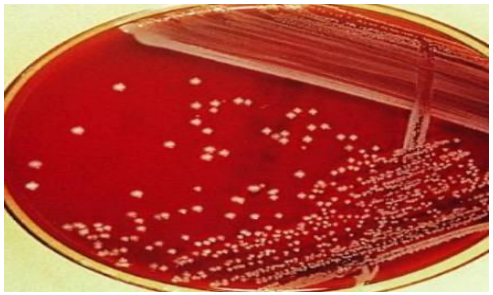
Types of culture media

2. Classification based on the ingredients

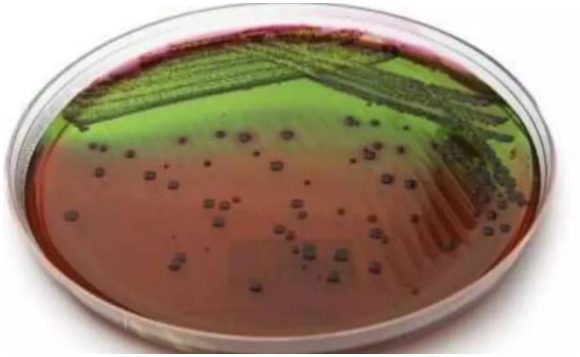
- A) simple medium e.g Nutrient agar ,Nutrient broth
- B) complex medium e.g Blood agar
- C) synthetic or defined medium e.g Peptone water
- D) special media include:

- Special media
- Enriched media
- Selective media
- Differential media
- Transport media
- Anaerobic media

special media



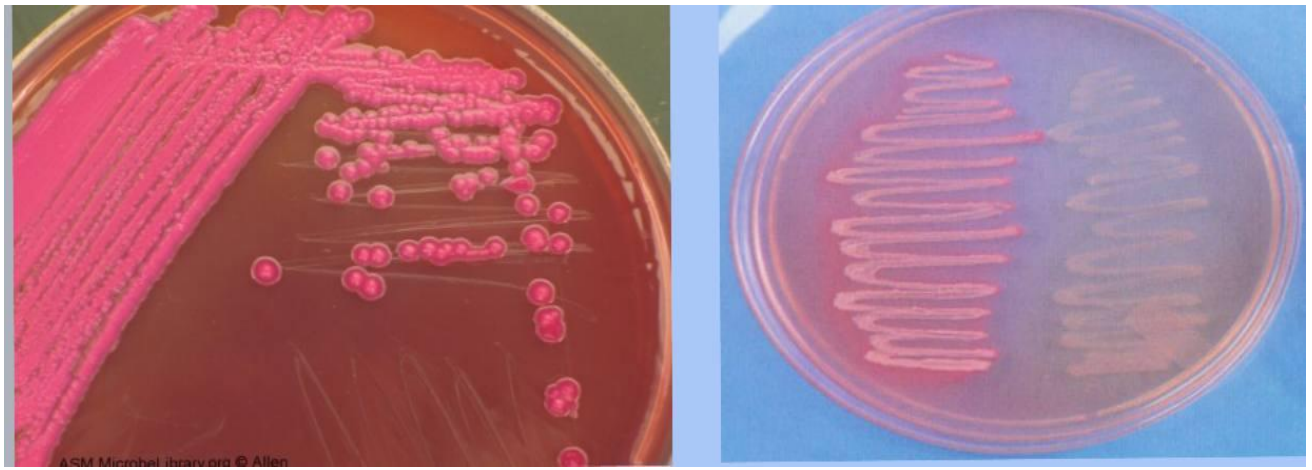
- Enriched media :Substances like blood, serum, egg are added to the simple medium. Used to grow bacteria that are exacting in their nutritional needs.eg: Blood agar(Enrichment and differential because it different between B haemolysis Str. Pyogens and α – haemolysis Str), Chocolate agar.



- Selective media : The inhibitory substance is added to a solid media to inhibit commensal or contaminating bacteria . Eosin methylene blue selective for gram negative bacteria .The dye methylene blue in the medium inhibit the growth of gram positive bacteria

special media

Differential media :are designed in such a way that different bacteria can be recognized on the basis of their colony color. Dyes and metabolic substrates are incorporated so that those bacteria that utilize them appear as differently colored colonies .e.g MacConkey agar :To differentiate between lactose fermentation and Non lactose fermentation bacteria.



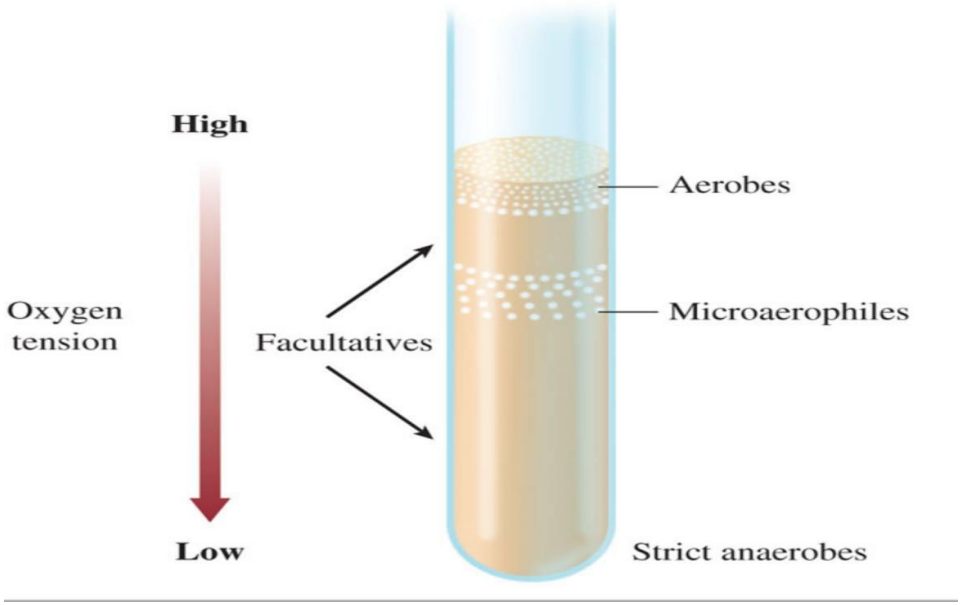
special media

- Transport media :Media used for transporting the samples.Delicate organisms may not survive the time taken for transporting the specimen without a transport media.eg :Stuart's medium

Classification according to O2 requirments:

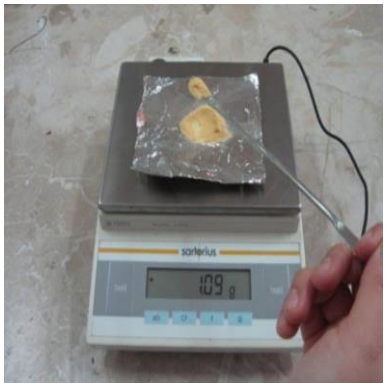
1- Aerobic media :All types of media, Which incubate in incubator in aerobic condition.

2- Anaerobic media :These media are used to grow anaerobic organisms.Ex: Thioglycolate medium,



Preparation of media

- Dissolving powder media in distilled water and mixing of heater to boiling .



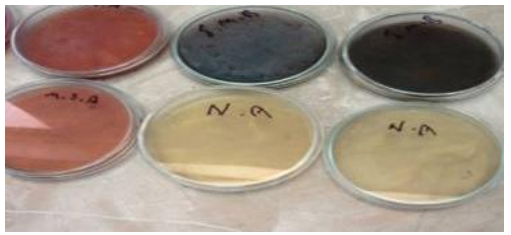
- Sterilization of media in autoclave at 121°C and pressure 15 psi for 15-20 min.

Preparation of media

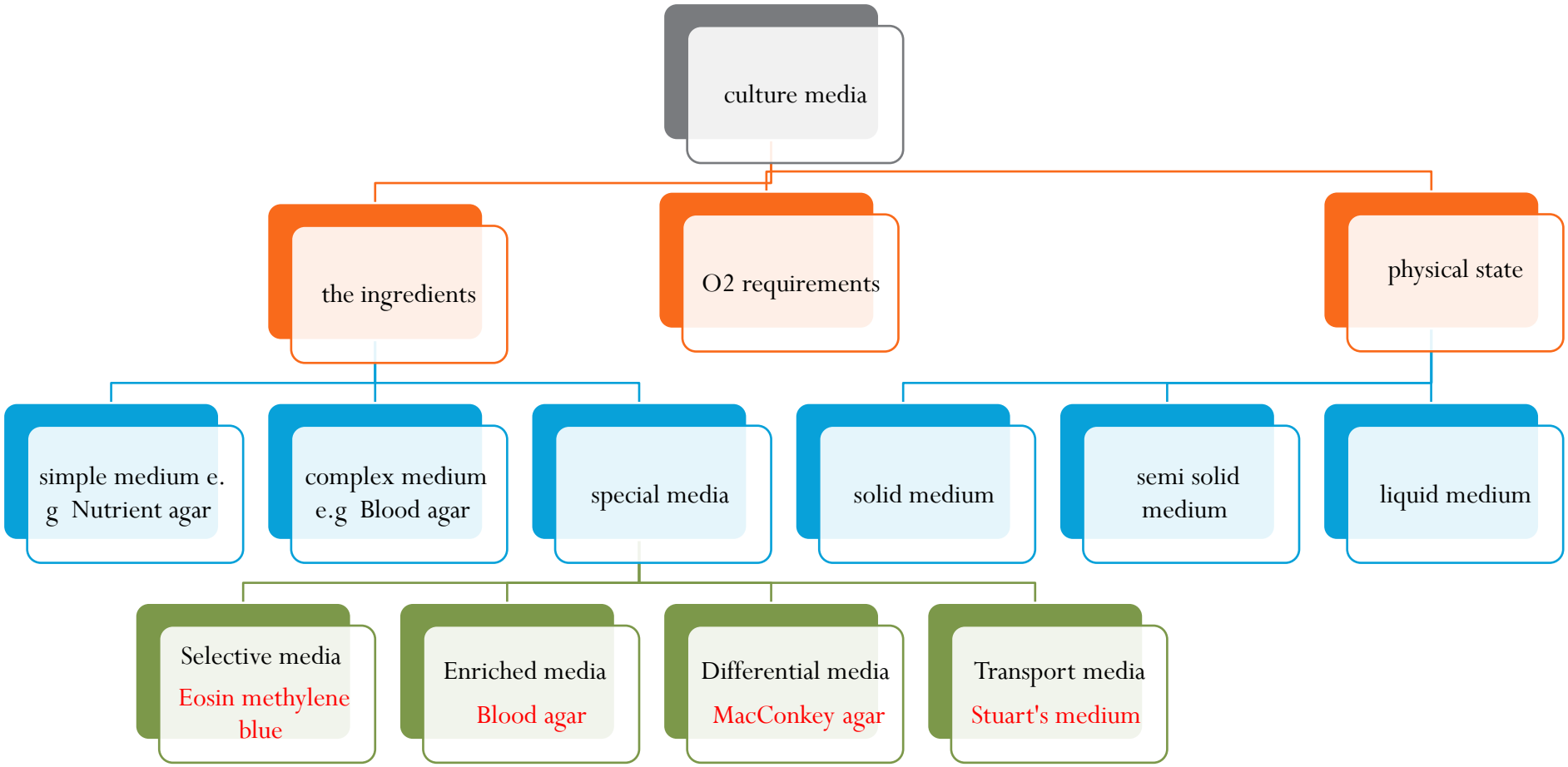
- 3- Cooling the media by air

- 4- adding the media to petridishes .

- 5- Wait until solidify



- 6- plates are kept inverted in cold place


culture media
the ingredients
O2 requirements
physical state
simple medium e.g Nutrient agar
complex medium e.g Blood agar
special media
solid medium
semi solid medium
liquid medium
Selective media
Eosin methylene blue
Enriched media
Blood agar
Differential media
MacConkey agar
Transport media
Stuart's medium


THANK YOU
FOR
LISTENING

Laboratory cultivation of microorganisms

LAB 4

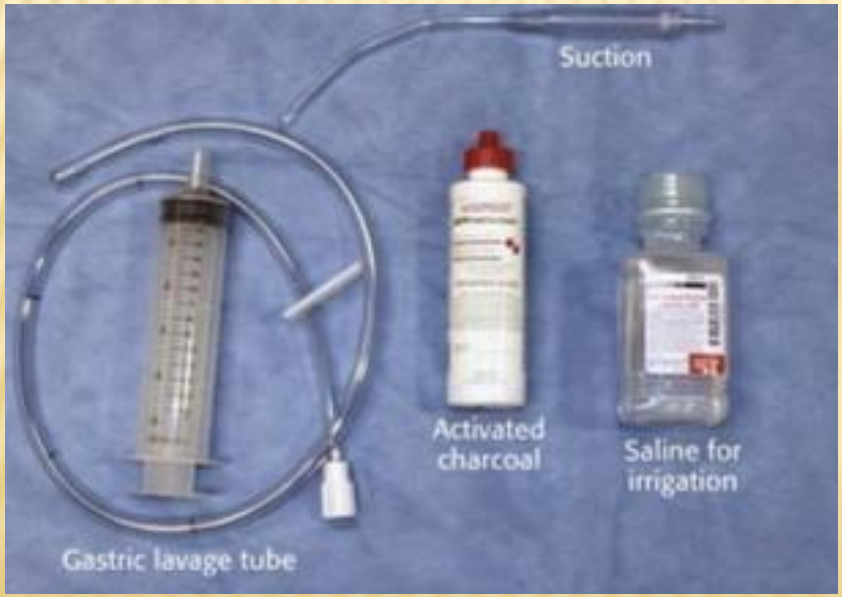
ZAINAB SAHIB

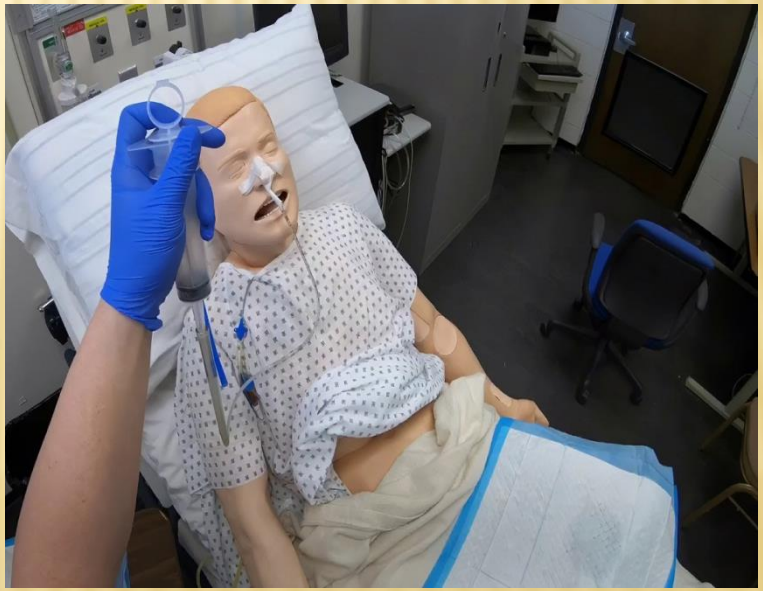
SAMPLING AND TRANSPORT OF TEST MATERIAL

- The aim of this subject is to provide the laboratory with a sample that contains the same types of microorganisms that were present in the sampled site. Most clinical specimens are collected in one of the following ways:-

- 1- Materials: such as saliva, sputum, feces, urine and discharging pus can be collected directly into sterile containers or screw-cap

- Washing: with physiological slain like throat washing and gastric washing for patient cannot produce sputum .

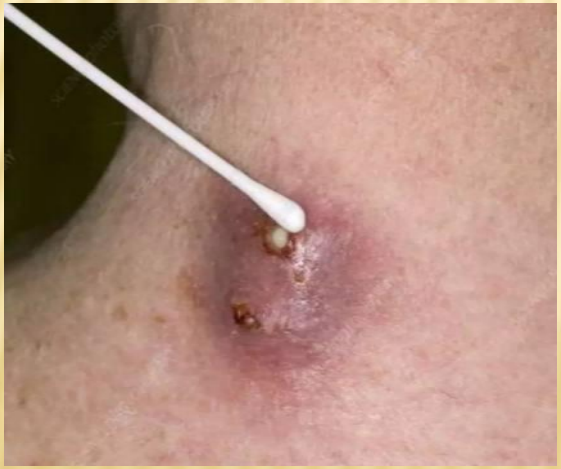




- Aspiration: needle syringe is used to collect materials inside patient body such as blood, cerebrospinal fluid (CSF), joints, fluid and closed abscesses.

- Biopsies: organ tissue collect in the same way of aspiration .

- Swabbing: a sterile cotton-wool mounted on a thin wire or wooden stick suitable for taking specimens of exudates from throat,skin,ear, wounds and lesions fig.



ISOLATION TECHNIQUES

- 1. Pour - plate method
- 2. Spread - plate method
- 3. Streak - plate method

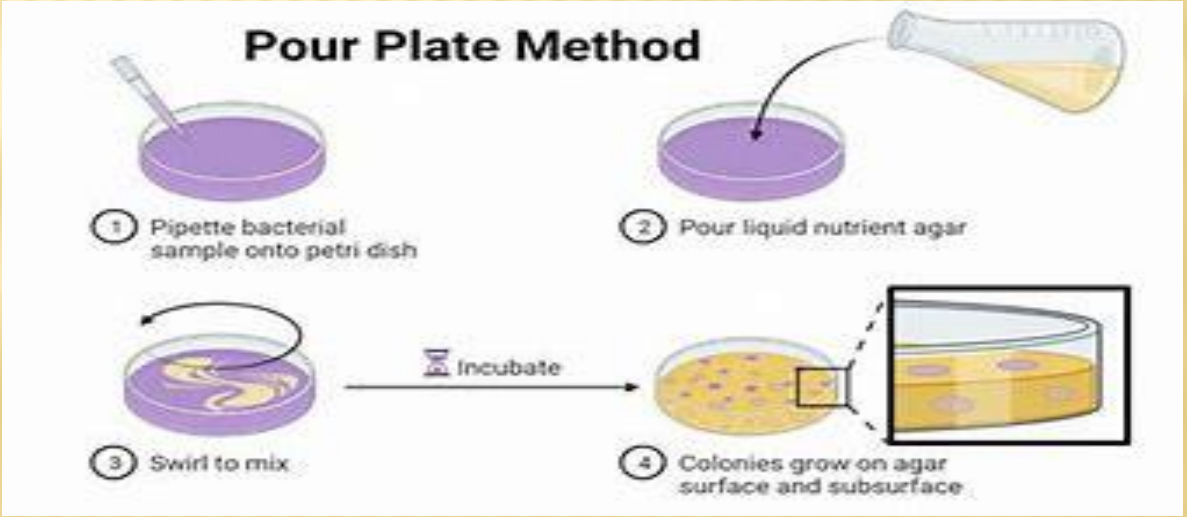
POUR - PLATE METHOD

1- sample is pipetted onto sterile plate

2- sterile medium is added and mixed well with inoculum

3- Incubate the plate at 37C° for 24 hours.

4- typical pour - plate results

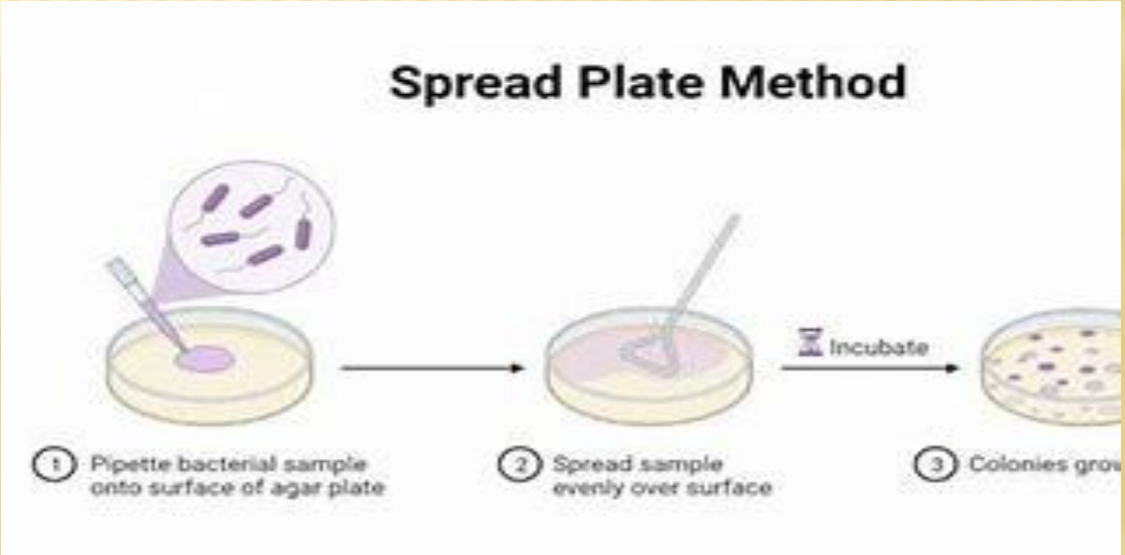


SPREAD - PLATE METHOD

1- sample is pipetted onto surface of agar plate.
2- sample us spread evenly over surface of agar using sterile glass spreader .

3- Incubate the plate at 37C° for 24 hours.

4- typical spread plate results .



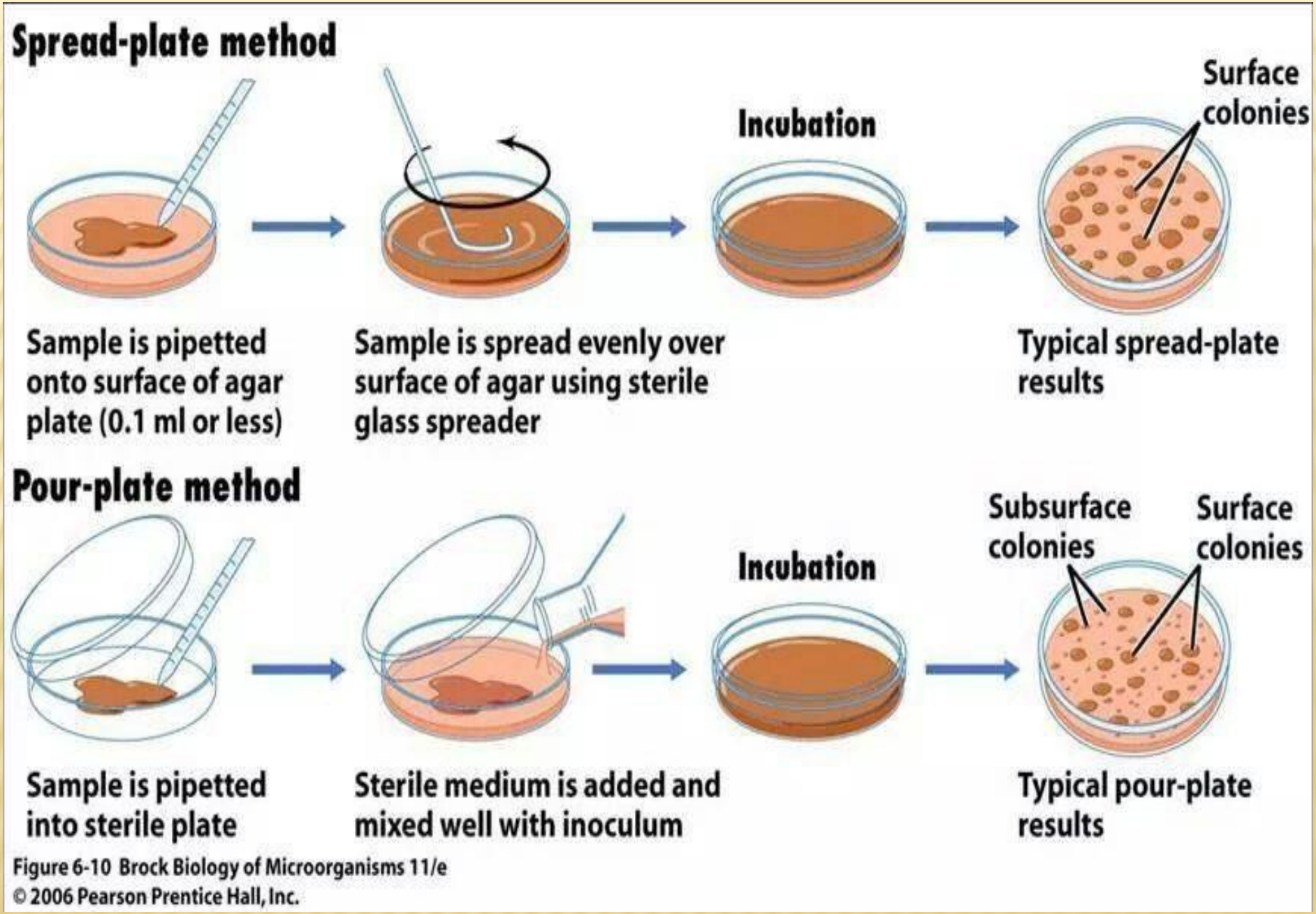


Figure 6-10 Brock Biology of Microorganisms 11/e

STREAKING METHOD

- streaking method: - Streaking a broth culture for colony isolation on solid media in Petri -dish.

1- place a loop full of broth culture on the surface of agar in the Petri dish , near but not touching the edge , lightly streak the inoculums back and forth over an area about 1 1/2 cm, do not dip up the agar .

2- Flame the loop and let it cool in air.

STREAKING METHOD

3- Rotate the plate in your left hand so that you can streak a series of four . parallel lines, each passing through the inoculum and extending across one side of the plate.

4-flam the loop again and let it cool in air.

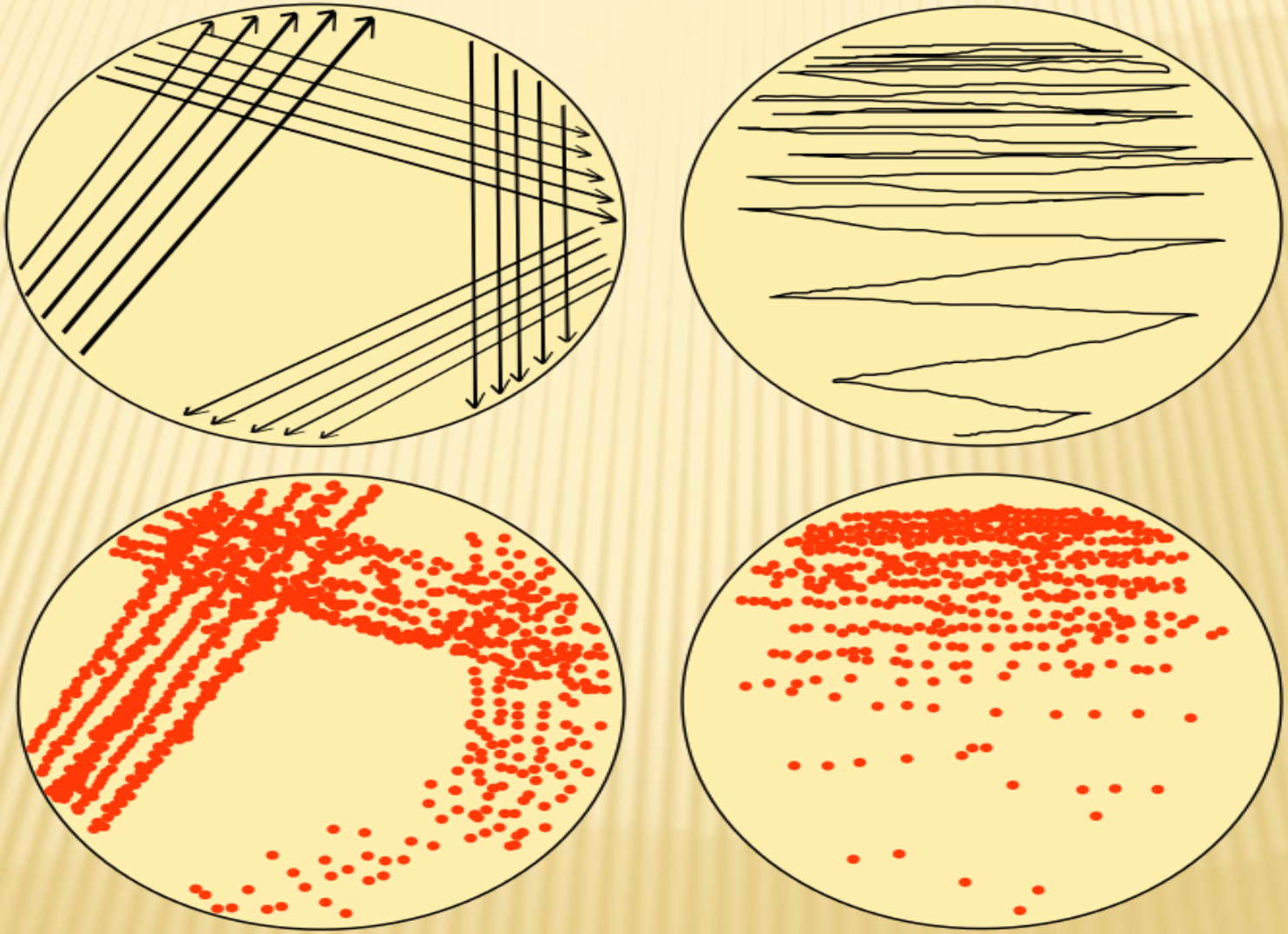
5- Rotate the plate and streak another series of four parallel lines, each crossing the end of the last four streaks and extending across the adjacent side of the plate.

STREAKING METHOD

6- Rotate the plate and repeat this parallel streaking once more.

7- Finally, make a few streaks in the untouched center of the plate, do not touch the original inoculums .

8-Incubate the plate at 37C° for 24 hours.



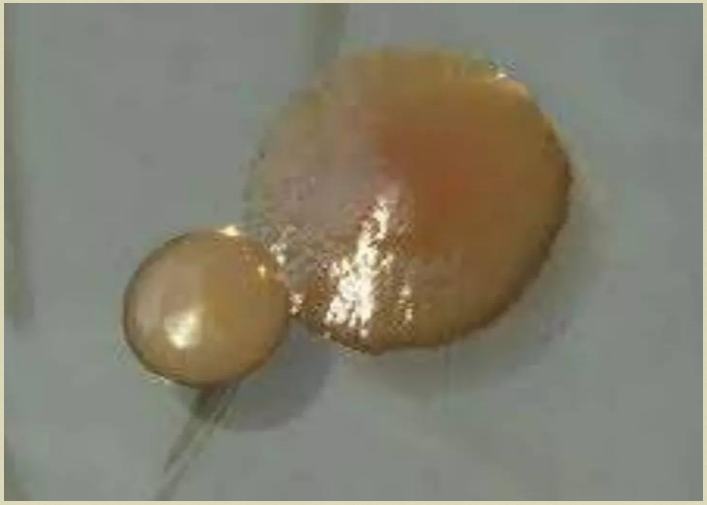
Lab 5
COLONY CHARACTERISTICS

Zainab Sahib

Colony Morphology

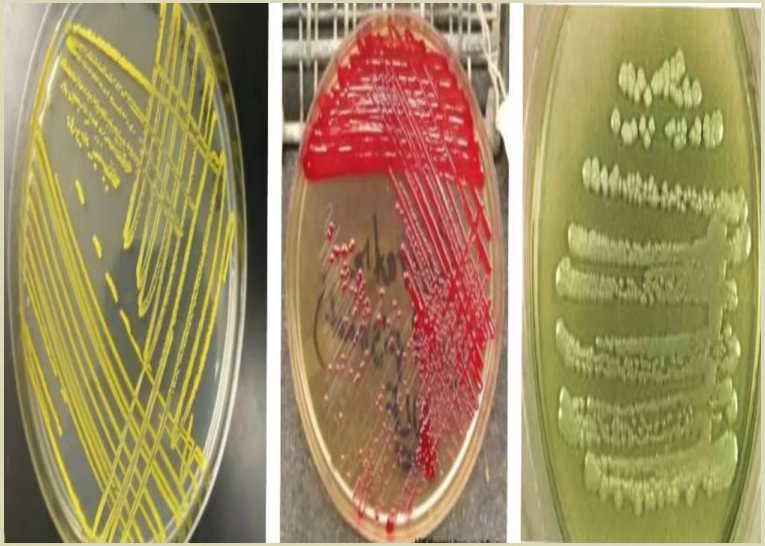
These characters used to accurately describe the morphology of a bacterial colony:

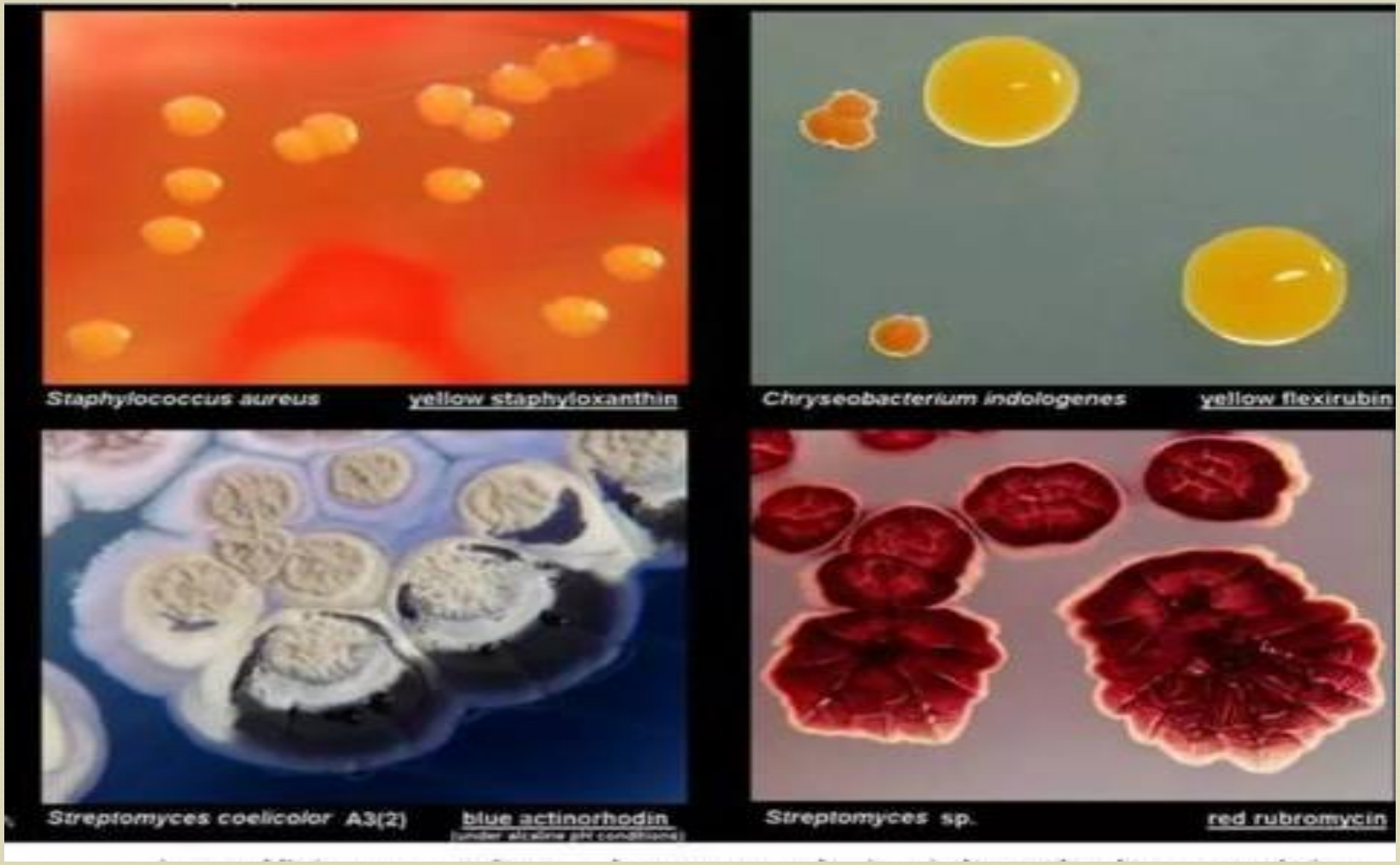
- Size :of the bacterial colony (Small : shigella - Medium : streptococcus - large :staphylococcus)



Colony Morphology

- Color : of the colonies (pigmentation) Some bacteria produce pigment when they grow in the medium . Staphylococcus aureus, Stretomyces sp., Bacillus Chryseobacterium indologenes, etc.




Staphylococcus aureus
yellow staphyloxanthin
Chryseobacterium indologenes
yellow flexirubin
Streptomyces coelicolor A3(2)
blue actinorhodin
Streptomyces sp.
red rubromycin

Colony Morphology

Texture :The colony surface may be smooth (shiny, gelatinous); rough(dull, granular or matte) or mucoid(slimy or gummy)

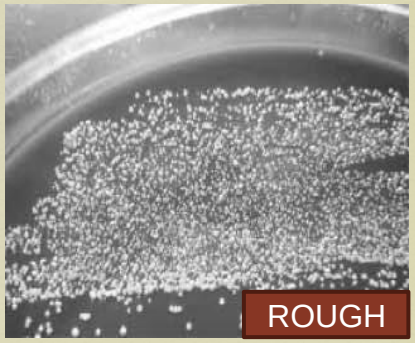
- Smooth

Streptococcus pneumoniae

- Rough

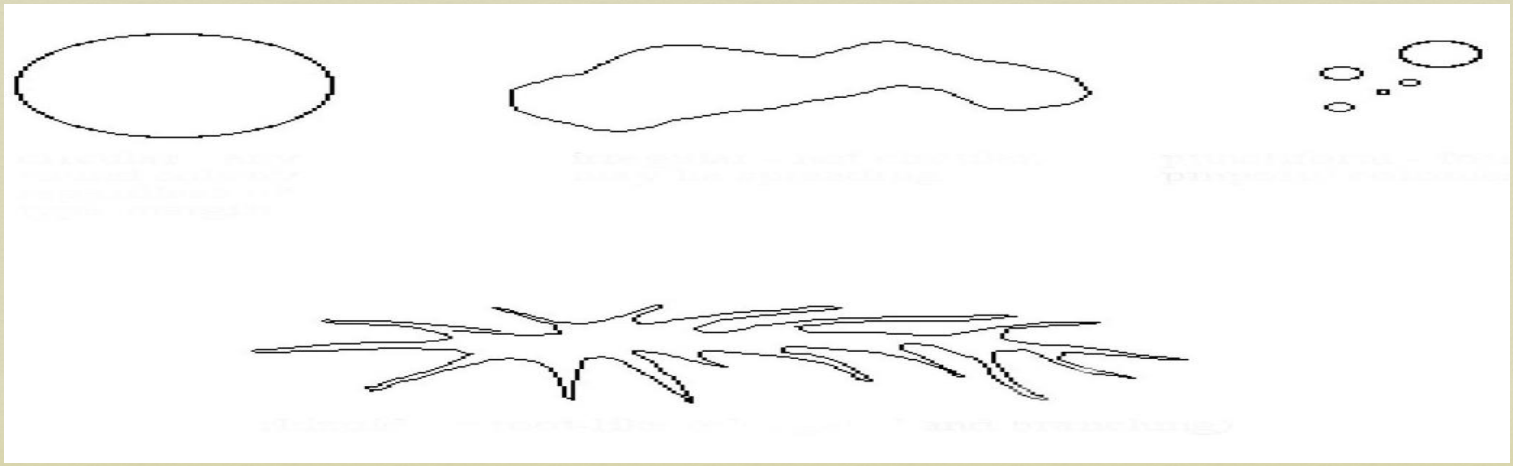
Mycobacterium tuberculosis

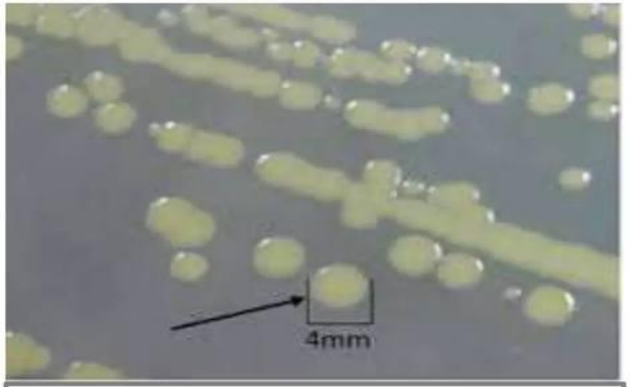
- Mucoid
- Klebsiella pneumoniae



COLONY CHARACTER ON AGAR PLATE

- Colony Shape on agar plates :
- circular any round colony regardless of type margin
- irregular - not circular, may ay be spreading
- punctiform - forming pinpoint colonies.
- rhizoid, or root-like (elongated and branching)





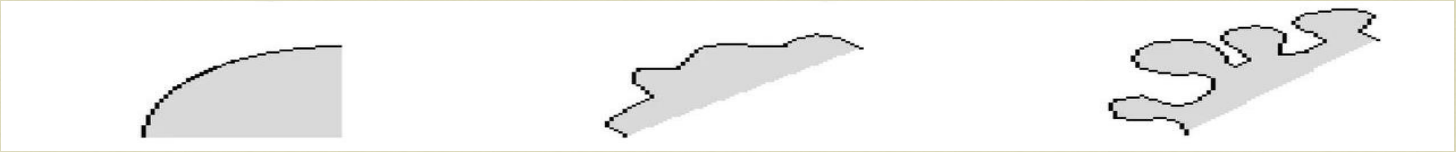
Circular

Pinpoint

Rhizoid

Colony shape on agar plates

- Margin (edges) of bacterial colony :
- Entire (smooth) - Undulate (wavy) - Lobate



- Filamentous - Curled (concentric) - Scalloped

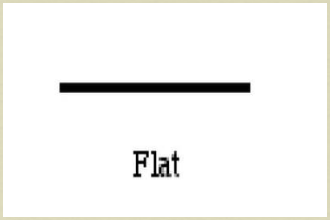
Undulate

filamentous

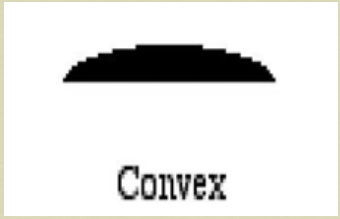
Colony shape on agar plates

- (Height) Elevation of bacterial colony

Flat

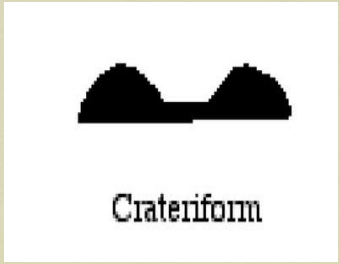


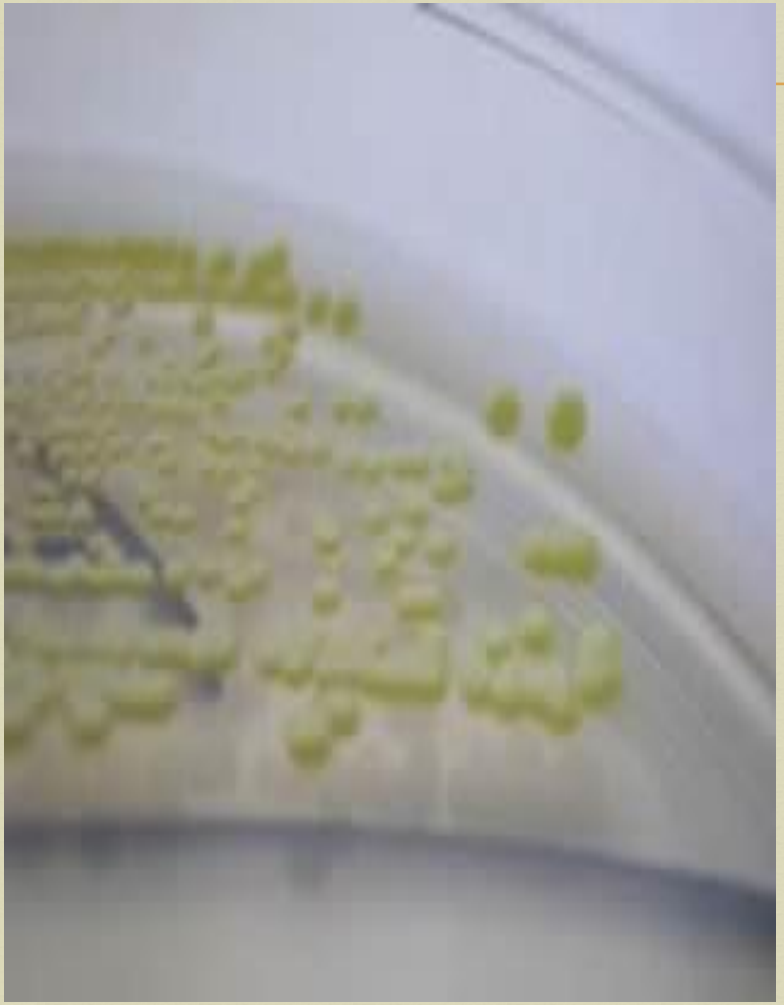
Convex



Umbonate

Crateriform





morphology of bacterial cell

Morphology of bacterial cell

- Bacteria is unicellular, free-living, microscopic microorganisms capable of performing all the essential functions of life.
- They possess both deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).
- Bacteria are prokaryotic microorganisms that do not contain chlorophyll.
- They occur in water, soil, air, food, and all natural environment.
- They can survive extremes of temperature, pH, oxygen, and atmospheric pressure.

BACTERIA CHARACTERISTICS

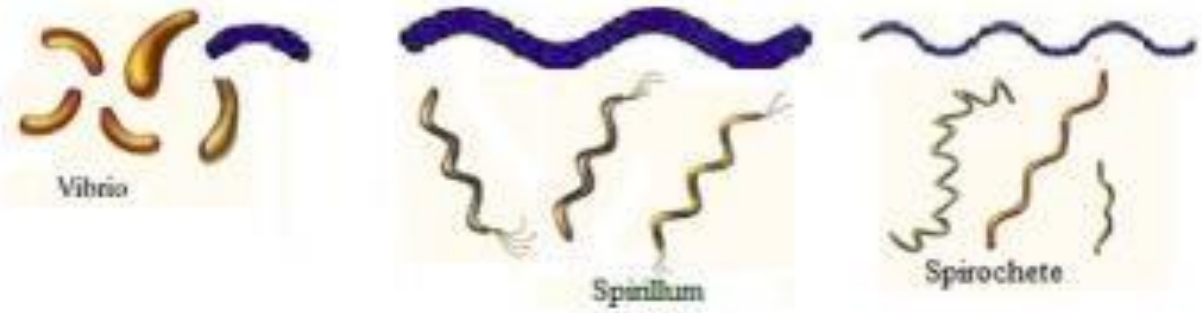
- Unicellular
- Circular DNA
- No organelles
- 1/10th the size of eukaryotic cells
- Flagella-long hair-like structure used for movement
- Reproduce asexually -Binary Fission

Shape of Bacteria

- Cocci: Spherical or oval shape
- Bacilli: Rod shaped cells
- Vibrios: comma shaped, curved rods possessing vibratory motility
- Spirilla: rigid forms
- Spirochete:flexous spiral forms
- Actinomycetes: branching filamentous bacteria
- Mycoplasma: cell wall deficient bacteria

(a) Coccus bacterial shapes

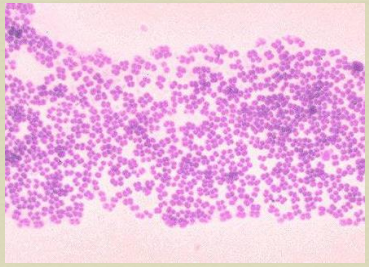
(b) Bacillus bacterial shapes



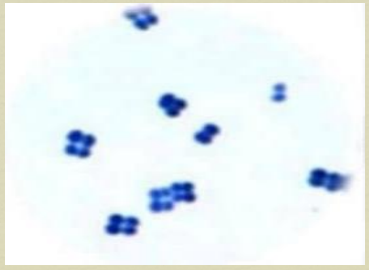
(c) Spirilla bacterial shapes

Cellular Arrangement

- Cocci arranged in;
- In pairs: Diplococci Eg: diplococcus pneumoniae.



- Group of Four : Tetrad Eg: Gaffyka tetragena.



Cellular Arrangement

- Group of eight: Sarcina Eg: Micrococcus tetragena.
- In chains: Streptococci Eg: streptococcus lactis.

- In clusters: Staphylococci Eg: staphylococcus aureus

Bacilli arranged

Arrangement of grouping formed by bacilli species

1. Diplobacilli
2. Streptobacilli
3. Trichomes

Diplobacilli

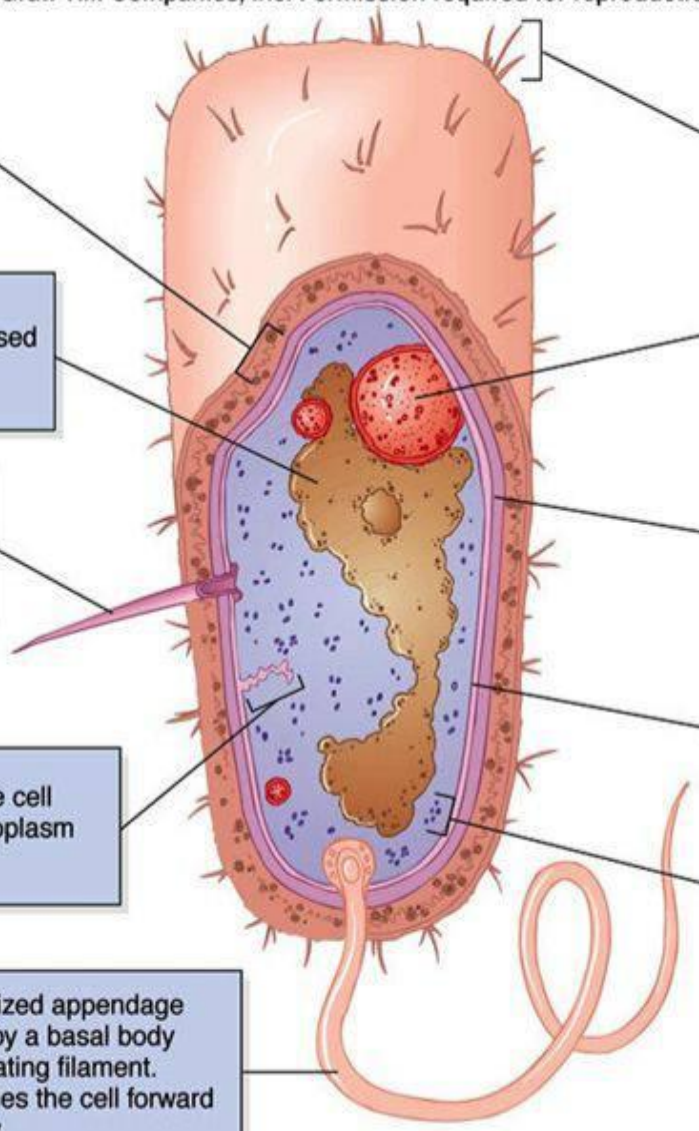
Trichomes

streptobacilli

Bacterial Cell structure

Ass.Lect. Zainab Sahib

Bacterial Cell Structure



Glycocalyx—A coating or layer of molecules external to the cell wall. It serves protective, adhesive, and receptor functions.

Bacterial chromosome or nucleoid—The site where the large DNA molecule is condensed into a packet. DNA is the code that directs all genetics and heredity of the cell.

Pilus—An elongate, hollow appendage used in transfers of DNA to other cells and in cell adhesion.

Mesosome—An extension of the cell membrane that folds into the cytoplasm and increases surface area.

Flagellum—Specialized appendage attached to the cell by a basal body that holds a long rotating filament. The movement pushes the cell forward and provides motility.

Fimbriae—Fine, hairlike bristles from the cell surface that help in adhesion to other cells and surfaces.

Inclusion/Granule—Stored nutrients such as fat, phosphate, or glycogen deposited in dense crystals or particles that can be tapped into when needed.

Cell wall—A semirigid casing that provides structural support and shape for the cell.

Cell membrane—A thin sheet of lipid and protein that surrounds the cytoplasm and controls the flow of materials into and out of the cell pool.

Ribosomes—Tiny particles composed of protein and RNA that are the sites of protein synthesis.

Fig. 4.1

Bacteria structure

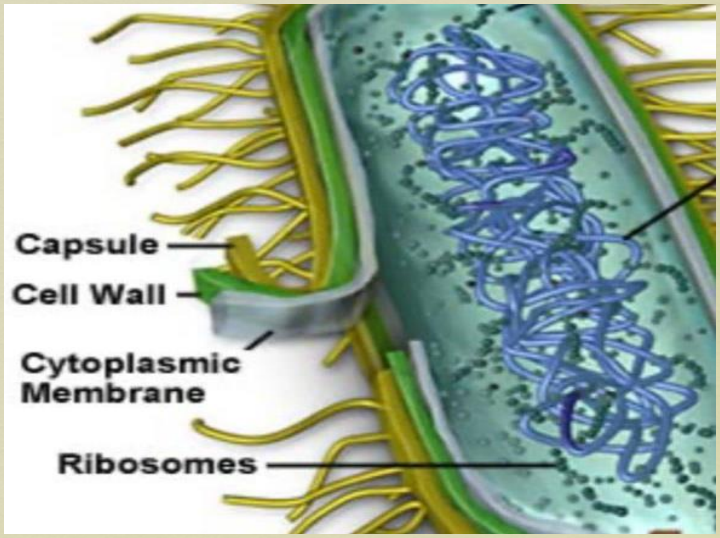
- I- Extracellular structure (Cell Wall Cell membrane Cytoplasmic or Plasma membrane) .

Functions of cell wall:

- Protects the bacteria.
- Allows them to live in “extreme” environments.
- Give it their external shape.

The function of cell membrane:

- uptake of nutrients
- excretion of waste products
- secrets the enzymes .



Bacteria structure

Cytoplasmic (inner) membrane

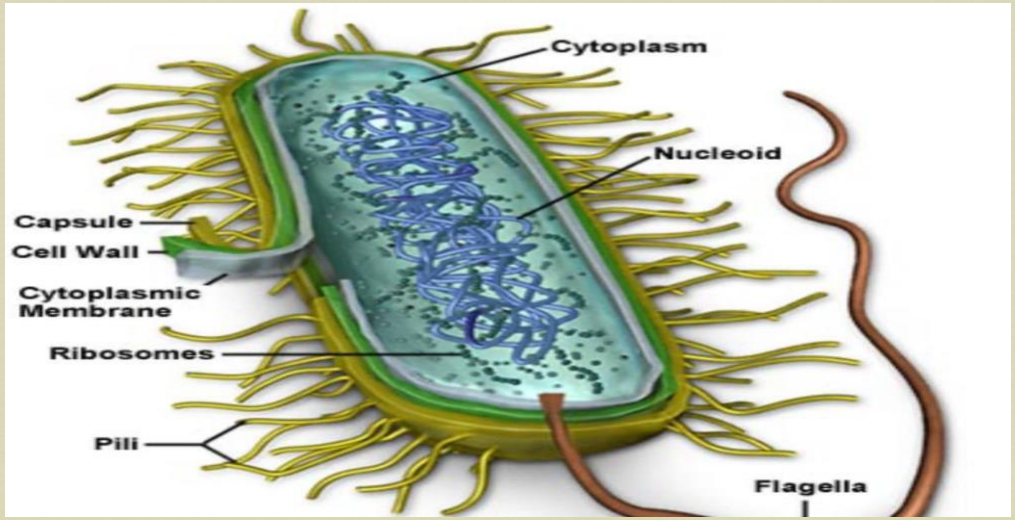
- – Feature of both Gram-positive and Gram-negative cells
- – allows the passage of membrane components through
- – has peripheral or integral proteins associated with it

- 2- Internal structure of bacteria:
- Cytopasm
- Ribosomes (for protein synthesis)
- Mesosome (contributed with replications)
- Volutin granules (source of energy)
- 3-Genetic Material

  - bacterial genes are contained on two kinds of DNA:
- a. chromosomal DNA (double strand od DNA)
- b. plasmids (composed of 100 copies of extrachromosomal DNA)
- Genetic materials have the all genes and coding of bacterial feature and antibiotic resistance.

Bacterial appendages: (Special structures)

- A- Pilli (fimbriae)
- Pilli are short, hair-like, protein: function “adherence” – stick to each other, stick to surfaces.
- Specialized “sex” pilus – conjugation



Bacterial appendages: (Special structures)

- Flagella:
- Organ of motility, a "movement“
- A = monotrichous
- B = amphitrichous
- C = lophotrichous
- D = peritrichous

Bacterial appendages: (Special structures)

- Capsule
- Some bacteria produce a capsule = a gelatinous, sticky layer that allows
- bacteria to
- - attach to substrates
- - make “colonies” together
- - also increases pathogenic bacteria’s resistance to host’s defenses
- Slime layer
- loosely associated with the bacteria, that is
- help the bacterial cell to adherence with the
- surfaces.

Bacterial appendages: (Special structures)

- Endospores (bacterial spores)
- some bacteria can form endospores to survive adverse conditions
- - very resistant to destruction
- - withstand desiccation and harsh conditions
- - endospore not for reproduction

Staining

- INTRODUCTION OF STAINING
- Microbial Staining giving color to microbes. Because microbes are colorless and highly transparent structures. Staining – process in which microbes are stained.(Stains/dyes): - organic compounds which carries either positive charges or negative charges or both .
- Stains classified in many ways
- Based on the charges:
- A-Basic stains/dyes – stain with +ve charge.
- B-Acidic stains/dyes – stain with –ve charge.
- C-Neutral stains/dyes – stain with both charges.

Staining

- Based on function of stain:
- A. Simple staining – only one dye is use dedifferentiation among bacteria is impossible Eg. Simple Staining.
- B. Differential staining- more than one dye is used- Differentiation among bacteria is possible- Eg. Gram's staining, Acid-fast staining.
- C. Special staining – more than one dye used - Special structures are seen. Eg. Capsule staining, Spore staining.

Principle of staining:

- Each staining methods have own principles but the following steps may be common:
- 1-Basic stain(+ve charge) :To stain -ve charged molecules of bacteria Mostly used because cell surface is –ve charge.
- 2- Acidic Stain(-ve charge) : To stain +ve charged molecules of bacteria. Used to stain the bacterial capsules. ((NOTE)): -As cell surface is –ve charged- Basic dyes mostly used.

Basic requirements for staining:

- •Clean grease-free slide.
- • Bacteria to be stained.
- • Inoculating loops- to transfer bacterial suspension to slide.
- • Bunsen burner – to sterilize inoculating loops before and after smear preparation.
- • Pencil marker – to mark (particularly central portion of slide) where bacterial smear is applied.

The Gram staining method

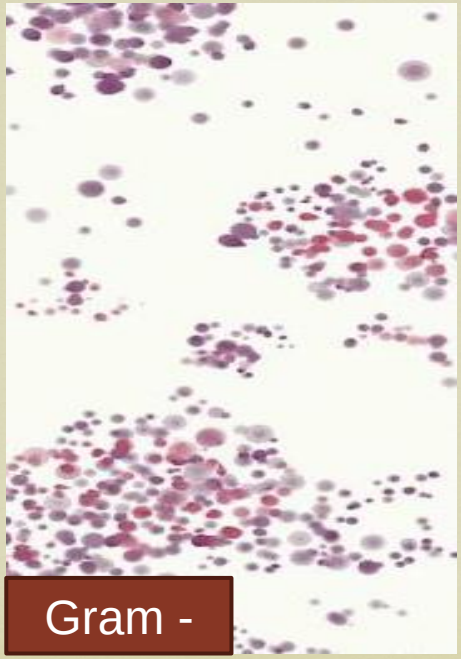
- 1.A small sample of a bacterial culture is removed from a culture. In this example it is being taken from a broth culture of the pure microbe but it could be removed from a culture on solid medium.
- 2. The bacterial suspension is smeared onto a clean glass slide. If the bacteria have been removed from a culture on solid media it will have to be mixed with a drop of distilled water.
- 3. The bacterial dried slowly at first and then, when dry, heated for a few seconds for fixation

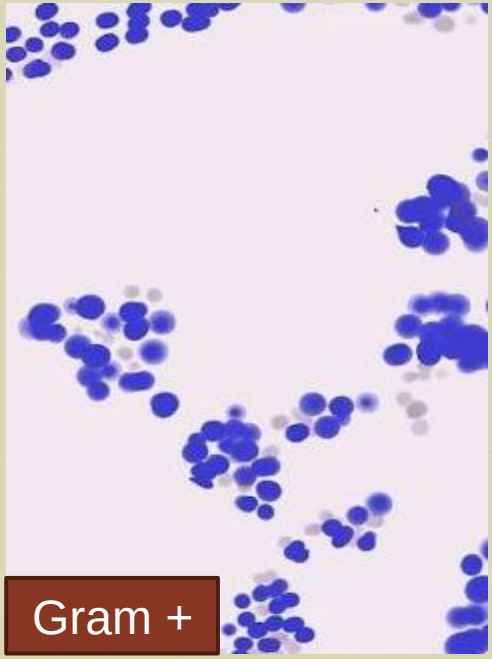
Gram staining

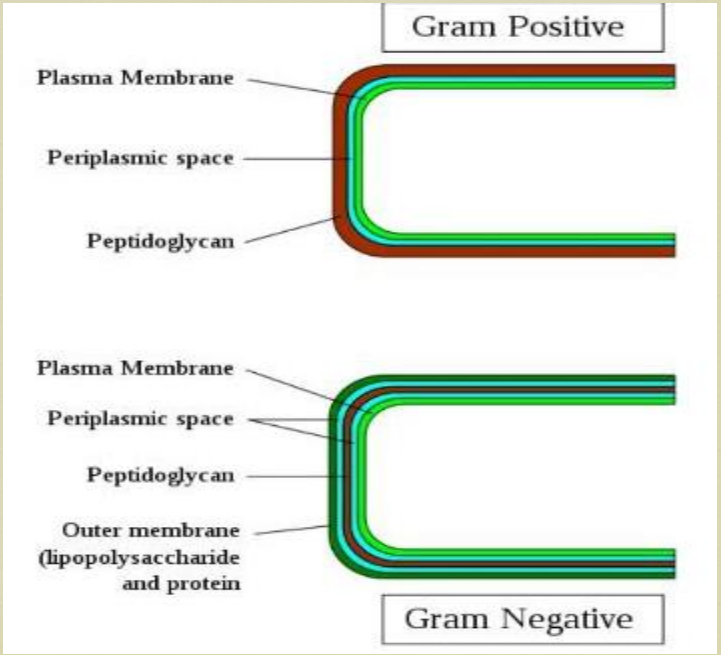
- 4. Cover the surface of the slide with Crystal Violet stain and let sit for (one minute).
- 5. Washing the slide with water.
- 6.Cover the slide with Gram's lodine and time for (one minute). Then Washing with water.
- 7. Cover the slide with Gram's decolorizer and time for 10-20 seconds. And wash with water.
- 8. Cover the slide with the counterstain, Safranin, and let sit for 30-60 seconds .
- 9. washing with water

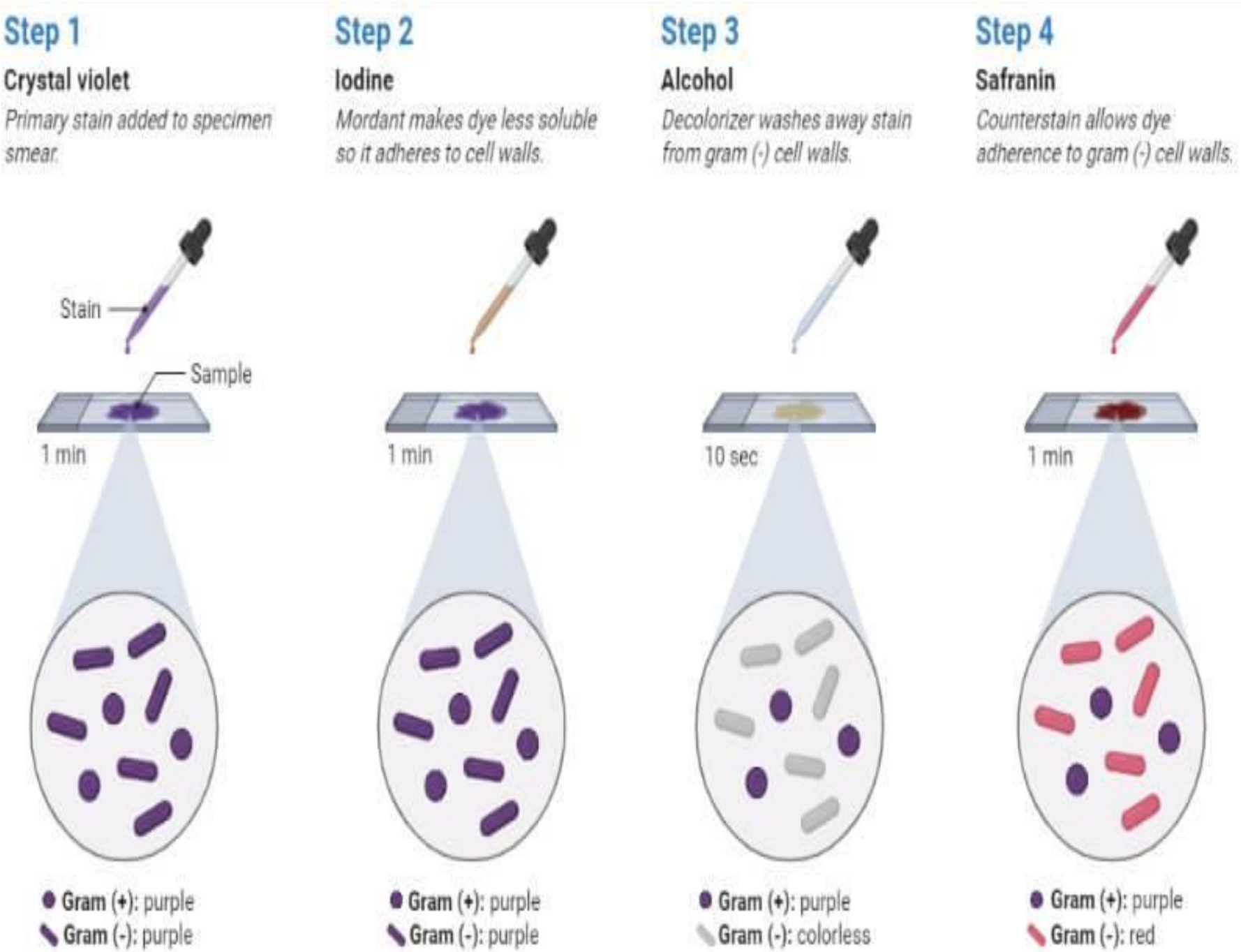
The Gram staining method

- 10.drying the slide and read with the oil immersion lens of the microscope. Look for Gram- negative and Gram- positive bacteria.








Step 1
Crystal violet
Primary stain added to specimen smear.
Stain
Sample
1 min
Gram (+): purple
Gram (-): purple
Step 2
Iodine
Mordant makes dye less soluble so it adheres to cell walls.
1 min
Gram (+): purple
Gram (-): purple
Step 3
Alcohol
Decolorizer washes away stain from gram (-) cell walls.
10 sec
Gram (+): purple
Gram (-): colorless
Step 4
Safranin
Counterstain allows dye adherence to gram (-) cell walls.
1 min
Gram (+): purple
Gram (-): red