Lec Fatma mustafa,

Asst Lec.Sura Mustafa & Renin Ibrahim

Practical microbiology

Biochemical Tests

Biochemical tests **are** laboratory procedures to differentiate various bacterial species by analyzing their biochemical activities.Biochemical tests frequently detect specific enzymes or metabolic pathways in microorganisms, helping differentiate them from other organisms.

Biochemical tests play an essential role in medical and biological sciences. These tests are among the most important methods for microbial identification.

Biochemical tests are one of the traditional methods for the identification of microorganisms, usually performed with phenotypic identification.For many years these methods were employed extensively, and they continue to be used nowadays,

biochemical tests used to identify gram positive and negative bacteria

1- Catalase Test

2- Coagulase Test

3- Oxidase Test

4- Indole Test

5- Urease Test

6- Sulfur Test

7- Triple sugar iron test

8- Nitrate Test

9- Starch Hydrolysis Test

10- Carbohydrate Fermentation Test

11- Methyl Red Test

12- Voges-Proskaur Test

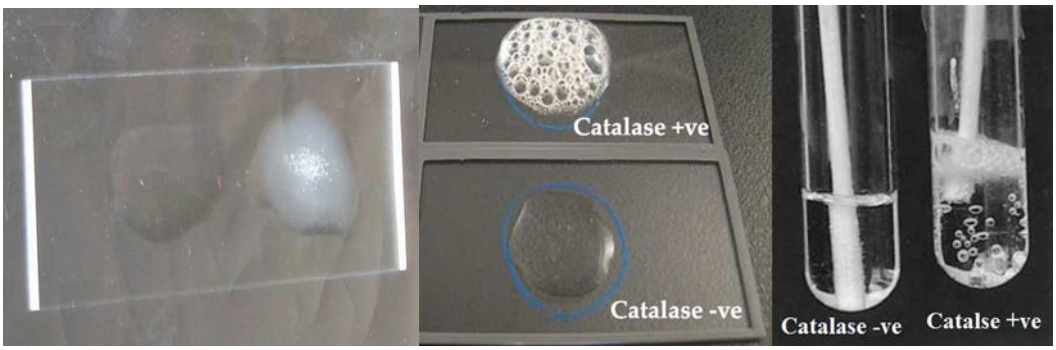
13- Citric Acid Utilization Test

14- Bile Esculin Agar Test

1 -Catalase Test

This test is can be used to detect the enzyme catalase. This enzyme is responsible for protecting bacteria from hydrogen peroxide (H2O2) accumulation, which can occur during aerobic metabolism. If hydrogen peroxide accumulates, it becomes toxic to the organism. Catalase breaks H2O2 down into water and O2.

Method: On the surface of clean slide mix few colonies with a drop of hydrogen peroxide, the formation of bubbles indicate a positive test. The test is done on a slide or in a test tube It is an essential test to differentiate between genus of Staphylococcus and Streptococcus, Staphylococcus gives a positive result while Streptococcus gives a negative result.



2 -Coagulase Test

The following test is used to identify microorganisms that can manufacture the coagulase enzyme. Mostly, it aids in the identification of Staphylococcus aureus, which is a coagulase and catalase test positive bacteria. Coagulase is one of the virulence factors found in S. aureus. During the reaction phase, the coagulase enzyme will coagulate the blood plasma. This test is carried out by combining blood plasma with a bacterial colony. Bacteria generate the coagulase enzyme, which causes the blood plasma to coagulate, indicating a positive reaction

Method:

A- Bound coagulase (detected in Slide method): Homogenous

suspension of the test organism is made in a drop of saline on a clean

slide then mixed with a drop of undiluted human or rabbit

plasma.Examine it under the microscope and look for clumping as

positive result, as the enzyme will precipitate the fibrin in the plasma

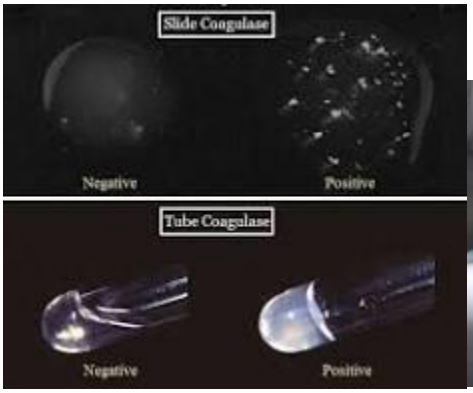
on the cell surface.

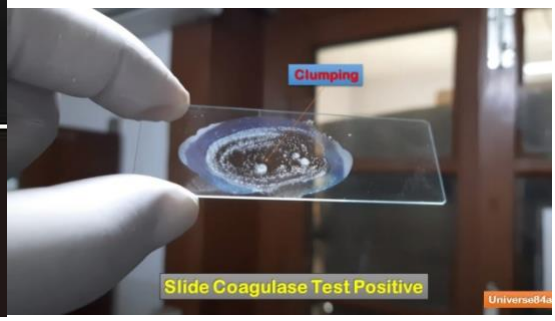
B-Tube method (detected in Free coagulase): It is done by adding 5

drops of an overnight broth culture of the test organism to l ml of human

or rabbit plasma diluted l:6 in sterile saline. The tubes are incubated for 4

hours at 37 °C in water bath and inspected hourly for clot formation by tilting the tube. Clot will float in the fluid or the whole plasma converts into gel





3-Oxidase test

The oxidase test is used to identify bacteria that produce cytochrome c

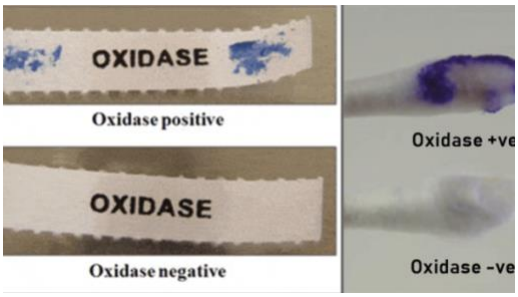
Procedure of Oxidase test:

1.Take a filter paper soaked with the substrateTetramethyl-p-phenylenediamine dihydrochloride)TMPD oxidase reagent

2.Moisten the paper with a sterile distilled water

3. Pick the colony to be tested with wooden or platinum loop and smear in the filter paper

4.Observe inoculated area of paper for a color change to deep blue or purple within 10-30 seconds



6-Urease Test

To determine the ability of microorganism to degrade urea by

means of the enzyme urease The presence of urease is detectable

when the organisms are grown in a urea broth medium containing the pH indicator phenol red. As the substrate urea is split into its products, the phenol red to turn to a deep pink. This is a positive reaction for the presence of urease. Failure of deep pink colour to develop is evidence of negative reaction.

Procedure of urease Test

Streak the surface of a urea agar slant with a portion of a well-isolated

colony.

Incubate the tube at 37°C for 48 hours to 7 days.

Examine for the development of a pink color

Example: Proteus mirabilis

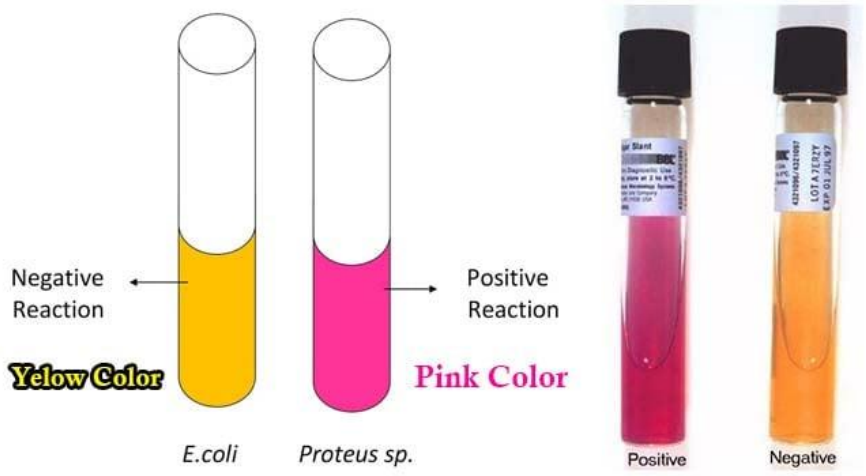
Positive Reaction: Development of a pink

color

Negative Reaction: No color change.

Examples: Escherichia, Shigella,

Salmonella



4-IMViC is a series of tests including the following tests:

Indole

Methyl Red (MR)

Voges- Proskauer (VP)

Citrate.

IMViC: These are a group of biochemical test that help in the identification and differentiation between enteric G-ve bacilli (enterobacteriaceae).

A-Indole production test:

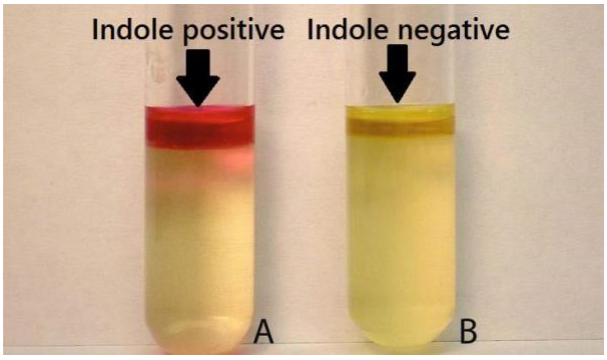
It tests for the bacterial ability to produce

indole. Bacteria use an enzyme, tryptophanase to break down the amino acid (tryptophan) to give indole, ammonia and pyruvic acid.

Tryptophan — Tryptophanase —> Indole + ammonia + pyruvic acid

Peptone liquid medium containing tryptophan is inoculated the- tested bacteria and incubated at 37 °C for 24 hrs. Few drops of kovac's reagent are added to the bacterial growth. The presence of red rig in the superficial layer of the medium indicate +ve result of indole production

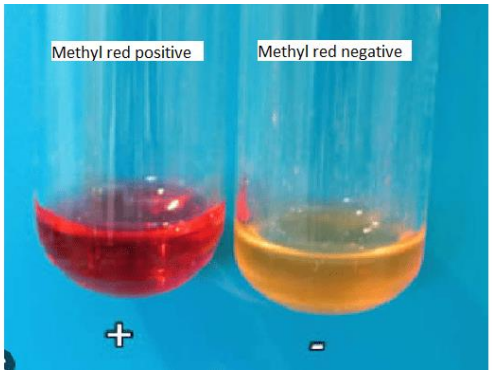
. Results: Indole-Positive reaction: red color ex. E.coli; Negative reaction: yellow color ex. Klebsiella



Methyl Red Voges-Proskaur Test (MR-VP test)

Methyl Red MR test

Principle to test the ability of the organism to produce acid end product from gl Inoculate the medium (MRVP broth (pH 6.9) with bacteria



MR results: Red: Positive MR (E. coli); Yellow: Negative MR (Klebsiella)

Voges-Proskaur Test VP test

To determine the ability of the organisms to produce neutral end product (acetoin) from glucose fermentation.

procedure

1. Inoculate the tested organism into 2 tubes of MR-VP broth

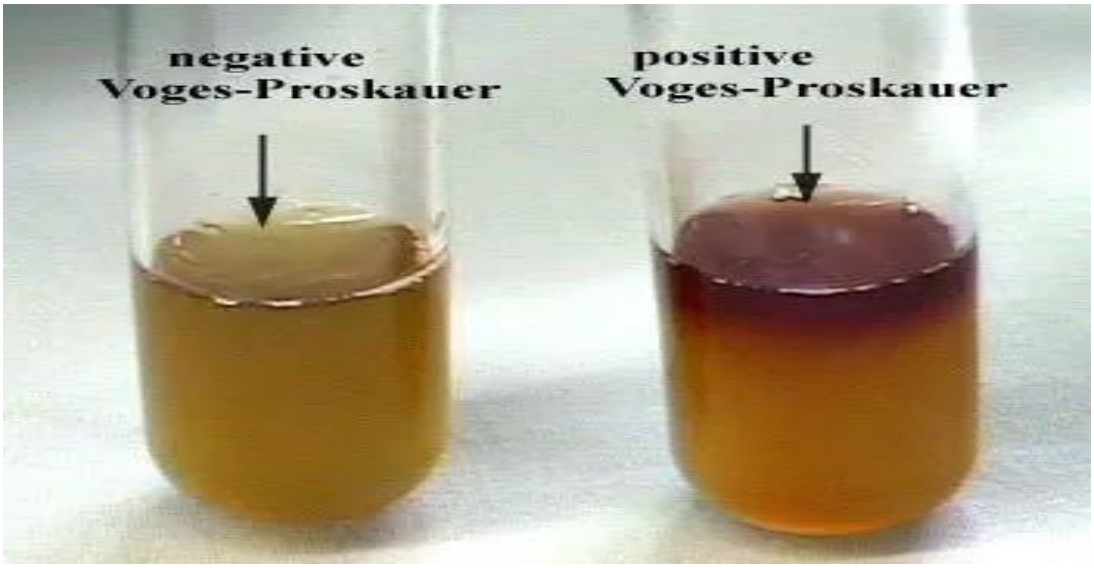
2. Incubate the tubes at 37°C for 24 hours

3. After incubation: Run the MR test in the tube 1, and the VP test in tube 2.

– For methyl red: Add 6-8 drops of methyl red reagent.

– For Voges-Proskauer: Add 12 drops of Barritt's A (□-naphthol), mix, 4 drops of Barritt's B (40% KOH), mix

– Let sit, for at least 1hour

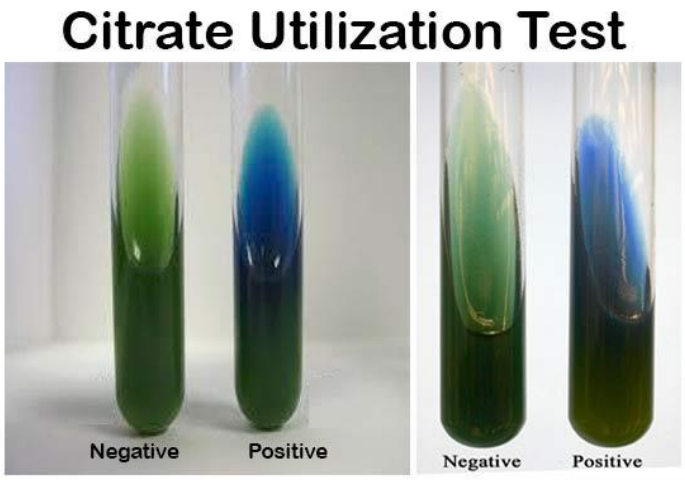


Voges-Proskauer results

Pink: Positive VP (Klebsiella), yellow: Negative VP (E. coli)

5-Citrate Utilization Test:

Simmons Citrate agar is a defined medium containing sodium citrate as the sole carbon source. The pH indicator, bromthymol blue, will turn from green at neutral pH (6.9) to blue when a pH higher than 7.6 is reached (alkaline). If the citrate is utilized, the resulting growth will produce alkaline products changing the color of the medium from green to blue. (Blue color= positive reaction eg; Klebsiella) ;(green color=negative reaction eg; E.coli)



Analytical Profile Index (API):

It is an miniaturized panel of biochemical tests compiled foridentification of groups of closely related bacteria.

Introduction of API Test for Bacteria

API test for bacteria as shown above picture is a 20-jumbo tests kit for a biochemical panel for the identification and differentiation of members of the family Enterobacteriaceae. API stands for Analytical Profile Index. It is an API 20E Test kit that is quick, safe, and easy to perform and it is hence a well-established method for manual microorganism identification to the species level. Modified API test kits are also available for the identification of microorganisms covering Gram-positive and Gram-negative bacteria and yeast. API strips give accurate identifications based on extensive databases and are standardized

API 20E test

VITEK 2 System for Rapid Identification of Clinical Isolates

The fully automated VITEK 2 system (bioMérieux) can provide identification results for microbial identification (bacteria and yeast identification) rapidly, accurately and reliable species-level identification in a few hours. It improved microbial identification and antibiotic susceptibility testing (AST) for all microbial isolates which isolated from different clinical specimens (blood, CSF, urine, stool, wound, burns, and others…).

The VITEK 2 system can:Reduce time to microbial identification and antibiotic susceptibility testing results

Lec Fatma mustafa,

Asst Lec.Sura Mustafa & Renin Ibrahim

Bacterial staining

Staining is a technique used in microscopy to enhance contrast in the microscopic image in biology and medicine to highlight structures in cell populations or organelles within individual cells

Simple Stains: The simple stain can be used to determine cell shape, size, and arrangement. The simple stain is a very simple staining procedure involving only one stain e.g. crystal violet.

Differential Stains: is a staining process which uses more than one chemical stain. Using multiple stains can better differentiate between different microorganisms. One commonly recognizable use of differential staining is the Gram stain.

Special Stains - These are stains that include the acid-fast, endospore, capsule ,flagellar stains.

Acid-Fast Stain - is used for staining cells of ***Mycobacterium*** and ***Nocardia.***

Albert stain: a stain for diphtheria bacilli and their metachromatic granules.

Gram stain

Gram stain is a common technique used to differentiate two large bacterial species into two large groups (Gram-positive and Gram- negative). Based on

their different cell wall constituents. The **Gram stain** procedure distinguishes between **Gram** positive and **Gram** negative groups by coloring these cells red or violet.

Gram stain

• One of the most important biological staining technique in bacteriology

• Differential stain

• Used to separate all known bacteria into 2 groups

– Gram positive

– Gram negative

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram-positive bacteria stain violet or Purple due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram-negative bacteria stain pink , which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process

The Gram Stain Procedure

Step 1 - Prepare a Smear

1. Using a sterile pipet, add a drop of sterile water so there is a film/bubble across the loop.

2. Transfer the water to the center of the slide.

3. Sterilize the loop.

4. Transfer bacteria from a plate to the slide. Touch the colony lightly to prevent from transferring too much bacteria to the slide. If you can see the colony on your loop, you probably have too much.

5. Sterilize the loop.

6. Let the liquid dry completely on the slide leaving behind a dry spot. This should take about 10 minutes

Once the liquid has evaporated, heat fix the bacteria to the slide by quickly passing the slide through the flame 3 times.

step2- Flood the Smear with Crystal Violet. Crystal violet is a basic dye, stains all the cells purple.Allow to stand for 1 min, rinse with water to remove excess stain

Step 3 - Flood the Smear with Iodine solution.The iodine forms an insoluble complex

with the crystal violet to anchor it into the cell wall.Allow to stand 1 min Rinse with

water to remove excess Iodin

step4- Drip Decolorizer (80% Methanol +20% Acetone) across the slide about 30 sec

This removes the outer membrane of the gram negative bacteri, Rinse immediately with water to remove excess alcohol

Step5- Counterstain

Flood the slide with Safranin solution Let stand for 1 minute

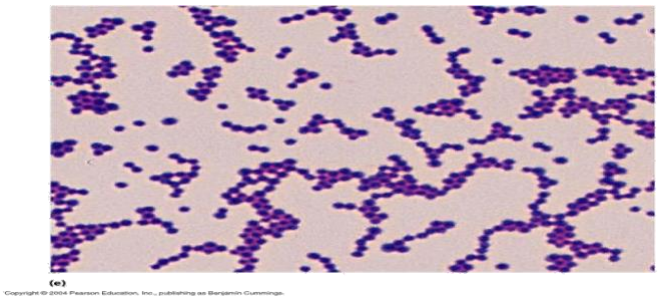
Rinse with water to remove excess stain Blot dry

Observe under Oil Immersion

Gram positive

Shape: Cocci

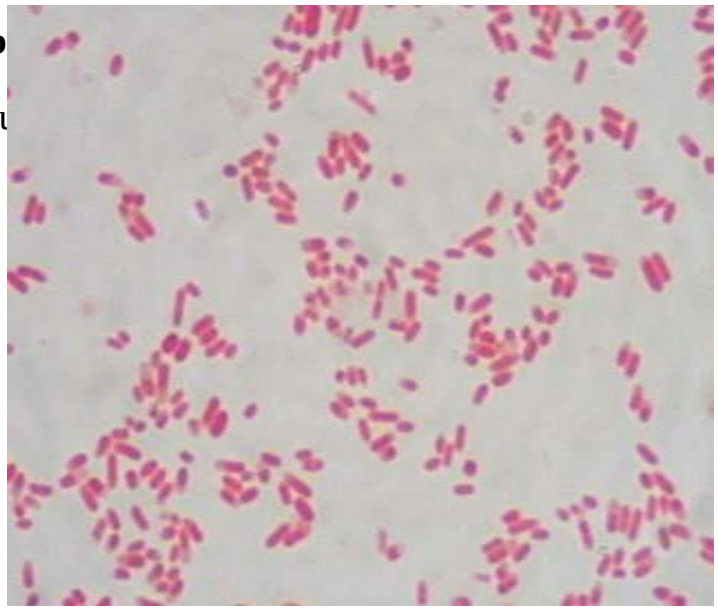
Colour: Violet (Purple)

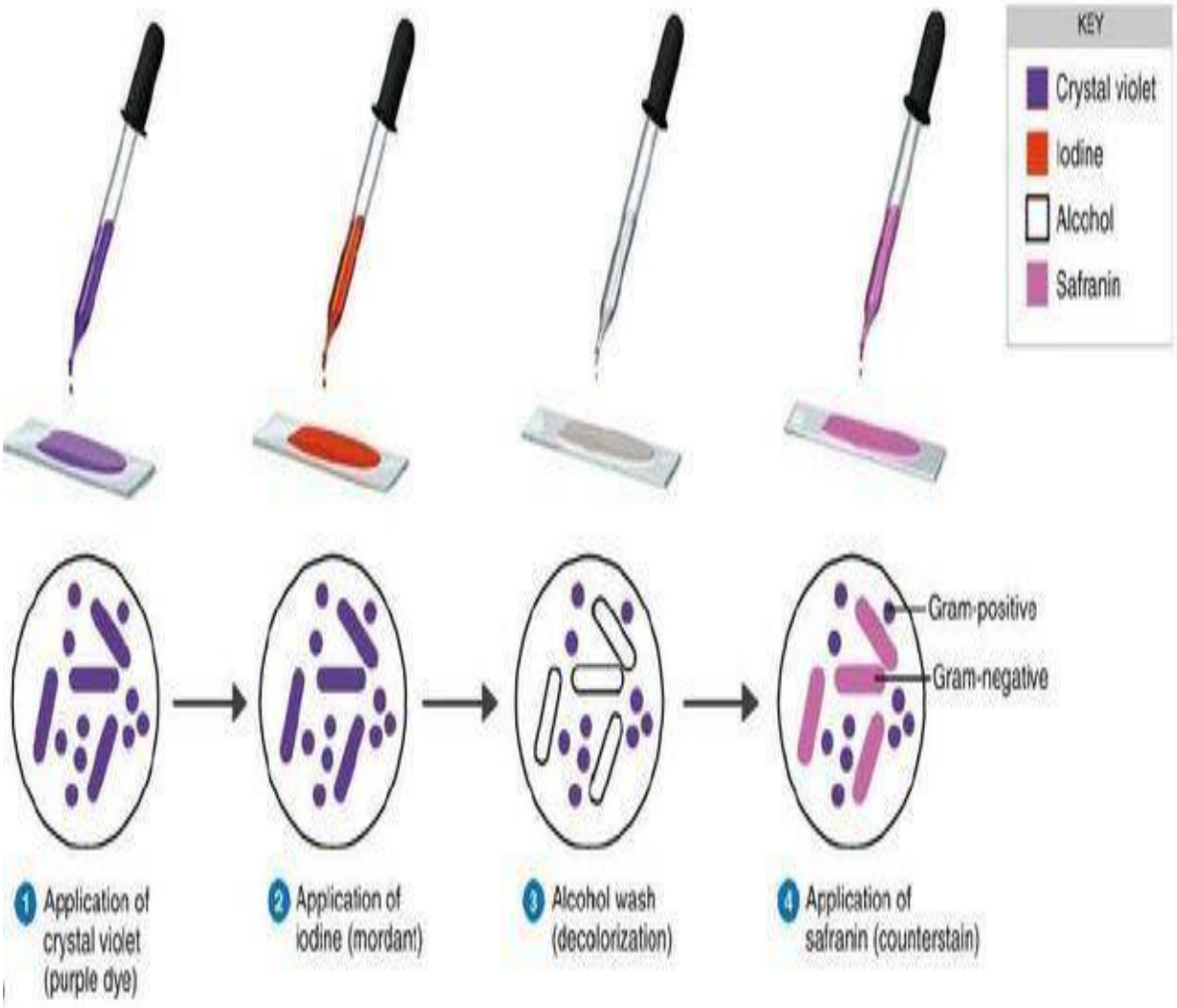


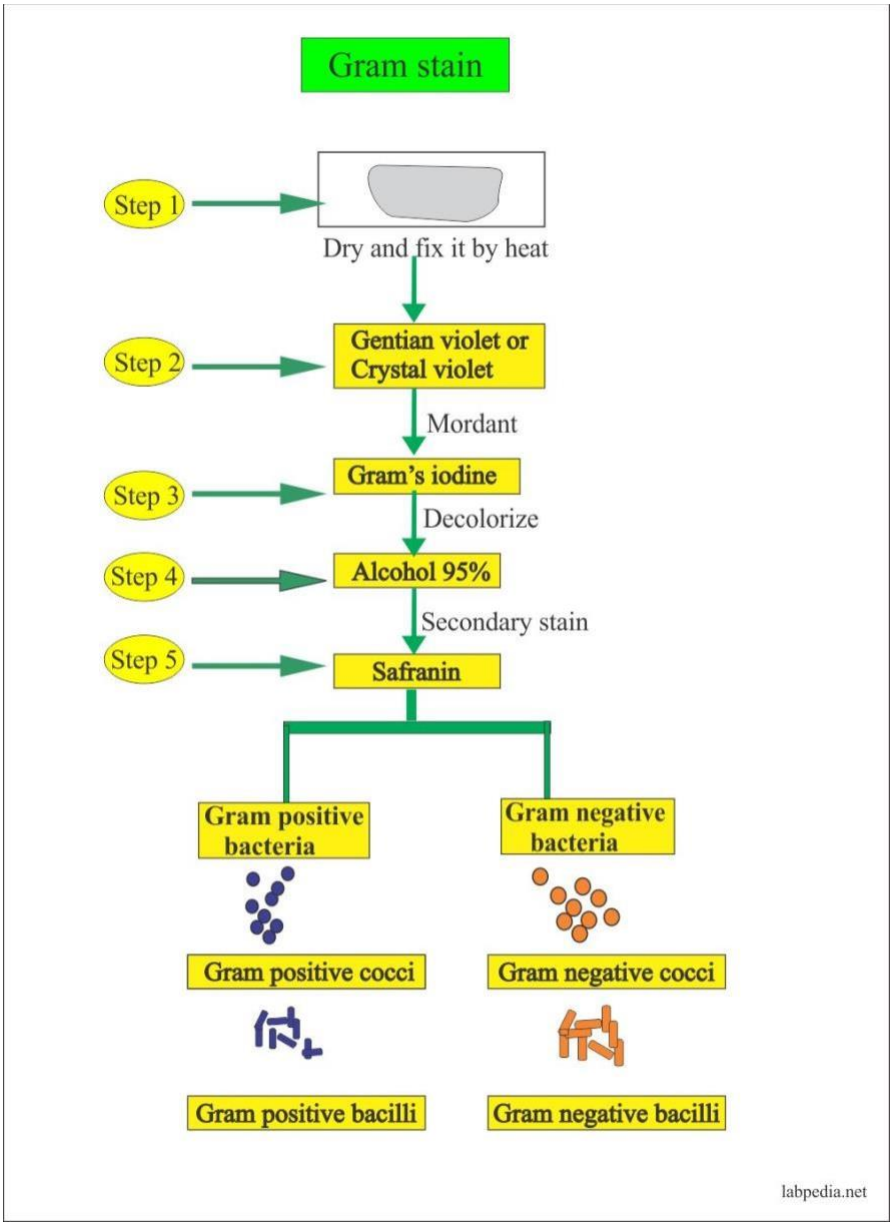
Gram negative

Shap

Colou




KEY
Crystal violet
Iodine
Alcohol
Safranin
Gram-positive
Gram-negative
1 Application of crystal violet (purple dye)
2 Application of iodine (mordant)
3 Alcohol wash (decolorization)
4 Application of safranin (counterstain)


Gram stain
Step 1
Dry and fix it by heat
Step 2
Gentian violet or Crystal violet
Mordant
Step 3
Gram's iodine
Decolorize
Step 4
Alcohol 95%
Secondary stain
Step 5
Safranin
Gram positive bacteria
Gram negative bacteria
Gram positive cocci
Gram negative cocci
Gram positive bacilli
Gram negative bacilli
labpedia.net

Lec Fatma Mustafa

Asst Lec.Sura Mustafa & Renin Ibrahim

Microscope

is an instrument used to see objects that are too small to be seen by the naked eye. And is an instrument that magnifies objects otherwise too small to be seen, producing an image in which the object appears larger

Types of Microscopes

Various types of microscopes are available for use in the microbiology laboratory.

.

dark-field microscope, which is used to observe live spirochetes, such as those that cause syphilis. This microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. A light object is seen on a dark background

Stereo microscopes provide lower magnification than compound microscopes. Stereo microscope magnification typically ranges from 5x-80x and the images seen are three-dimensional images rather than a flat images.

Ultraviolet microscopes. utilize the shorter wavelength of ultraviolet **.** electromagnetic energy to improve the image resolution beyond that of the diffraction limit of standard optical microscopes

phase-contrast microscope. This microscope also contains special condensers that throw light "out of phase" and cause it to pass through the object at different speeds. Live, unstained organisms are seen clearly with this microscope, and internal cell parts such as mitochondria, lysosomes, and the Golgi body can be seen with this instrument

The fluorescent microscope uses ultraviolet light as its light source. When ultraviolet light hits an object, it excites the electrons of the object, and they give off light in various shades of color. Since ultraviolet light is used, the resolution of the object increases

Electron microscopy. The energy source used in the electron microscope is a beam of electrons. . Viruses and some large molecules can be seen with this instrument

The more traditional form of electron microscope is the transmission electron microscope (TEM) and The scanning electron microscope (SEM).

light microscope

The common light microscope used in the laboratory is called a compound light microscope

A compound light microscope.

The common light microscope used in the laboratory is called a compound microscope because it contains two types of lenses that function to magnify an object. The lens closest to the eye is called the ocular, while the lens closest to the object is called the objective. Most microscopes have on their base an apparatus called a condenser, which condenses light rays to a strong beam. A diaphragm located on the condenser controls the amount of light coming through it. Both coarse and fine adjustments are found on the light microscope

Immersion oil has the same light-bending ability as the glass slide, so it keeps light in a straight line as it passes through the glass slide to the oil and on to the glass of the objective, the oil-immersion lens. With the increased amount of light entering the objective, the resolution of the object increases

Parts Of Compound Microscope

. **Eyepieces**: The eyepieces are the lenses at the top that the viewer looks through; they are usually 10X or 15X. To get the total magnification level, multiply the magnification of the objective used (ex: 10X eyepiece * 40X objective = 400X total magnification)

.

Objective Lenses: Usually you will find 3 or 4 objective lenses on a microscope. The most common ones are 4X (shortest lens), 10X, 40X and 100X (longest lens). The higher power objectives (starting from 40x) are spring loaded. Spring loaded objective lenses will retract if the objective lens hits a slide.

Arm: Structural element that connects the head of the microscope to the base. The entire microscope is handled by a strong and curved structure known as the arm.

Stage: The flat platform that supports the slides. Stage clips hold the slides in place. If your microscope has a mechanical stage, the slide is controlled by turning two knobs instead of having to move it manually. One knob moves the slide left and right.

Base: The bottom of the microscope—what the microscope stands on. It is a U-shaped structure and supports the entire weight of the compound microscope

Clips;The upper part of the stage is connected to two clips. The slide can be held in its position with the help of the clips.

Diaphragm;The diaphragm is fastened below the stage. It controls and adjusts the intensity of light that passes into the microscope.

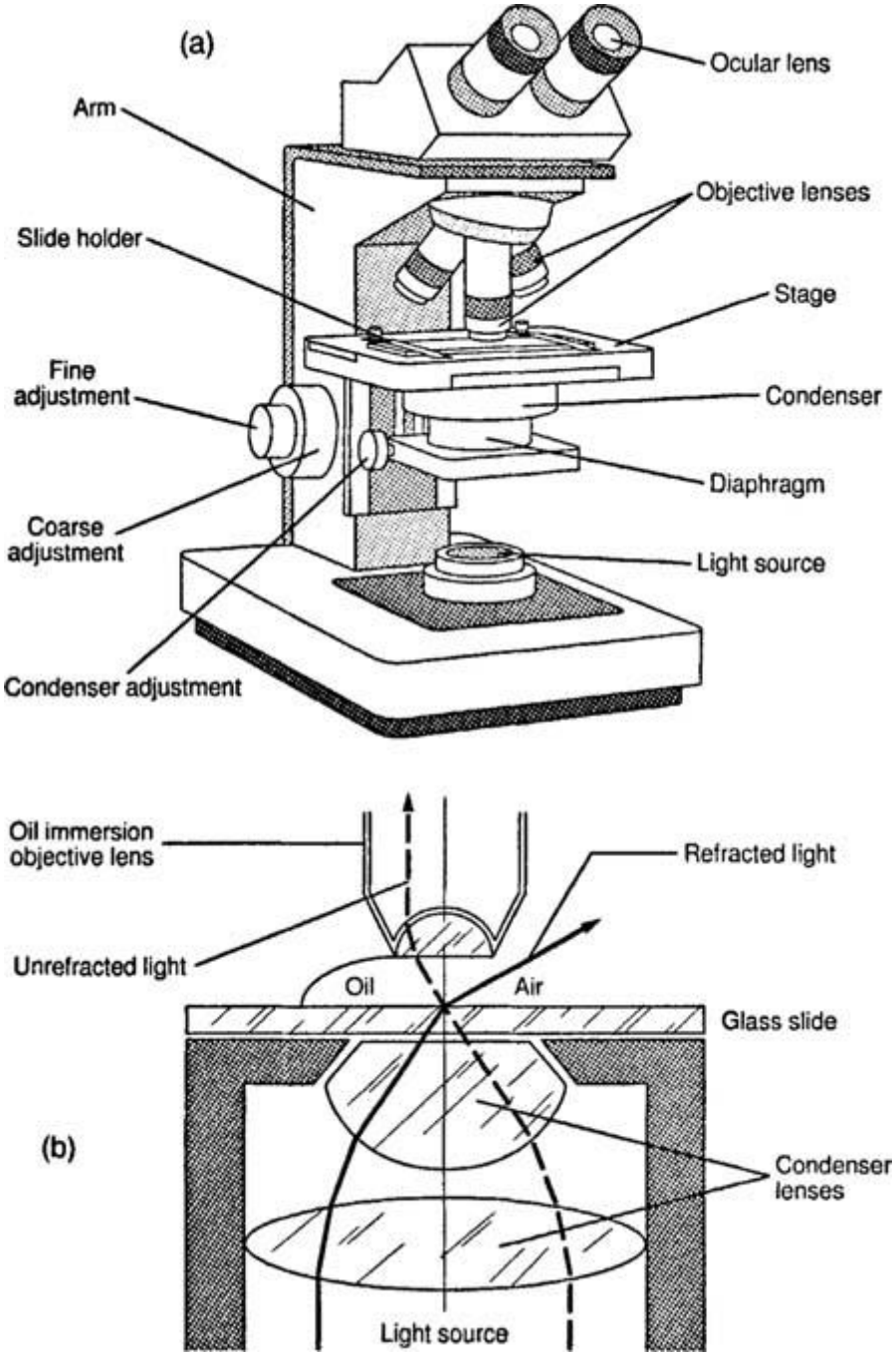
Illuminator: A steady light source that shines up through the slide. Mirrors are sometimes used in lieu of a built-in light. If your microscope

has a mirror, it is used to reflect light from an external light source up through the bottom of the stage.

Condenser Lens: Condenser lenses focus the light that shines up through the slide, and are useful for attaining sharp images at magnifications of 400X and above. Most microscopes that go up to 1000X come equipped with an Abbe condenser, which can be focused by moving it up and down. The Abbe condenser should be set closest to the slide at 1000X, and moved further away as the magnification level gets lower.

Fine adjustment knob;It is the small knob which is used for sharp and fine focusing of the object. For accurate and sharp focusing, this knob can be used.

Coarse adjustment knob;It is a large knob that is used for moving the body tube down and up for bringing the object to be examined under exact focus.


(a)
Ocular lens
Arm
Objective lenses
Slide holder
Stage
Fine adjustment
Condenser
Diaphragm
Coarse adjustment
Light source
Condenser adjustment
Oil immersion objective lens
Refracted light
Unrefracted light
Oil
Air
Glass slide
(b)
Condenser lenses
Light source

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Bacterial shape

• can usually be determined with appropriate staining and a light microscope

• may be pleomorphic with some species, such as Bacteroides

. • is used, along with other properties, to identify bacteria

Depending on their shape, bacteria are classified into several varieties

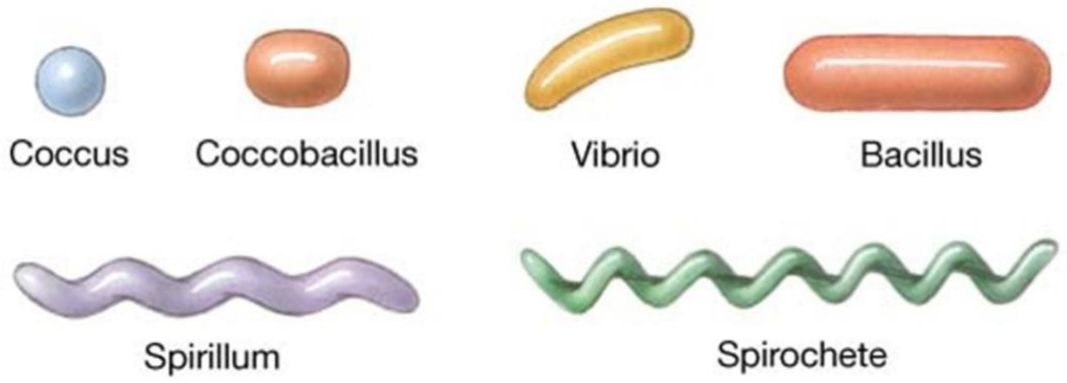
1. Cocci are spherical or oval cells

2. Bacilli are rod shaped cells

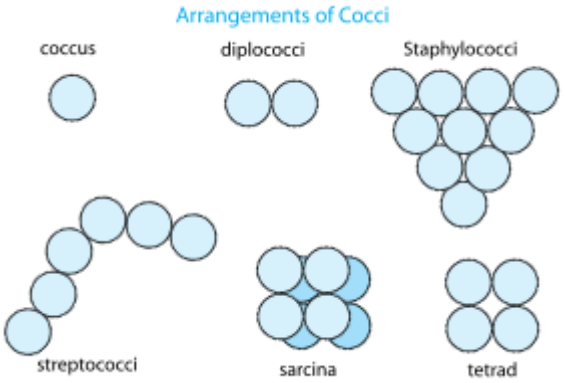
3. Vibrios are comma shaped curved rods and derive their name from their characteristics vibratory motility

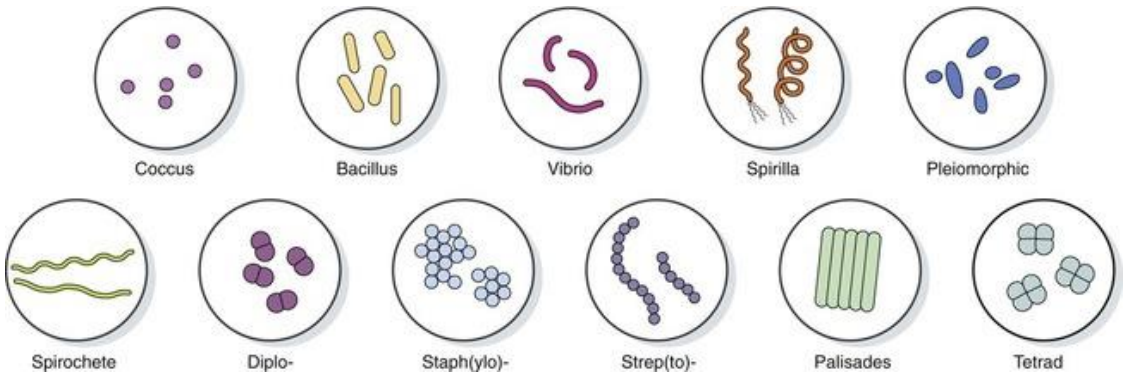
4. Spirilla are rigid spiral forms The spiral shape can appear in several forms: vibrio, spirillum, and spirochete.

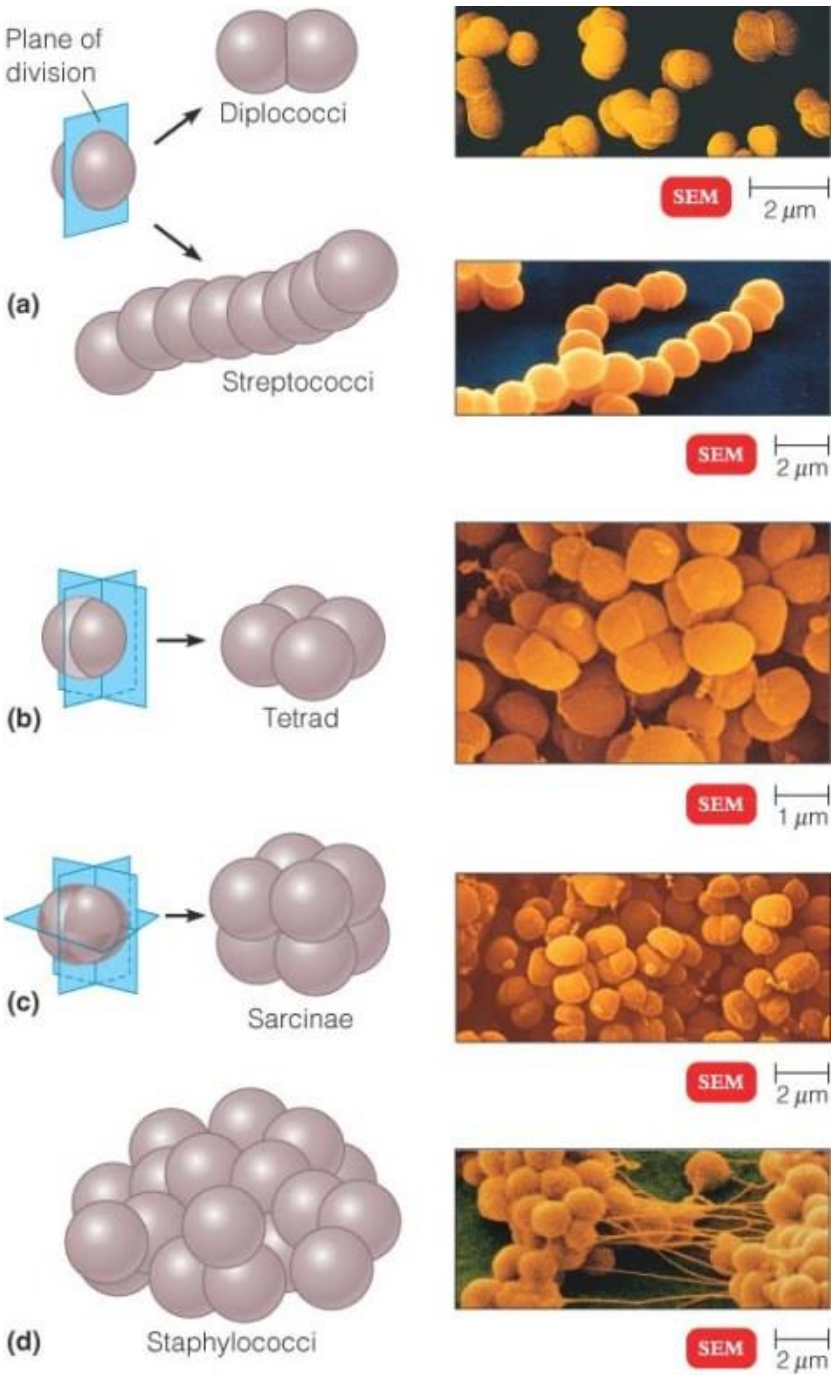
5. Mycoplasmas are bacteria that are cell wall deficient and hence do not possess a stable morphology. They occur as round or oval bodies



Bacteria sometime show characteristic cellular arrangement or grouping. According to the plane of cellular division, cocci may be arranged in pairs (diplococci), chains (streptococci), groups of four (tetrads) or eight (sarcina), or grape like clusters (staphylococci The bacillus shape can appear as a single bacillus, a streptobacillus, or a coccobacillus






Plane of division
Diplococci
(a)
Streptococci
SEM
2 μm
SEM
2 μm
(b)
Tetrad
SEM
1 μm
(c)
Sarcinae
SEM
2 μm
(d)
Staphylococci
SEM
2 μm

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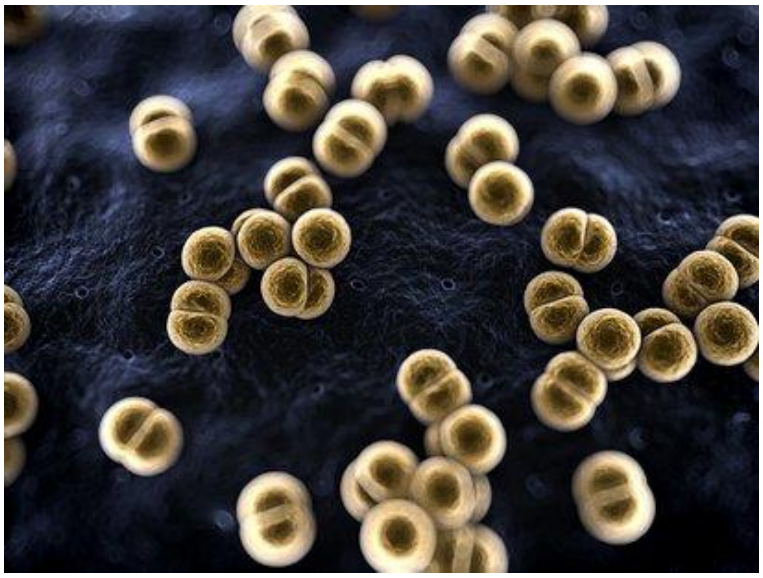
Neisseria

Gram-negative cocci often arranged in pairs(diplococcic) Catalase test-positive, Oxidase test-positive



General characteristics

*** G –ve diplococcic, kidney shape, with parallel long axis.**

*** Oxidase positive.**

*** Ferment carbohydrates.**

*** Non -haemolytic**

Media used:

Blood agar, chocolate agar, modified Thayer-Martin agar

Thayer-Martin agar is a selective and enriched medium for the isolation and cultivation of Neisseria sp. from mixed flora with suppression of

most other gram-negative diplococcic, gram-negative bacilli, gram-.positive organisms, and yeast

Typical colonial morphology or Colony characteristics on these media is as follows

Neisseria gonorrhea: Small, grayish-white to colorless, mucoid with a .smooth consistency and defined margins

.Neisseria meningitides: Medium to large, blue-gray, mucoid

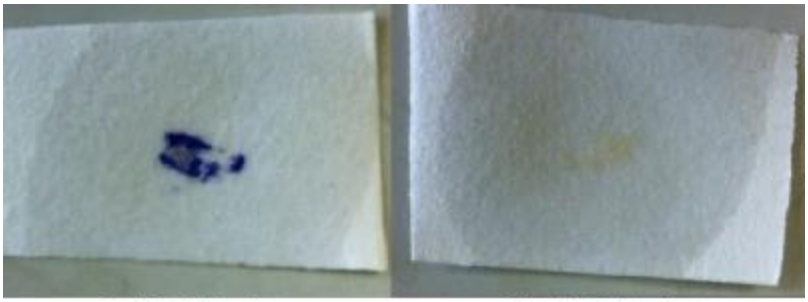
All the medically significant species of Neisseria are positive for both catalase and oxidase

Oxidase test

The oxidase test is used to identify bacteria that produce cytochrome c

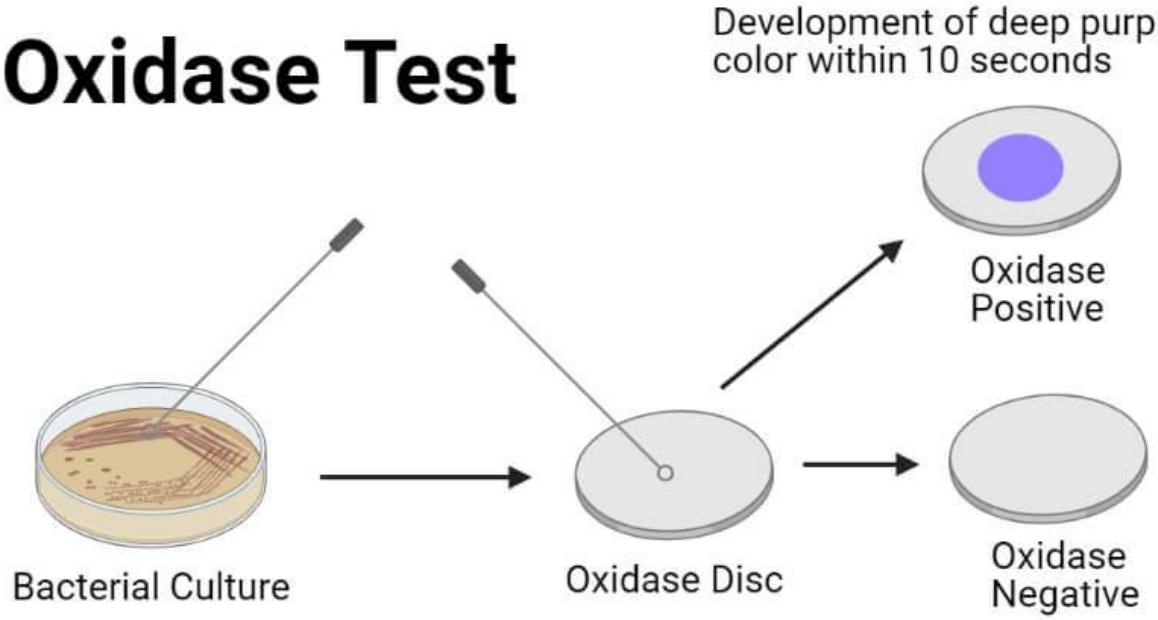
Procedure of Oxidase test:

1. Take a filter paper soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride
2. Moisten the paper with a sterile distilled water
3. Pick the colony to be tested with wooden or platinum loop and smear in the filter paper
4. Observe inoculated area of paper for a color change to deep blue or purple within 10-30 seconds



Positive Oxidase Test

Negative Oxidase Test
(Read within 30 seconds)



Neisseria. gonorrhoeae

. Diagnosis

Swab sample. A swab sample from the part of the body likely to be infected (cervix, urethra, rectum, or throat) can be sent to a lab for testing

1- Gram stain.

2- Urine test Gonorrhoea in the cervix or urethra can also be diagnosed with a urine sample sent to a lab.

3- Oxidase Test

4- Culture; Thayer-Martin agar or Thayer-Martin medium

Neisseria meningitidis

. Diagnosis

; Specimen; The gold standard of diagnosis is isolation of N. meningitidis from sterile body fluid. Blood, CSF specimen is sent to the laboratory immediately for identification of the organism

. 1- Gram stain; show adjacent coffee bean-shape, Gram negative cells (diplococci) with flattened surfaces facing each other.

2- Culture; culturing the organism on Thayer-Martin agar

. 3- Oxidase test

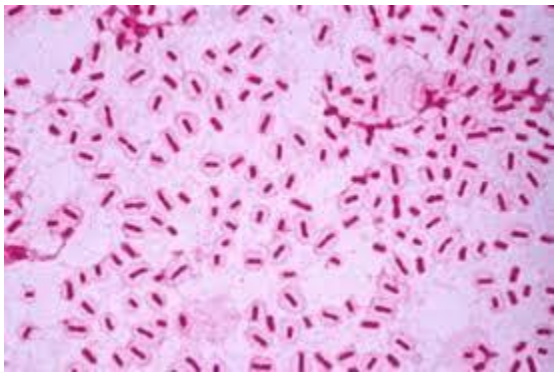
4- Sugar fermentation tests

. 5- Serology

Haemophilus influenza

- Morphology Slender, short, gram-negative rods or coccobacillus
- Non-Motile, No flagella or pilli
- Capsules are present and important in pathogenicity.
- Fastidious Microorganism
- Oxidase and Catalase positive

H. influenzae are small, pleomorphic, gram-negative bacilli or coccobacilli with random arrangements. H. influenzae is a fastidious organism which grows best at 35-37°C with ~5% CO2 (or in a candle-jar) and requires hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD, also known as V factor) for growth



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Cultural characteristics

On Blood Agar

- Translucent, low, convex or flat pinpoint colonies
- Satellitism

On Chocolate Agar

- Grayish, Transparent, smooth, low, convex or flat with a slightly splayed out, entire edge, mucoid, pale

Bacterial culture of H. influenzae is performed on agar plates, the preferable one being chocolate agar, with added X (hemin) and V (nicotinamide adenin dinucleotide) factors at 37 °C in a CO2-enriched incubator. Blood agar growth is only achieved as a satellite phenomenon around other bacteria. Colonies of H. influenzae appear as convex, .smooth, pale, grey, or transparent colonies,

Most strains of Haemophilus spp does not grow on 5% Sheep Blood .)Agar, which contains hemin (factor X) but lacks NAD (factor V)

Staphylococcus aureus produce NAD as a metabolic by product when grow in a culture media containing blood. Therefore, Haemophilus spp may grow on sheep blood agar very close to the colonies of Staphylococcus aureus (as it produces NAD-factor V); this phenomenon is known as satelliting

Satellitism

- On blood agar colonies of S Aureus release V-Factor during growth
- Which defuses into the surrounding medium
- Enhancing the growth of H. influenzae.
- Influenzae requires pantothenic acid, thiamine and uracil.

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Haemophilus influenzae on Chocolate Agar

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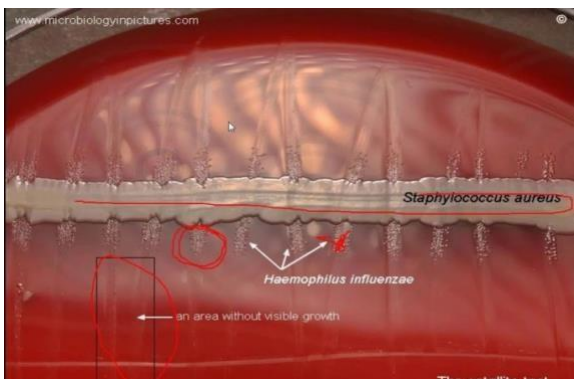
Satellite phenoenon of Haemophilus influencae with Staphylococcus aureus on blood agar

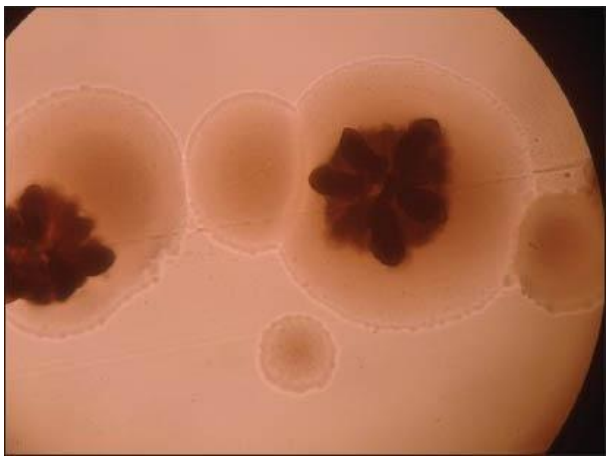


Aggregatibacter actinomycetemcomitans

(*Actinobacillus actinomycetemcomitans*)

General Characteristics

Aggregatibacter actinomycetemcomitans is a fastidious, facultatively anaerobic, non-motile, small Gram-negative rod, 0.4–0.5 μm × 1.0–1.5 μm in size. Microscopically, the cells may appear as cocci in broth and in clinical samples. It grows poorly in ambient air, but well in 5% CO_2 . Colonies on chocolate agar are small, with a diameter of ≤0.5 mm after 24 h, but may exceed 1–2 mm after 48 h. Primary colonies are rough-textured and adhere strongly to the surface of agar



Lec Fatma Mustafa

Asst Lec.Sura Mustafa & Renin Ibrahim

Biochemical Tests

Biochemical tests **are** laboratory procedures to differentiate various bacterial species by analyzing their biochemical activities.Biochemical tests frequently detect specific enzymes or metabolic pathways in microorganisms, helping differentiate them from other organisms.

Biochemical tests play an essential role in medical and biological sciences. These tests are among the most important methods for microbial identification.

Biochemical tests are one of the traditional methods for the identification of microorganisms, usually performed with phenotypic identification.For many years these methods were employed extensively, and they continue to be used nowadays,

biochemical tests used to identify gram positive and negative bacteria

1- Catalase Test

2- Coagulase Test

3- Oxidase Test

4- Indole Test

5- Urease Test

6- Sulfur Test

7- Triple sugar iron test

8- Nitrate Test

9- Starch Hydrolysis Test

10- Carbohydrate Fermentation Test

11- Methyl Red Test

12- Voges-Proskaur Test

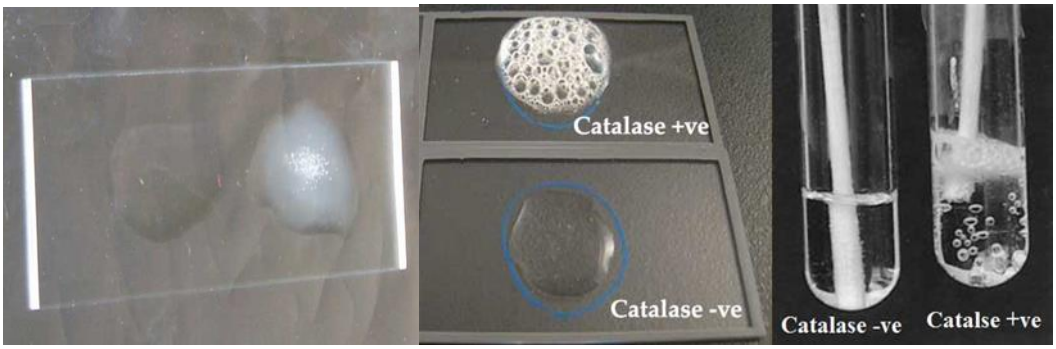
13- Citric Acid Utilization Test

14- Bile Esculin Agar Test

1 -Catalase Test

This test is can be used to detect the enzyme catalase. This enzyme is responsible for protecting bacteria from hydrogen peroxide (H2O2) accumulation, which can occur during aerobic metabolism. If hydrogen peroxide accumulates, it becomes toxic to the organism. Catalase breaks H2O2 down into water and O2.

Method: On the surface of clean slide mix few colonies with a drop of hydrogen peroxide, the formation of bubbles indicate a positive test. The test is done on a slide or in a test tube It is an essential test to differentiate between genus of Staphylococcus and Streptococcus, Staphylococcus gives a positive result while Streptococcus gives a negative result.



2 -Coagulase Test

The following test is used to identify microorganisms that can manufacture the coagulase enzyme. Mostly, it aids in the identification of Staphylococcus aureus, which is a coagulase and catalase test positive bacteria. Coagulase is one of the virulence factors found in S. aureus. During the reaction phase, the coagulase enzyme will coagulate the blood plasma. This test is carried out by combining blood plasma with a bacterial colony. Bacteria generate the coagulase enzyme, which causes the blood plasma to coagulate, indicating a positive reaction

Method:

A- Bound coagulase (detected in Slide method): Homogenous

suspension of the test organism is made in a drop of saline on a clean

slide then mixed with a drop of undiluted human or rabbit

plasma.Examine it under the microscope and look for clumping as

positive result, as the enzyme will precipitate the fibrin in the plasma

on the cell surface.

B-Tube method (detected in Free coagulase): It is done by adding 5

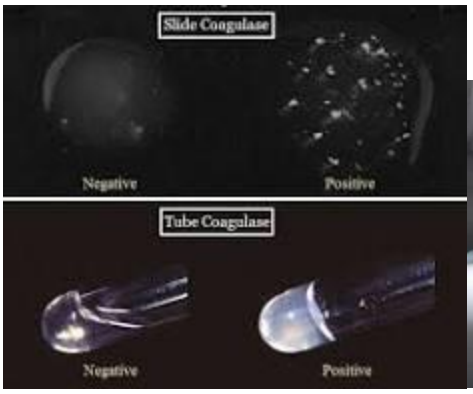
drops of an overnight broth culture of the test organism to l ml of human

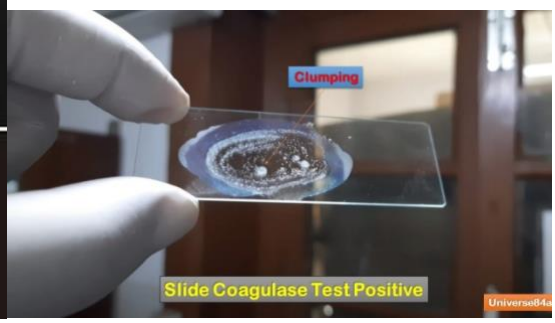
or rabbit plasma diluted l:6 in sterile saline. The tubes are incubated for 4

hours at 37 °C in water bath and inspected hourly for clot formation by

tilting the tube. Clot will float in the fluid or the whole plasma converts

into gel





3-Oxidase test

The oxidase test is used to identify bacteria that produce cytochrome c

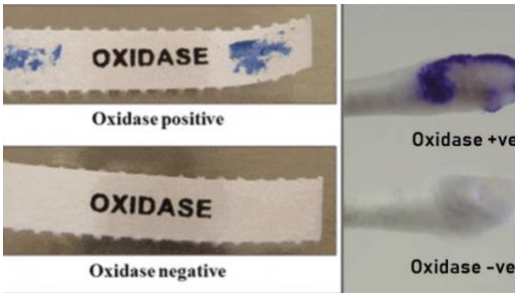
Procedure of Oxidase test:

1.Take a filter paper soaked with the substrateTetramethyl-p-phenylenediamine dihydrochloride)TMPD oxidase reagent

2.Moisten the paper with a sterile distilled water

3. Pick the colony to be tested with wooden or platinum loop and smear in the filter paper

4.Observe inoculated area of paper for a color change to deep blue or purple within 10-30 seconds



6-Urease Test

To determine the ability of microorganism to degrade urea by

means of the enzyme urease The presence of urease is detectable

when the organisms are grown in a urea broth medium containing the pH indicator phenol red. As the substrate urea is split into its products, the phenol red to turn to a deep pink. This is a positive reaction for the presence of urease. Failure of deep pink colour to develop is evidence of negative reaction.

Procedure of urease Test

Streak the surface of a urea agar slant with a portion of a well-isolated

colony.

Incubate the tube at 37°C for 48 hours to 7 days.

Examine for the development of a pink color

Example: Proteus mirabilis

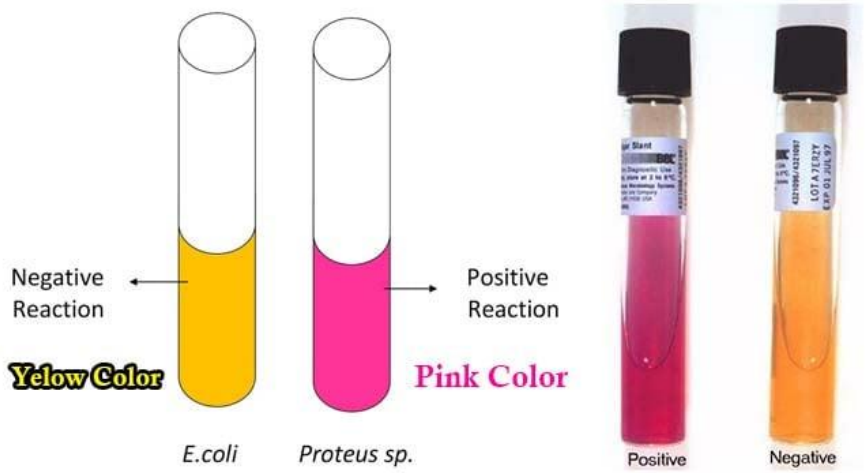
Positive Reaction: Development of a pink

color

Negative Reaction: No color change.

Examples: Escherichia, Shigella,

Salmonella



4-IMViC is a series of tests including the following tests:

Indole

Methyl Red (MR)

Voges- Proskauer (VP)

Citrate.

IMViC: These are a group of biochemical test that help in the identification and differentiation between enteric G-ve bacilli (enterobacteriaceae).

A-Indole production test:

It tests for the bacterial ability to produce indole. Bacteria use an enzyme, tryptophanase to break down the amino acid (tryptophan) to give indole, ammonia and pyruvic acid.

Tryptophan — Tryptophanase —> Indole + ammonia + pyruvic acid

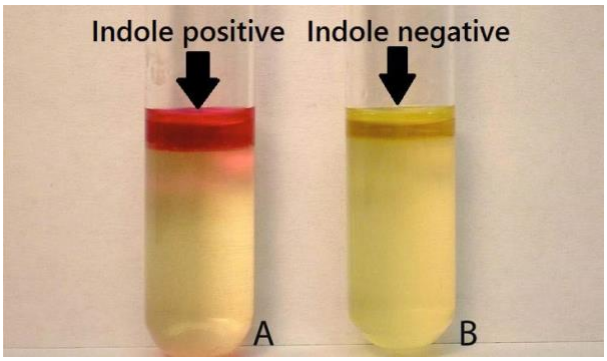
Peptone liquid medium containing tryptophan is inoculated the- tested

bacteria and incubated at 37 °C for 24 hrs. Few drops of kovac's reagent

are added to the bacterial growth. The presence of red rig in the

superficial layer of the medium indicate +ve result of indole production

. Results: Indole-Positive reaction: red color ex. E.coli; Negative reaction: yellow color ex. Klebsiella

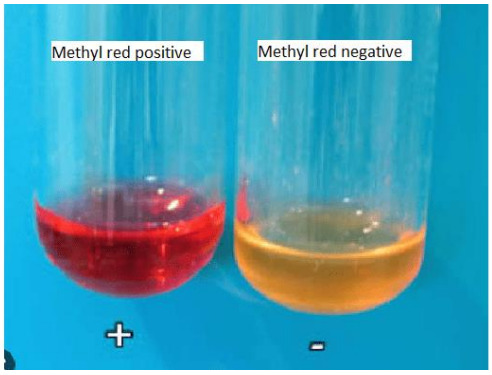


Methyl Red Voges-Proskaur Test (MR-VP test)

Methyl Red MR test

Principle to test the ability of the organism to produce acid end product from

gl Inoculate the medium (MRVP broth (pH 6.9) with bacteria



MR results: Red: Positive MR (E. coli); Yellow: Negative MR (Klebsiella)

Voges-Proskaur Test VP test

To determine the ability of the organisms to produce neutral end product (acetoin) from glucose fermentation.

procedure

1. Inoculate the tested organism into 2 tubes of MR-VP broth

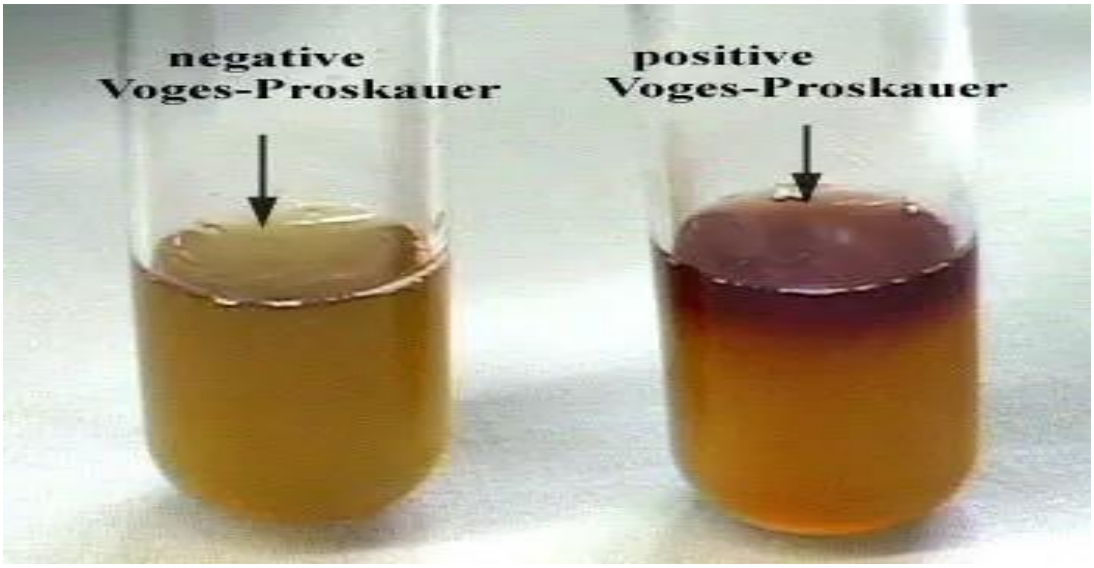
2. Incubate the tubes at 37°C for 24 hours

3. After incubation: Run the MR test in the tube 1, and the VP test in tube 2.

– For methyl red: Add 6-8 drops of methyl red reagent.

– For Voges-Proskauer: Add 12 drops of Barritt's A (□-naphthol), mix, 4 drops of Barritt's B (40% KOH), mix

– Let sit, for at least 1hour

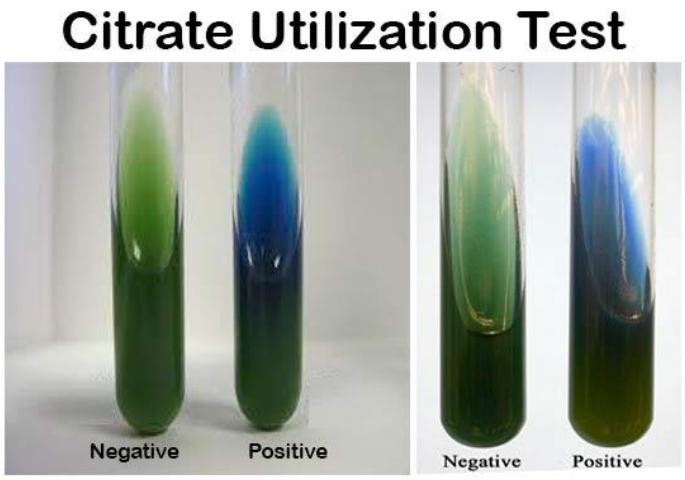


Voges-Proskauer results

Pink: Positive VP (Klebsiella), yellow: Negative VP (E. coli)

5-Citrate Utilization Test:

Simmons Citrate agar is a defined medium containing sodium citrate as the sole carbon source. The pH indicator, bromthymol blue, will turn from green at neutral pH (6.9) to blue when a pH higher than 7.6 is reached (alkaline). If the citrate is utilized, the resulting growth will produce alkaline products changing the color of the medium from green to blue. (Blue color= positive reaction eg; Klebsiella) ;(green color=negative reaction eg; E.coli)



Analytical Profile Index (API):

It is an miniaturized panel of biochemical tests compiled foridentification of groups of closely related bacteria.

Introduction of API Test for Bacteria

API test for bacteria as shown above picture is a 20-jumbo tests kit for a biochemical panel for the identification and differentiation of members of the family Enterobacteriaceae. API stands for Analytical Profile Index. It is an API 20E Test kit that is quick, safe, and easy to perform and it is hence a well-established method for manual microorganism identification to the species level. Modified API test kits are also available for the identification of microorganisms covering Gram-positive and Gram-negative bacteria and yeast. API strips give accurate identifications based on extensive databases and are standardized

API 20E test

VITEK 2 System for Rapid Identification of Clinical Isolates

The fully automated VITEK 2 system (bioMérieux) can provide identification results for microbial identification (bacteria and yeast identification) rapidly, accurately and reliable species-level identification in a few hours. It improved microbial identification and antibiotic susceptibility testing (AST) for all microbial isolates which isolated from different clinical specimens (blood, CSF, urine, stool, wound, burns, and others…).

The VITEK 2 system can:Reduce time to microbial identification and antibiotic susceptibility testing results

,Lec.Raneen Ebraheem AL-Jubori
sura Mustafa,
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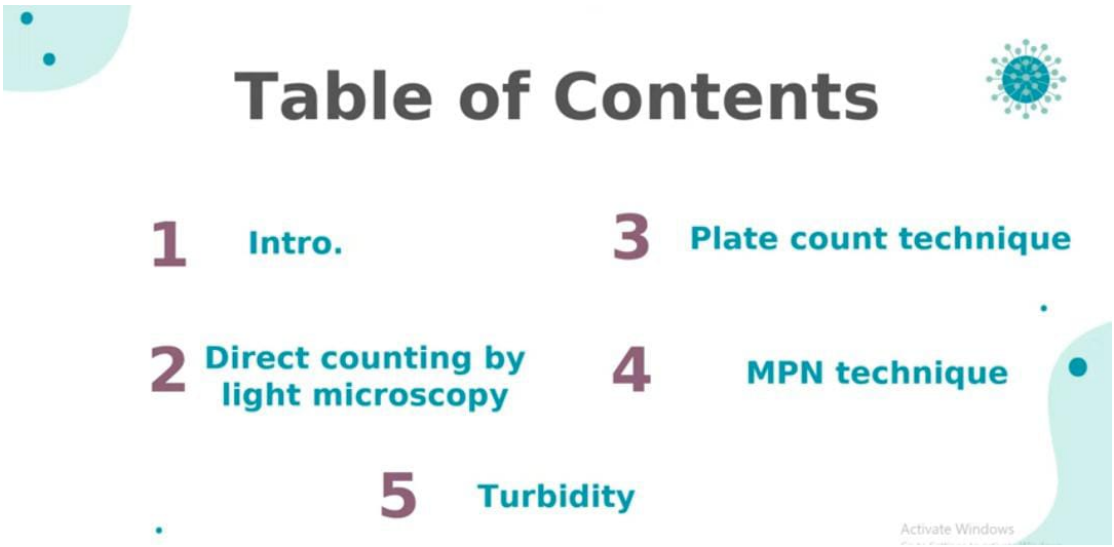
Enumeration of Microorganisms

In this lecture, the number of bacteria or any microorganisms, which is known as bacterial counting, will be calculated or estimated, There are different ways to count, including those shown in the chart below:



a- Plate count

b- Spectrophotometer (turbidity)

Direct microscopic method

Many studies require the quantitative determination of bacterial populations. The two most widely used methods for determining bacterial numbers are the standard, plate count method and spectrophotometric (turbidity) analysis Although the two methods are similar in the results, but there are distinct differences. For example, the standard plate count method is anindirect measurement of the cell density and reveals information related only to live bacteria. The

spectrophotometric analysis is based on turbidity and indirectly measures all bacteria (cell biomass) dead and a live

The standard plate count method:

This method consist of diluting a sample with sterile saline diluent until the bacteria are dilute enough to count accurately That is, the final plates in the series should have between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample) and designated too few to count (TFTC), more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as a distinct plates with more than 300 colonies cannot be counted and are designated too many to count (TMTC). Count the colonies on each plate, the assumption is that each viable bacterial cell is separate from all others and will develop into a single discrete colony.

*Counting using farm methods (the dish method):

An example of this is the plate of eggs when they are placed in the incubator while providing all the appropriate conditions for their hatching. Then, when the young offspring are formed and then turn into young girls, I will count the total number in addition to noting the difference in appearance or shape, but one of the disadvantages of this method is that it It is expensive, laborious, and takes a long time. In addition, it does not give the true number of microbes, meaning that the number is less than expected. One of the advantages of this method is that it studies the variation, that is, the similarity and microbial species. difference between the

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sura Mustafa,
Fatima Mustafa

Bioassay method:

Sometimes a microbe is invisible, that is, it cannot be seen under a microscope, and it cannot be grown in culture media. Therefore, we resort to the bioassay method.

Example:

1-Juice sample: First, the workplace is sterilized with 70% alcohol. Open a gasoline flame for 10 minutes. Then the experiment is done near the flame to ensure the availability of a clean, sterile area free of contaminants. Then close all windows and doors and reduce air currents. We take 1 ml of the juice and put it in the dish, making three steps. We repeat each sample and add the flask containing the culture medium, then we take the sample average.

2- Sewage water: when planted directly, the microbes will grow abundantly and we cannot count them due to their large number, so we resort to the dilution method by using 4 to 6 dilutions.

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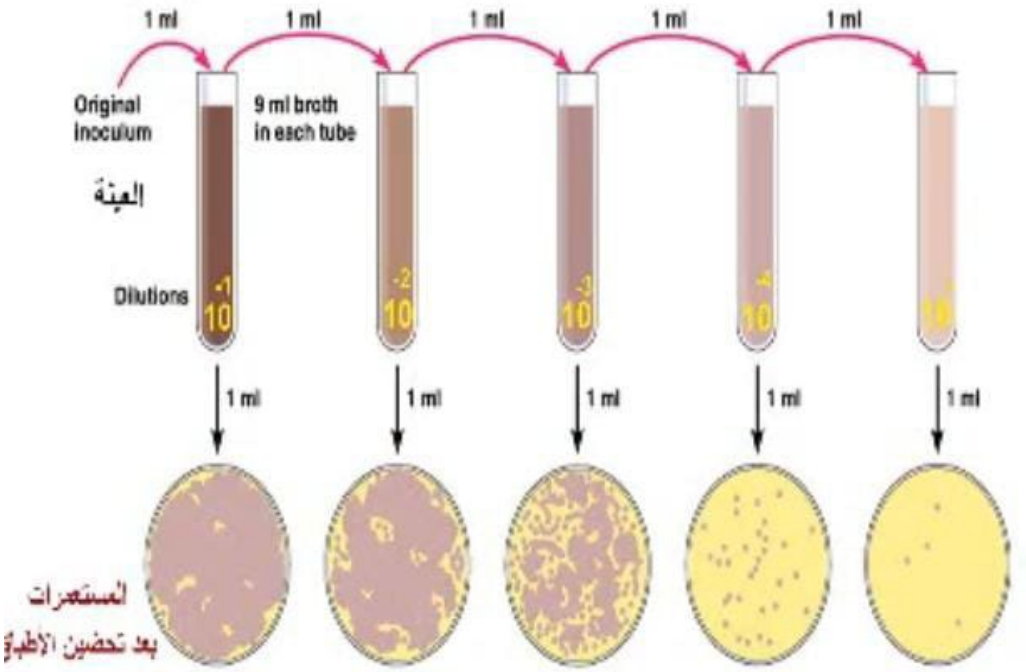

3-Soil sample: We sterilize the weighing device, then we take a 10-gram soil sample when measuring it with the weighing scale. Then we take a flask and put 100 ml of distilled water in it and add 10 grams to it, then we put it on the shaking device for 5 to 10 minutes, then we make the dilutions mentioned in the method above.

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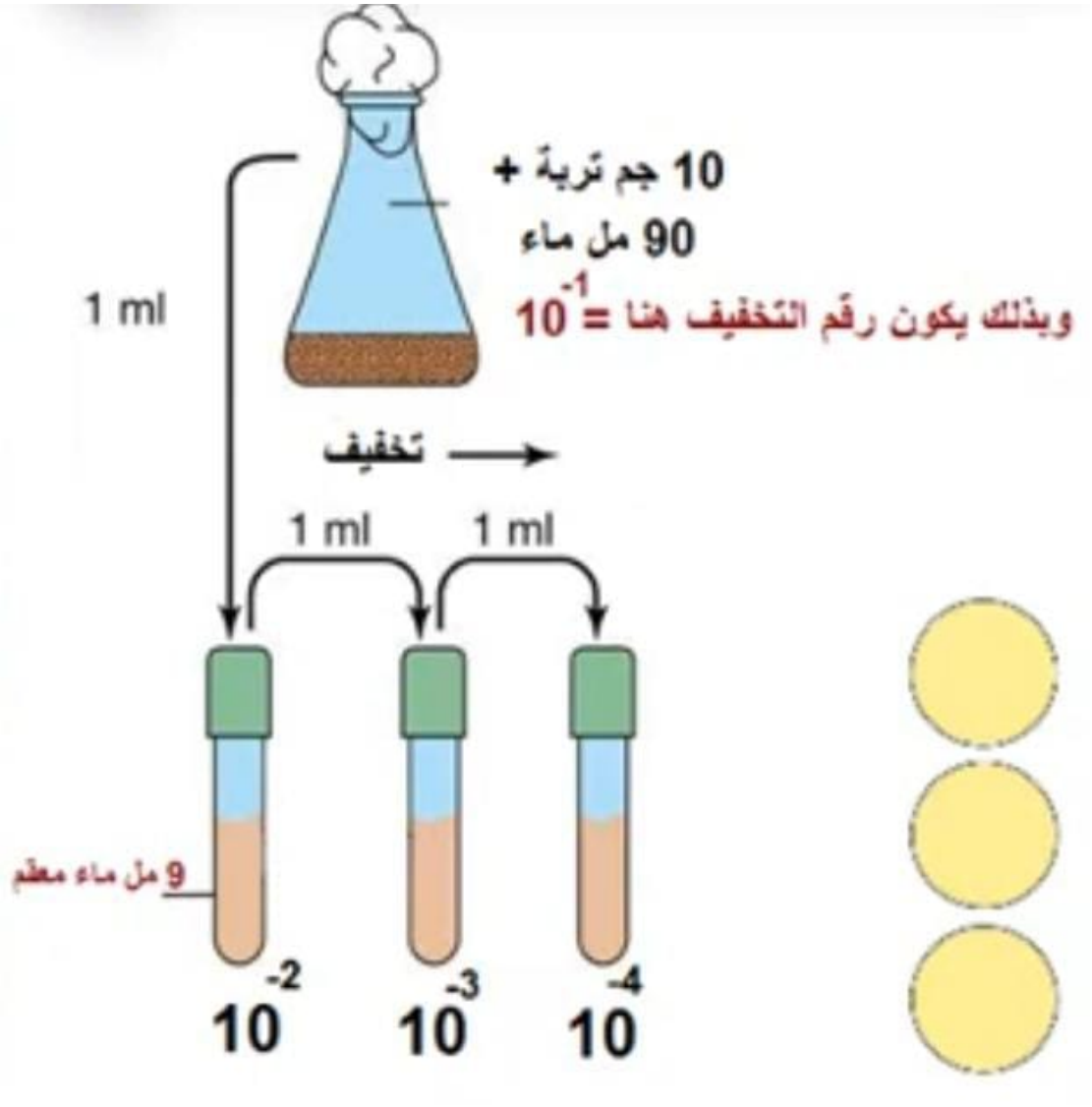

Subject teacher

Assistant teacher Raneen Ibrahim Al-Jubori

Lec.Raneen Ebraheem
Sura Mustafa
Fatima Mustafa

Taxonomy of Enterobacteriaceae

- **Domain: Bacteria**
- **Phylum: Proteobacteria**
- **Class: Gammaproteobacteria**
- **Order: Enterobacterales**
- **Family: Enterobacteriaceae**

N.B. Enterobacteriaceae can be roughly referred to as "enterobacteria " or "enteric bacteria", as several members live in the intestines of human and animals.

This family is considered one of the most important bacterial families and the largest and most important of the negative families, as it contains more than 110 different species, and the species within this family are considered among the most famous bacterial species .that are of very great importance, such as E.Colli, Sheglla.

It is found in the intestines of humans and animals. It may exist in a normal flora or in a pathogenic form. This family was given this name as evidence of its presence inside the intestines. During the making of gram stains, the species of this family appear in the form of thin threads of a pale red color.Facuitative anaerobic bacteria,oxidase negative,fermentation sugar(glucose) such as E.Colli ,enterobactar and non lactose fermentation entero bacteriaceae such as Salmonella, Sheglla and proteus.

Medically Important Enterobacteriaceae

- *Escherichia*
- *Shigella*
- *Salmonella*
- *Edwardsiella*
- *Citrobacter*
- *Yersinia*
- *Klebsiella*
- *Enterobacter*
- *Serratia*
- *Proteus*
- *Morganella*
- *Providencia*

Lec.Raneen Ebraheem
Sura Mustafa
Fatima Mustafa

One of the important media in diagnosing lactose is as described above

Lec.Raneen Ebraheem
Sura Mustafa
Fatima Mustafa

One of the important media in diagnosing lactose is as described above

Enterobacteriaceae: Types of Infectious Diseases

I. Intestinal (diarrheal) infection

II. Extraintestinal infection

- Urinary tract (primarily cystitis)
- Respiratory (nosocomial pneumonia)
- Wound (surgical wound infection)
- Bloodstream (gram-negative bacteremia)
- Central nervous system (neonatal meningitis)

Classification of Enterobacteriaceae

1. **According to their Cultural Characters:**
   (Growth on MacConkey's agar medium)
   a. Lactose –fermenters (rose pink colonies) include Coliform group: *Escherichia*, *Klebsiella*, *Citrobacter* and *Enterobacter*
   b. Non-lactose fermenters (pale yellow colonies) include: *Salmonella*, *Shigella*, *Yersinia*, and *Proteus*

2. **According to their pathogenicity:**
   a. Primary pathogens: *Salmonella*, *Shigella*, *Yersinia* and certain strains of *E. coli*
   b. Opportunistic pathogens: Coliform bacilli

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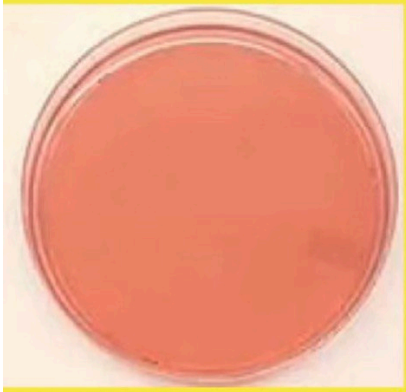
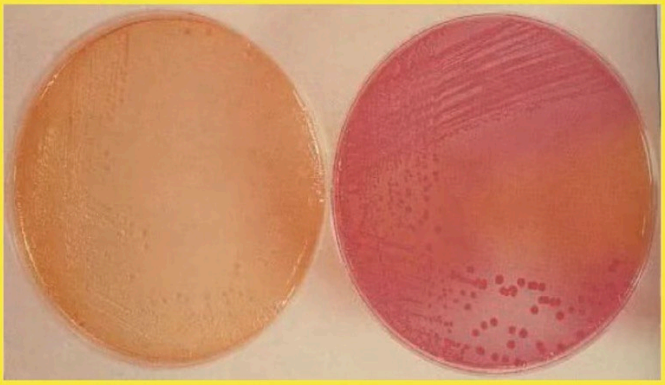
Growth of Enterobacteriaceae on MacConkey agar



Uninoculated plate



Colorless colonies

Lactose non feremters
Salmonella, Shigella, Proteus

Pink colonies

Lactose feremters
E. coli, Citrobacter Klebsiella, Enterobacter

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Sura Mustafa
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Methods of infection transmission:-

Finally, the methods of transmission of this bacteria are through contaminated food, using public toilets, and not taking care of personal hygiene. All of these factors can cause .infection with one or more of these types.

Lec.Raneen Ebraheem
Sura Mustafa
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-Sterilization and Disinfection-

Sterilization :is the process of killing or removal all microorganisms (bacterial, viral, and fungal) with the use of either physical or chemical agents.

Disinfection: removal or killing of disease-causing microorganisms, Eliminates most pathogens but not necessarily all types of microbes. Disinfection reduces the level of microbial contamination Disinfection and sterilization are essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients

Antiseptic: is a chemical that is applied to a living body to inhibit the growth of microorganisms. Hand sanitizers are antiseptics

Sterilization and Disinfection methods

A.Physical method

Heat method is the most common method of sterilization that kills the microbes directly.

Dry Heat Method.

Lec.Raneen Ebraheem
Sura Mustafa
Fatma Mustafa

Flaming_ the substance is exposed to the flame for just a few minutes. The flame will burn out the microbes directly.

Incineration - It is an effective method of sterilization in microbe cultures. The end of the microbe loop is exposed to red hot flame;thus, it kills microorganism. It is the easiest way to destroy microbes in metals.

Hot Air oven - The application of hot air oven is dry materials like glassware, heavy metals, thermostable materials etc. Here, hot air is allowed to circulate at a certain time and temperature. This way hot air oven works.

Moist heat Method

Boiling technique

is widely used for sterilization of metallic devices like scalpels, surgical scissors, needles etc. These substances are boiled to high temperatures to kill the disease-causing bacteria.

Pasteurization

It is the simple process of heating milk at high temperatures. Once the milk is boiled, it is again subjected to cooling. In this way, microbes in the milk get killed automatically.

Autoclaving

- It is a type of sterilization that uses steam sterilization equipment. substance is exposed to a temperature. It is a

powerful method for killing bacterial spores. Autoclaves operate at high temperature and pressure in order to kill microorganisms and Spores Sterilization refers to the complete killing of all living organisms, including spores

Filtration Method

Filtration involves removing microbes from a solution using a filter. When a liquid passes through the mechanical filter, microorganisms are trapped in the small pores of that filter. The fluid that a container receives has no bacteria as it is sterilized. Yes, the fluid is decontaminated by just using a filter. Since the filtration method is user-friendly, it is widely employed in beverages, bacteriological media, pharmaceuticals etc. There are several types of mechanical filters are available for this method.

Lec.Raneen Ebraheem
Sura Mustafa
Fatma Mustafa

Radiation method

Ultraviolet light is effective for controlling microbes in the food substance.ultraviolet is used to reduce the microbe population. It is also used to reduce the surface contamination in the morgue, pharmacy, hospital room. Ultrasonic waves The dentist uses the Cavitron device to clean teeth and destroy bacteria. The ultrasonic machines are used for cleaning dental plaques, coins, metals, etc.

Lec.Raneen Ebraheem
Sura Mustafa
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B-Chemical method

there are a lot of modern hospital and laboratory instruments and tools that are susceptible to heat. This means they have some components that should not be exposed to high temperature. like rubber, plastic, glass, and other similar elements. Chemical sterilization is the process of using to kill, eliminate, and remove all germs, viruses, and bacteria. This can be in the form of gas or liquid chemicals Chemical sterilization uses the following elements and compounds.

1- Silver

2-Alcoholes

3- Hydrogen Peroxide

4-Glutaraldehyde and Formaldehyde

5-Bleach

The above chemicals are used in many different ways and processes. Some can be mixed with other chemicals. Others are directly applied.

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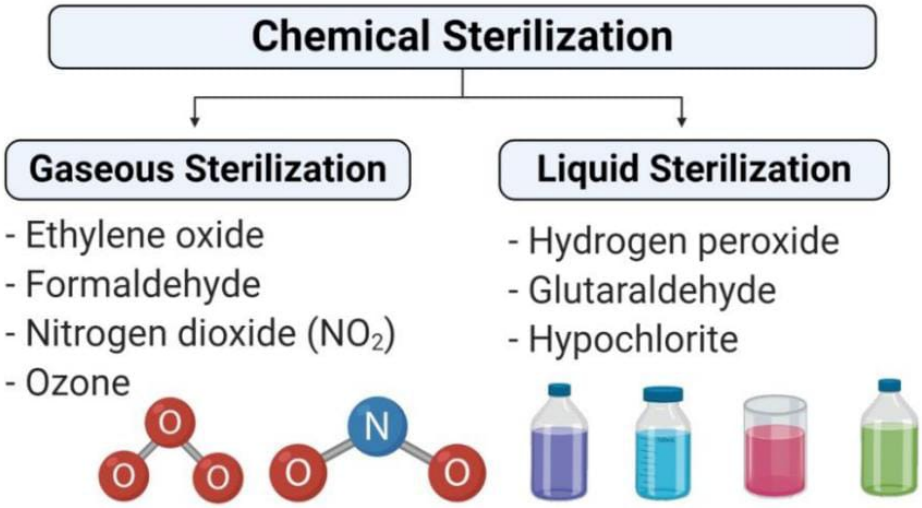

Microbiology Lab/1 *Sura Mustafa*
3rd class / Dentistry *Fatima Mustafa & Renan Ibrahim*

Laboratory safety

- All students must read and understand the information contained in this document with regarding laboratory safety and emergency procedures.
- Research and teaching workplaces are full of potential risks causing serious injury and/or equipment damage.
- two people it must be present so that the person can turn off the equipment and call for assistance case of emergency.
- The safety of your personal laboratory depends mostly on you .
- Uncontrolled use in laboratories is prohibited. Without prior approval.

Personal and General laboratory safety

1-Never eat, drink, or smoke while working in the laboratory.
2-Read labels carefully.
3-Do not use any equipment unless you are trained and approved as a user by your supervisor.
4-Wear safety glasses or face shields when working with hazardous materials and/or equipment.
5-Wear gloves when using any hazardous or toxic agent. Fatma Mustafa
6-Clothing: When handling dangerous substances, wear gloves, laboratory coats, and safety shield or glasses. Shorts and sandals should not be worn in the lab at any time. Shoes are required when working in the machine shops.
7-If you have long hair or loose clothes, make sure it is tied back or confined.
8-Keep the work area clear of all materials except those needed for your work. Coats should be hung in the hall or placed in a locker. Extra books, purses, etc. should be kept away from equipment, that requires air flow or ventilation to prevent overheating.
9-Disposal - Students are responsible for the proper disposal of used material if any in appropriate containers.
10-If leaving a lab unattended, turn off all ignition sources and lock the doors.
11-Never pipette anything by mouth.
12-Clean up your work area before leaving.
13-Wash hands before leaving the lab and before eating.

Chemical safety

1-Treat every chemical as if it were hazardous.
2-Make sure all chemicals are clearly and currently labeled with the substance name, concentration, date, and name of the individual responsible.
3-Never return chemicals to reagent bottles. (Try for the correct amount and share any excess.)
4-Comply with fire regulations concerning storage quantities, types of approved containers and cabinets, proper labeling, etc. If uncertain about regulations, contact the building coordinator.
5-Use volatile and flammable compounds only in a fume hood. Procedures that produce aerosols should be performed in a hood to prevent inhalation of hazardous material.
6-Never allow a solvent to come in contact with your skin. Always use gloves.
7-Never "smell" a solvent!! Read the label on the solvent bottle to identify its contents.
8-Dispose of waste and broken glassware in proper containers.
9-Clean up spills immediately.

Microbiology Lab/1 *Sura Mustafa*
3rd class / Dentistry *Fatima Mustafa & Renan Ibrahim*

Laboratory safety

Additional Safety Guidelines

1-Never do unauthorized experiments.
2-Never work alone in laboratory.
3-Keep your lab space clean and organized.
4-Do not leave an on-going experiment unattended.
5-Always inform your instructor if you break a thermometer. Do not clean mercury yourself!!
6-Never taste anything. Never pipette by mouth; use a bulb.
7-Never use open flames in laboratory unless instructed by TA
8-Check your glassware for cracks and chips each time you use it. Cracks could cause the glassware to fail during use and cause serious injury to you or lab mates.
9-Maintain unobstructed access to all exits, fire extinguishers, electrical panels, emergency showers, and eye washes. 10-Do not use corridors for storage or work areas.

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2-Sura Mustafa
3-Fatima Mustafa

Culture Medium or Growth Medium

The culture medium is the medium in which bacteria grow. It is a solution in which a number of different nutritional elements are combined in addition to the agar, and it is prepared under high pressure and temperature.

Types of culture media that differ according to the bacteria allocated to them:

1-General Media

2-Enriched Media

3-selective Media

4-Differentail Media

5-Transport Media

6-Anaerobic Media

Components of the food environment:-

Extract of some plants

Animal tissues

Yeast extract

Organic salt

Sugar

*In terms of the type of medium, media can be divided according to the state of matter into:

1- solid media

2- semisolid media

3- and liquid media

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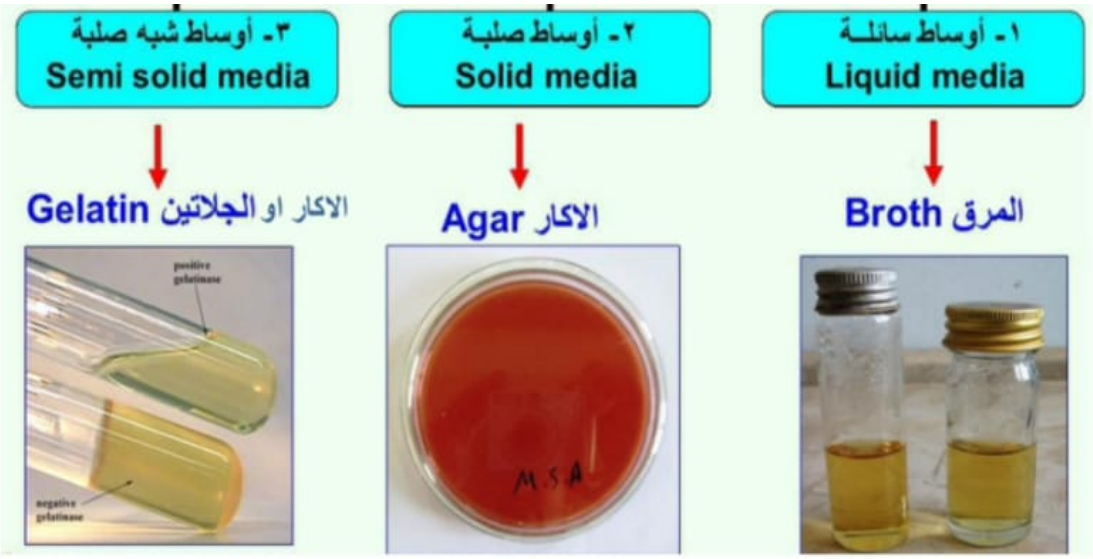

Or they are divided according to their chemical composition into simple and complex media

1- General Media: It is called the nutrient medium, which contains all the nutrients for the growth of various bacterial species. This type consists of sugar, water, necessary salts, beef extract or yeast extract, which is a source of protein.

2- Enriched Media: These are media that contain one specific nutritional component, for example blood agar and chocolate agar, which largely contain blood, which tends to grow specific types of bacteria or fungi.

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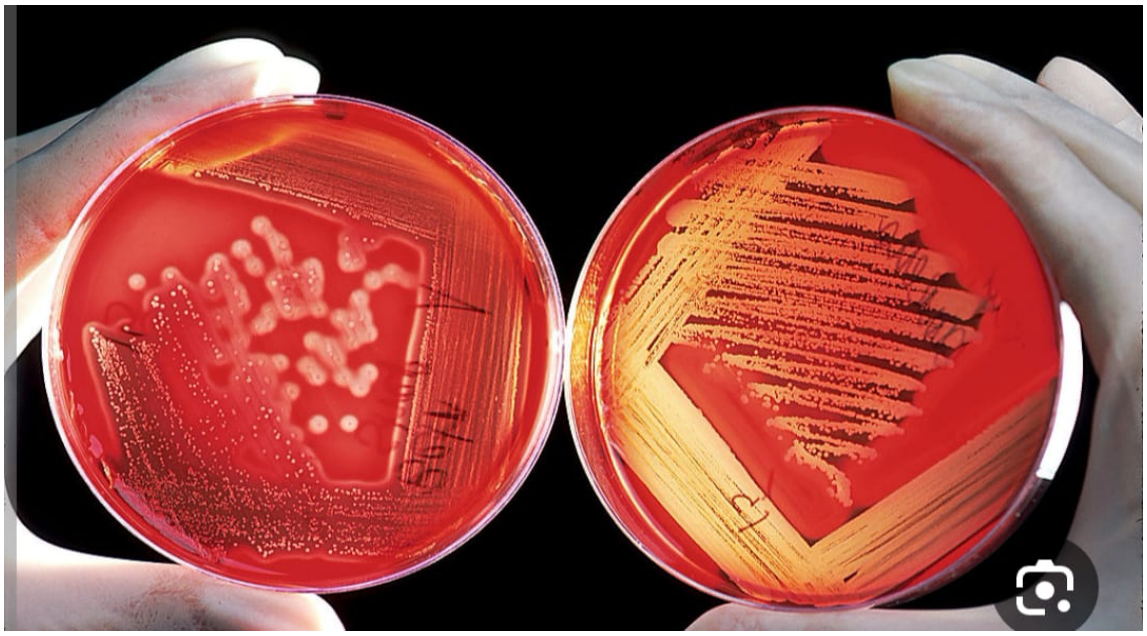

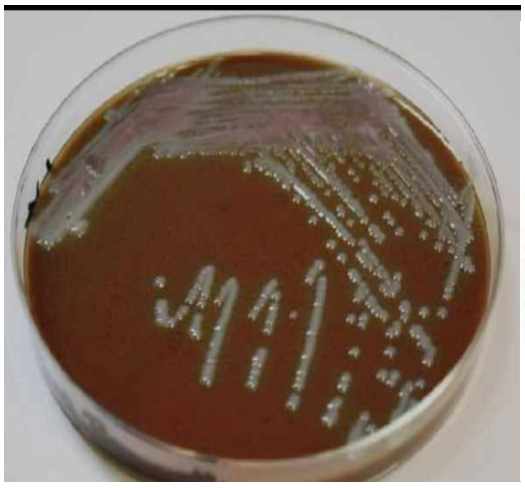

3- selective Media: It is a type of media that works to grow one specific type of bacteria, so it contains its own nutrients, such as:

MacConkey agar

Mannitol salt agar

Xylose agar

5- Transport Media: These are media used to quickly transfer samples to them in order to reach the laboratory. They are often sterile media to prevent contamination of samples and preserve them from drying out, for example glycerin saline solution in Pike's medium.

6-Anaerobic Media: Anaerobic bacteria, whose growth depends on the presence of a small amount of oxygen, need the presence of a nutrient medium that helps them do this by limiting oxidation and reduction reactions and containing an additional amount of food in return. An example of Robertson's medium for cooked meat(RCM) and

thioglycolate broth.

Bacterial growth: When cultivated in culture media, bacteria grow significantly while being provided with an abundance of nutrients, during which they produce different types of bacterial colonies with a different appearance. Some of them may be colored, some of them may be regular in shape, they may be circular, and others may be irregular in shape (these morphological characteristics enable people to recognize On bacteria and distinguishing their types.

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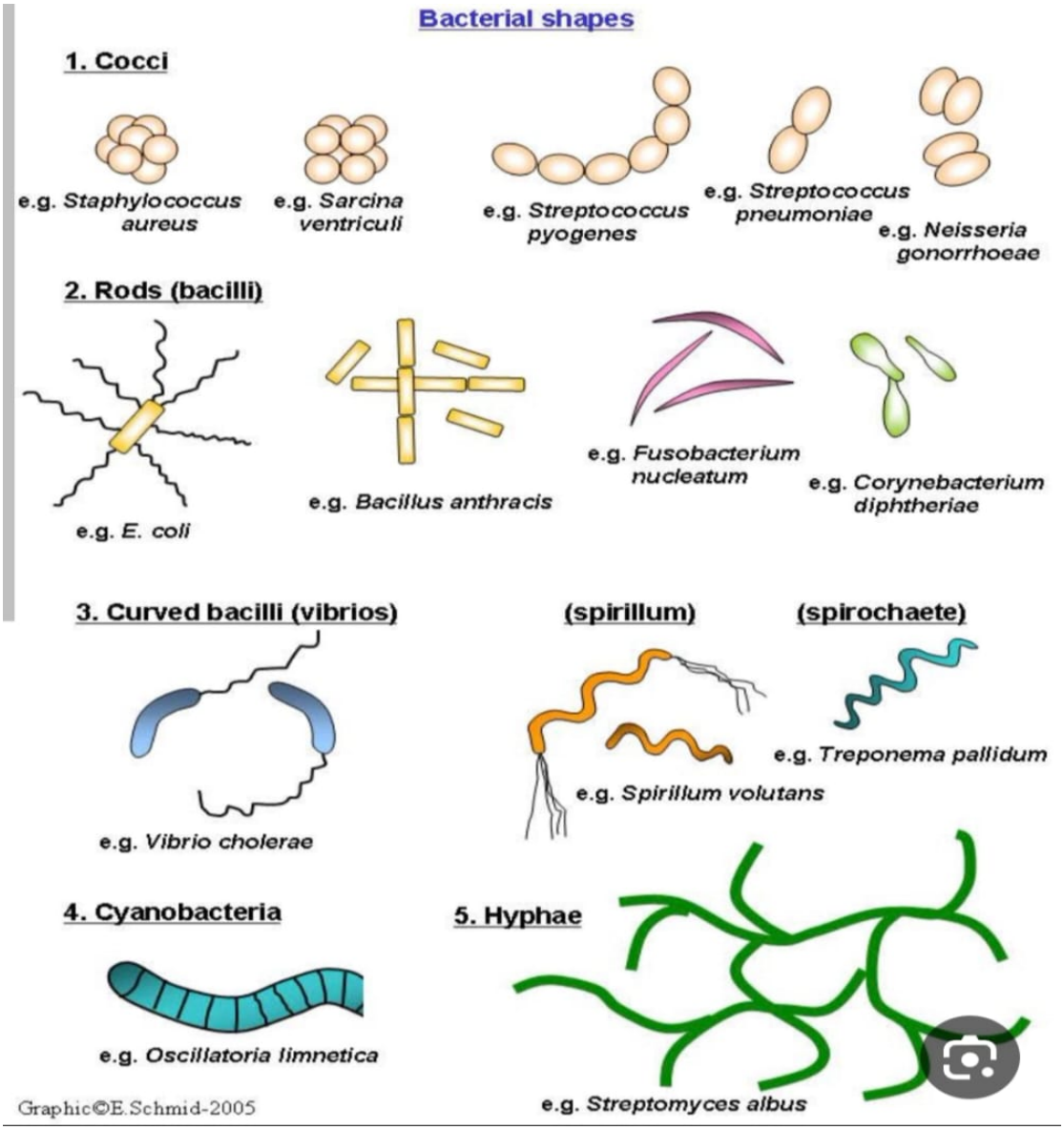

Method of preparing the culture medium:

*Weight of the planting medium

*Melt the medium using heat while stirring

*Autoclave sterilization

*Cooling the medium after sterilizing the flask starter before casting

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*Pour the medium into petri dishes

*Flaming the medium after casting

*Flaming the petri cap if it is glass after pouring

*Allow the agar to harden

*Place the dishes in bags until used

*Store in the refrigerator upside down

Microbiology Lab/ 2 *Fatima Mustafa*
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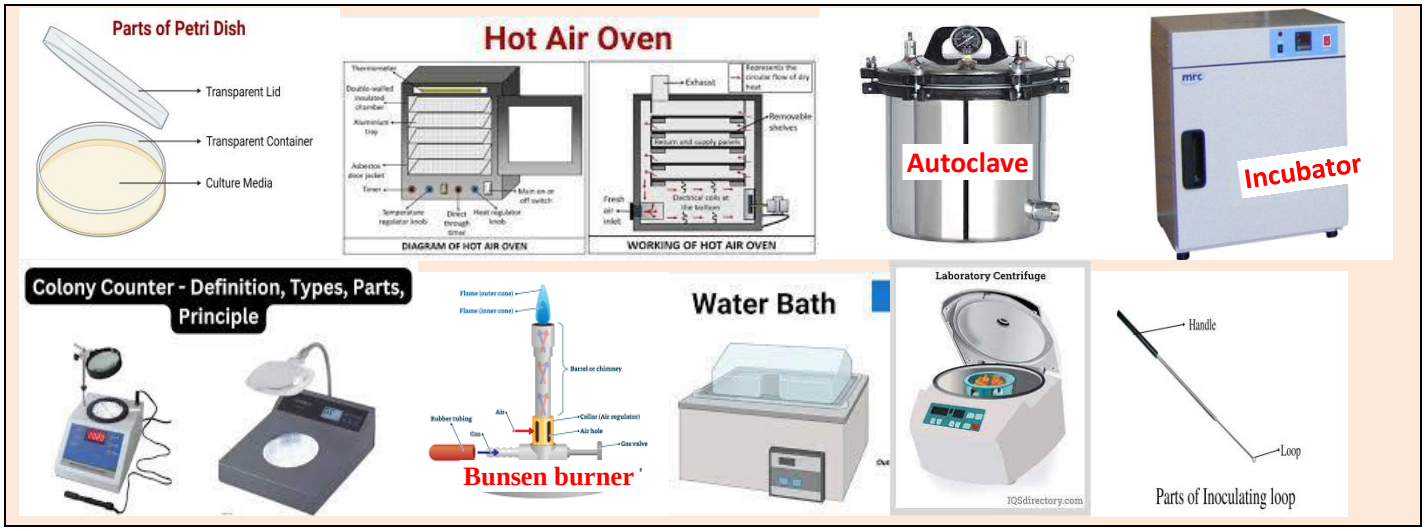
Equipment and Tools in Microbiology Laboratory

The instruments used in the microbiology labs include a bunch of different kinds of instruments required for a lot of different processes conducted within those laboratories.

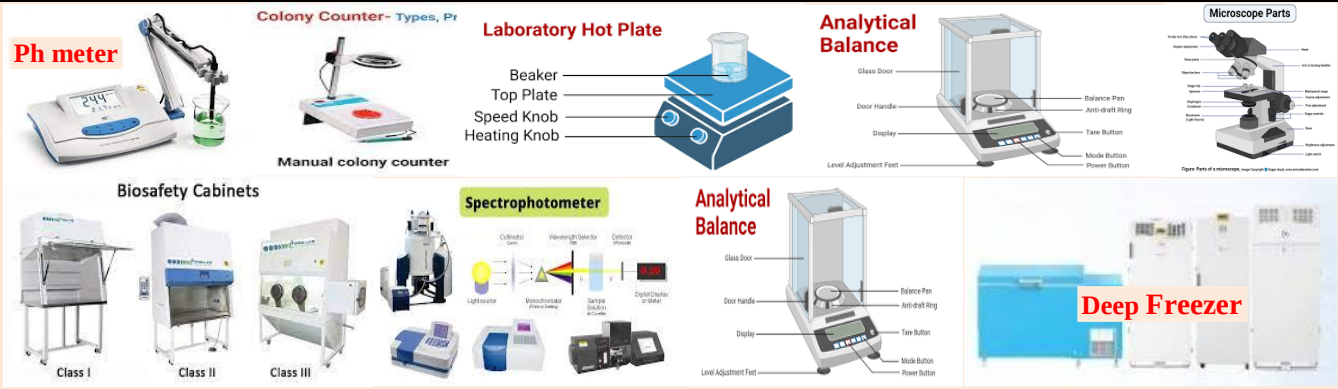
- **Petri dish** : is a flat dish made of plastic or glass with a cover that is used to grow microorganisms
- **Incubator**: is a warm cabinet that you can set it's temperature to a proper for microbes growth. As most of the microbes pathogenic to man grow profusely at body temperature of normal human being (i.e. 37°C), the usual temperature of incubation is 37°C.
- **BOD Incubator (Low Temperature Incubator**):Some microbes are to be grown at lower temperatures for specific purposes. The BOD low temperature incubator which can maintain temperatures from 50°C to as low as 2-3°C is used for incubation in such cases.
- **Hot air oven**: is similar to incubator in make except that it can operate at temperatures up to 300°C and has a fan for circulating hot air. It is used for sterilization of glassware and materials that are spoiled by moist heat. The death of cells occurs due to the oxidation of cellular constituents by the dry heat..
- **Autoclave** :sterilizes items by heating them with steam to a very high temperature. autoclave is a pressure chamber used to sterilize equipment and supplies by subjecting them to high-pressure saturated steam at 121 °C (249 °F) for around 15–20 minutes depending on the size of the load and the contents.
- **Bunsen burner :** It is a gas flame and common tool used in science lab. which used for heating sterilization inoculating loop, plating out cultures, transferring cultures, heat-fixing of smears and creating a sterile zone for aseptic operation.
- **Centrifuge**: is a laboratory device that is used for the separation of fluids, gas or liquid, based on density. Separation is achieved by spinning a vessel containing material at high speed; the centrifugal force pushes heavier materials to the outside of the vessel. There are multiple types of centrifuge.
- **Water Bath is** a conventional device that is used for chemical reactions that required a controlled environment at a constant temperature. Its uses for heating samples under a controlled temperature.
- **Distilled Water Plant**: Use to prepare a distilled water it is made of steel or brass. It is also called distilled water still. Water is used in the preparation of media and reagents.
- **pH Meter**: s an instrument for determining the pH of liquid media, liquid samples and buffers. It has a glass pH electrode. When not in use, it should be kept half immersed in water contained in a small beaker and .Before use, the meter is calibrated using two Practical 2 standard buffers of known pH. Usually buffers of pH 4.0, 7.0 and 9.2 are available commercially.
- **Analytical Balance:** is used in precise weighing of small amounts (upto miligrams) of samples and chemicals used for preparing media and stock solutions.
- **Refrigerator** : is used in microbiology laboratory for storing preserving cultures, media, many sensitive materials, stock solutions, chemicals, kits and nutrient media that should be maintained at certain temperatures.
- **Inoculating loop:** a tool for transferring and streaking cultures. It consists of a thin nichrome wire whose one end is twisted into a small loop while the other end is fixed to a thermoset plastic handle.

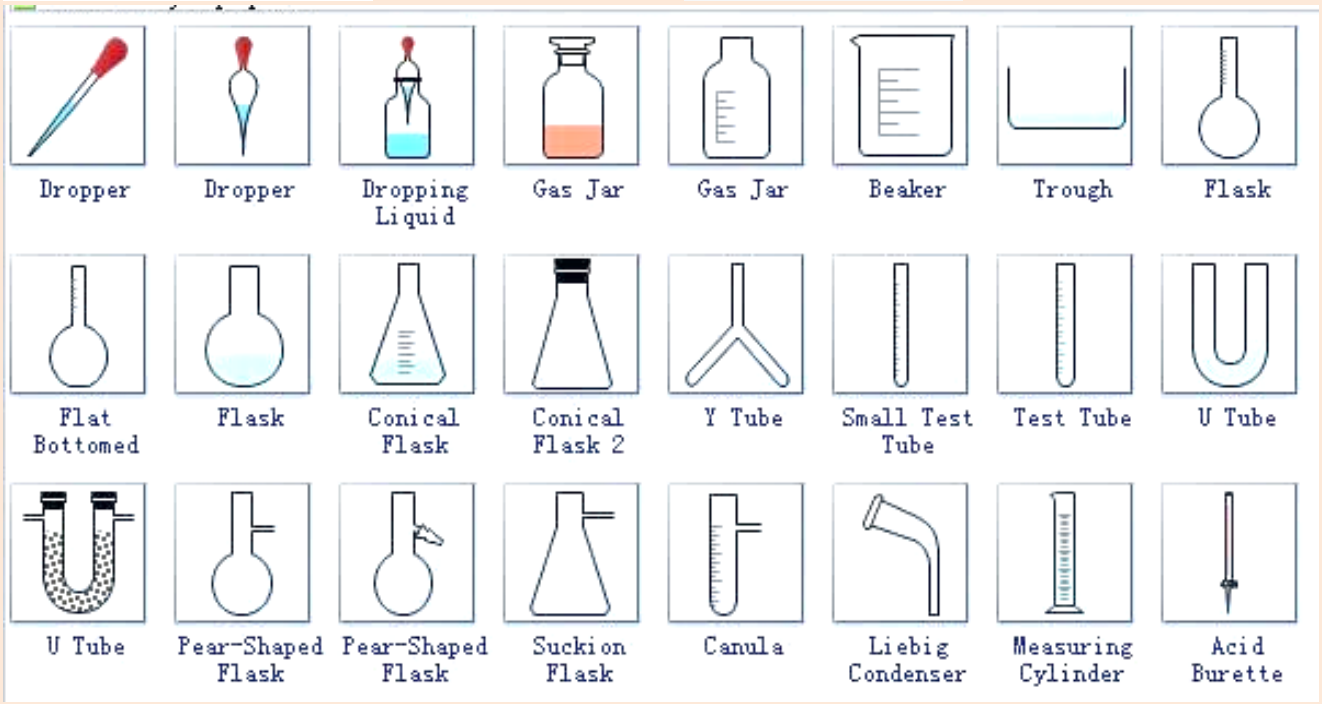
Equipment and Tools in Microbiology Laboratory

- **-Hot Plate:** Used to heat substances. This is an electrically powered equipment performs the dual function of heating and agitation. The agitation occurs by magnetic arrangement. Any type of glassware can be used for the heating and agitation. Magnetic beads are used for the agitation.
- **Colony counter :** It is used for counting microbial colony (bacterial and yeast). The instrument is equipped with a backlight source, gridlines and a magnifying lens. It also has a sensor for digitally registering the number of colonies counted
- **Magnetic Stirrer:** In the preparation of solutions, certain chemicals require stirring for long time, to be dissolved in certain solvents. Magnetic stirrer is used to dissolve such substances easily and quickly. A small teflon- coated magnet, called 'stirring bar', is put into a container containing the solvent and the solute.
- **Vortex Mixer:** It is an instrument used for thorough mixing of liquids in test tubes.
- **Deep Freezer (-86 ° C):** It is used to store stock cultures in microbiology. It is a device used to store materials which should be kept at low temperatures (cells, tissues, enzymes, proteins, etc.)
- **Microscopes:** Different types of microscopes are used for visual observation of morphology, motility, staining and fluorescent reactions of bacteria.
- **Spectrophotometer:** It is an instrument for measuring the differences in color intensities of solutions. A beam of light of a particular wavelength is passed through the test solution and the amount of light absorbed (or transmitted) is measured electronically.
- **shaker incubator** :It is used in cultivating, multiplying and in the characterization tests of microorganisms. This device provides the heat necessary for the growth of microorganisms.
- **BIOSAFTY CABINATE** It is used in microbial inoculation and isolation studies as well as sterile storage of materials. In addition, it is utilized for protection of user, samples and the environment from hazardous contamination.



Equipment and Tools in Microbiology Laboratory





Microbiology Lab/3 *Fatima Mustafa*

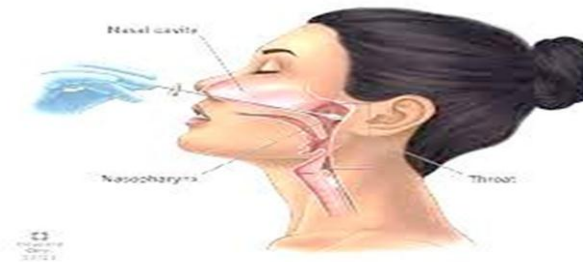
3rd class / Dentistry *Sura Mustafa & Renan Ibrahim*

Pathogenic bacteria Specimens: collecting and processing

1 Timing of collection: Sputum, urine, stool, etc. are best collected in early morning and sent to the laboratory the same day.

Targeted parts of body

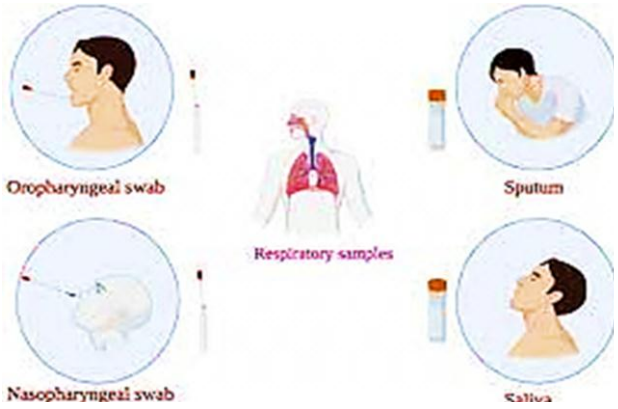
1- **Samples from Upper Respiratory Tract:**



These sections include specimens from the nasopharyngeal area and the throat.

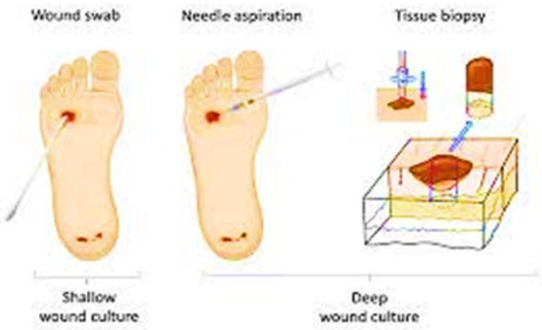
A nasopharyngeal culture is obtained by inserting a thin sterile swab gently through the nose to touch the pharynx; gently rotate and remove. A throat culture is obtained by introducing a sterile swab into the mouth.

2- **Samples from Lower Respiratory Tract**:



Sputum, small amount of sputum is all that is required, but it must be sputum and not oral secretions. - Rinsing the mouth with saline or water (but not mouthwash) may reduce contamination with normal or pharyngeal flora - Encourage deep cough with expectoration of the sputum into a sterile specimen collection cup that is labeled with the patient's name. - Do not send saliva (spit) for culture.

3- **Specimens of Wound Exudate** Using a sterile transport swab in collecting wound exudate specimens. Gently cleanse the area, using dry, sterile gauze to remove any contaminants. - Using a sterile bacterial culture collection system, introduce deeply enough to obtain a moist specimen; replace the swab in the container. Do not break the container.



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Pathogenic bacteria Specimens: collecting and processing

4- Urine for Culture: When a urine culture is ordered, follow these steps: - Explain carefully to patients the mechanics of midstream collection - The specimen must be free of any contaminating matter that might be present on the genital organs. Do not collect urine specimens from a urine drainage bag.

5- Stool for Culture: When collecting stool specimens, follow these guidelines. A small amount is all that is required, about the size of a walnut. Place the specimen in stool culture transport medium. When stool specimens are not readily obtainable, rectal swabs are acceptable; however, it must be indicated whether the specimen is a stool or a rectal swab. Place the swab in stool culture transport medium.

6- Blood: A blood culture requires two bottles of blood (one for aerobic and one for anaerobic culture. Each blood culture should be collected from a separate venipuncture. - Collect blood specimens before antimicrobial treatment is initiated, if possible.

Pathogenic Bacteria/ Lab 5 *Sura Mustafa*
3rd. class / Dentistry *Renin Ibrahim & Fatima mustafa*

Staphylococci Identification

Staphylococci Gram-positive, cocci, 0.5-1.5µm in diameter. Form irregular grapelike clusters. Non- motile, non- sporing. Often found in the human nasal cavity, mucous membranes and skin. There are 4 species of staphylococci commonly associated with clinical infections: *Staphylococcus aureus, S. epidermidis, S. haemolyticus and S. saprophyticus.*

Classification

1- Based on pathogenicity

S.aureus is pathogenic that causes superficial skin lesions, deep-seated infections, and nosocomial infection. *S saprophiticus* causes urinary tract infections, especially in girl. **Non-pathogenic** includes *S.epidermidis & S.hominis.*

2- Based on pigment production

S. auras: **golden-yellow** pigmented colonies ***S saprophytic:*** **gray colonies** ***S.albus:*** white colonies ***S. citrus***: lemon yellow colonies

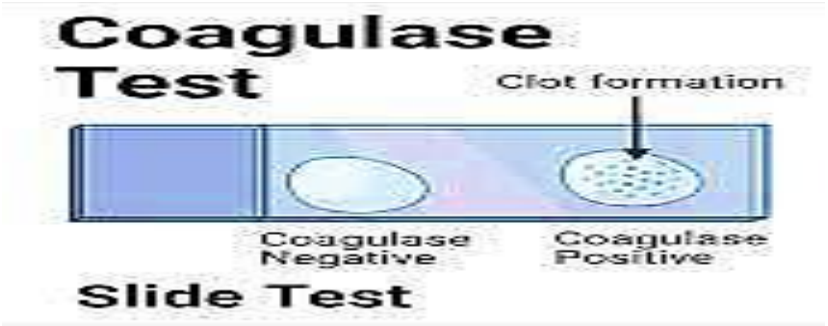
3- Based on Biochemical test

Test	Gram Stain	Coagulase	Manitol S.A	Novobiocin disc	Catalase	Hemolysis	DNase
S. aureus	G+ ve	+	+	**Sensitive**	+	**Beta**	+
S. saprophyticus	G+ ve	-	-	**Resistante**	+	Gamma	-
S. epidermidis	G+ ve	-	-	**Sensitive**	+	None	-
S.haemolyticu	G+ ve	-	-	**Sensitive**	+	**Beta**	-

A-Coagulase test:

Coagulase test is used to differentiate *Staphylococcus aureus* (positive) from Coagulase Negative Staphylococcus **(CONS). Coagulase Test Procedure including**:

1. Apply staphylococcal colony in a drop of water on a clean glass slide with a minimum of spreading
2. Mix undiluted plasma with staphylococcal suspension on the slide by straight inoculating wire.
3. Read as positive a clumping (agglutination) of cocci visible to the naked eye within 10 seconds. Read as negative the absence of clumping, but re-examine any slow reacting strains by the tube coagulase test.



Pathogenic Bacteria/ Lab 5 *Sura Mustafa*

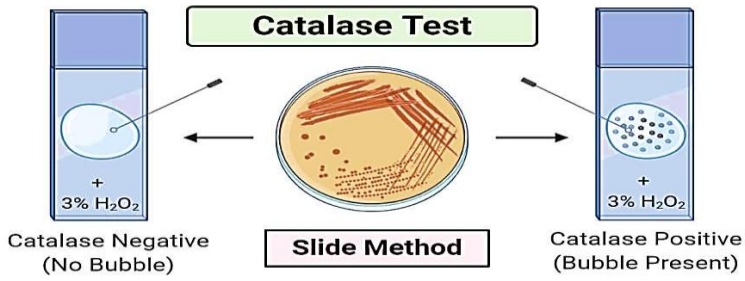
3rd. class / Dentistry *Renin Ibrahim & Fatima mustafa*

Staphylococci Identification

B- Catalase Test Slide Method

1. Use a loop or sterile wooden stick to transfer` a small amount of colony growth in the surface of a clean, dry glass slide. **2.** Place a drop of 3%Hydrogen peroxide in the glass slide.

3. Observe for the evolution of oxygen bubbles.



C- Mannitol salt agar

MSA: is a commonly used as selective and differential growth medium for many groups of staphylococci. The medium contains a high concentration of salt (7% - 10%), making the medium a limiting factor for the growth of Staphylococcus bacteria. It is also considered a divider between the types of Staphylococcus bacteria using manitol sugar and a specific use of pH (phenol red)

D- DNase test:

1-using a sterile loop, inoculate the DNase agar with the organism to be tested on the test area.

2-Incubate the plate at 35-37°C for 24 hours.

3-After incubation observes the color change in DNase with methyl green.

- Positive: Medium is colorless around the test organism.
- Negative: If no degradation of DNA occurs, the medium remains green

Streptococci

Streptococci is a genus of gram-positive coccus or spherical bacteria, non-motile, non-spore forming. They often occur as chains or pairs and are facultative or strict anaerobes. ***Group A*** *streptococci* have a hyaluronic acid capsule.

Classification of streptococci system includes:

1- Based on hemolysis reactions.

- β - Hemolytic (clear, complete lysis of red cells) → *S. pyogenes*
- α – Hemolytic (incomplete, green hemolysis) → *S. pneumoniae and viridans*
- γ – Hemolytic (no hemolysis) → *S. faecalis*

Different methods for laboratory diagnosis of

A- is isolated from samples such as skin, throat, sputum, urine, and blood.

Culture: The organism is cultured on blood agar with an added bacitracin antibiotic disk to show beta-hemolytic colonies and sensitivity (zone of inhibition around the disk) for the antibiotic. Best growth achieved at pH 7.4-7.6 and temperature 37^0C.

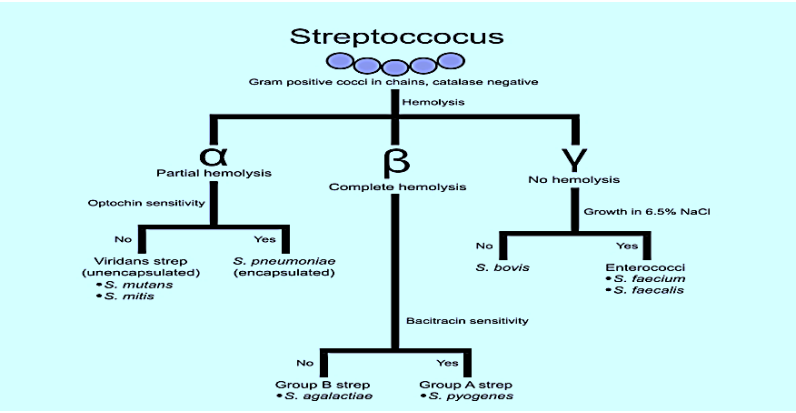
B- ***St. pneumonia:*** Its infection of lung, Otitis media. Isolated from samples such as sputum, blood, wound.

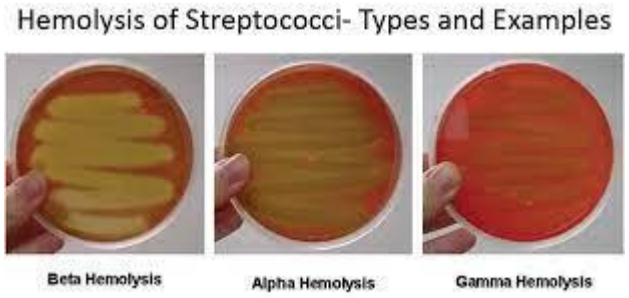
Culture: requires blood or chocolate agar. • Growth improved by 5-10% CO2. Best growth achieved temperature 25 - 40^0C. • Colonies are surrounded by greenish hemolysis, cultured on blood agar with an added optochin disk to show alpha- hemolytic colonies.

C- ***S. Faecalis:*** It is associated with urinary tract infections.

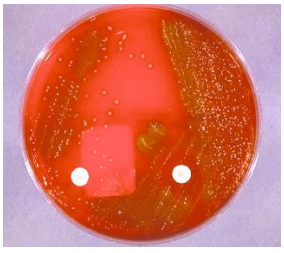
Culture: in MacConkey agar. Colonies are magenta in color and pin point. It can grow in the range of 10 to 45°C and survive at temperatures of 60°C for 30 min. It ferments glucose and does not produce a catalase.

Diagnosis plan for *Streptococci spp*





S. pneumonia



pyogenes

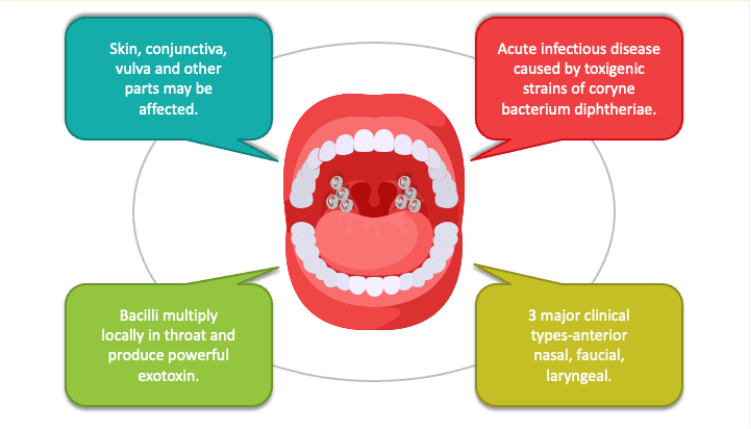
Mycobacterial Lab/7 *Sura Mustafa*
3rd class / Dentistry *Renin Ibrahim& Fatima Mustafa*

Corynebacterium diphtheriae

Corynebacterium diphtheriae is a Gram-positive bacillus, non-motile, non-capsulated, characteristic forms resembling „Chinese-letters" and club-shaped. Diphtheria is most commonly an infection of the upper respiratory tract and causes fever, sore throat and its ability to produce diphtheria toxin.

A thick, gray-green fibrin membrane, the pseudo-membrane, often forms over the site(s) of infection as a result of the combined effects of:

1-bacterial growth. 2-toxin production. 3-necrosis of underlying tissue. 4-host immune response.



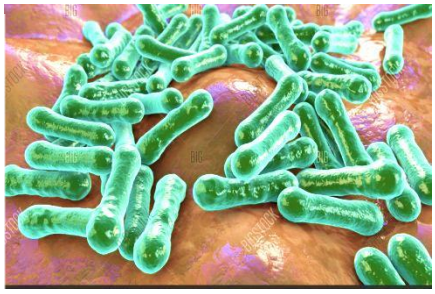
Different methods for laboratory diagnosis

A- They can swab of the throat or nose and test it for the bacteria that cause diphtheria.

B- ***Corynebacterium*** appears green colored rod shaped bacteria with bluish black metachromatic granules at the poles.

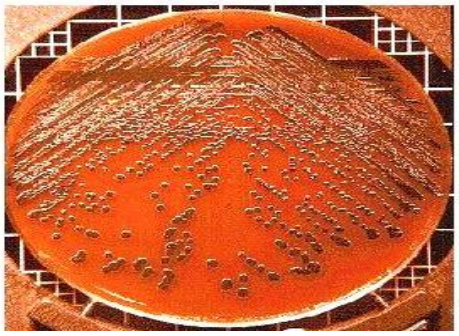
Abert´s stain Procedure

1. Prepare a smear on clean grease free slide.
2. Air dry and heat fix the smear.
3. Treat the smear with Albert's stain and allow it to react for about 7 mins.
4. Drain of the excess stain do not water wash the slide with water.
5. Flood the smear with Albert's iodine for 2 minutes.
6. Wash the slide with water, air dry and observe under oil immersion lens.



C- Telluride Blood Agar

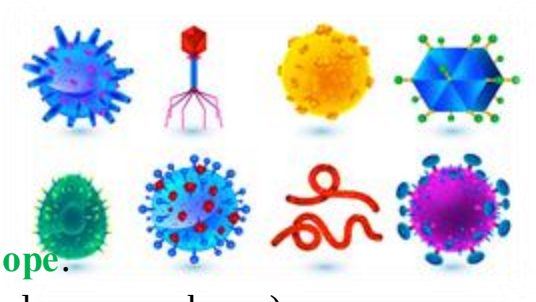
Potassium tellurite is the selective agent that turns the media brown-black as a result from the reduction of potassium tellurite to metallic tellurite. This differentiation is based on the ability of C. diphtheriae to produce black (or brown) colonies, surrounded by a brown/black halo. The dark halo is due to the production of H_2S from cystine, interacting with the tellurite salt



3rd Class /microbiology Sura Mustafa
Ranin Ibraheem &Fatima Mustafa

Key elements of a virology laboratory

Definition of viruses



- Are infectious agents
- No ATP generating metabolism.
- Do not undergo binary fission.
- Sensitive to interferon
- **Too small therefore don't see with a light microscope**.
- Acellular (absence of nucleus, organelles, cytoplasm, plasma membrane).

The key elements of a virology laboratory and diagnostic

- 1) Physical infra-structure
- (2) Human resources
- (3) Equipment and supplies.

Function of virus Partials

Virus isolation and a number of methods for detection of:

1. Viral antigens
2. Nucleic acids
3. Antibodies (serology)
4. core stock of techniques
5. Cell culture

Human resources

1. Enough staff for a diagnosis virology
2. Match virologist have advanced studies in virology with three to five years.
3. Two junior microbiologists having a Master's degree in Medical Microbiology with 1-2 years' experience in diagnostic virology.

Reagents and supplies

- Diagnostic kits as per requirements of the laboratory
- Tissue culture media
- Fetal bovine serum
- Fluorescent conjugates
- ELISA plates, antibodies and conjugates
- Analytical- fine chemicals for preparation of buffers
- Sterile tissue culture plastic

Key elements of a virology laboratory

- V-bottom polystyrene microtiter plates for haem-agglutination
- Serum storage cryovials and boxes
- Micropipette tips
- PCR tubes
- PCR reagents DNA and RNA extraction kit.

- Refrigerate centrifuge.
- Water bath.
- pH meter
- Magnetic stirrer.
- Vortex mixer.
- Electronic balance for weighing chemicals.
- Elisa Reader and washer.
- Micropipttes (100ul, 200 ul, 20 ul).
- Multi-channel pipettes – 8 and 12 channel pipettes (20-200 ul and 50-300 ul).
- Autoclave – Two (one for de-contamination and one for sterilization).
- Hot air oven for sterilizing glassware

\

- PCR machine (conventional and real-time).
- Gel electrophoresis apparatus.
- UV transilluminator.
- Ice-making machine.
- Liquid nitrogen containers.
- Water purification/distillation system for tissue culture work.
- Glassware such as volumetric flasks, measuring cylinders, pipettes (1 ml, 2 ml, 5 ml and 10
- Electric brushing machine and automatic pipette washer.
- Desirable equipment
- Shaker water bath.
- Ultracentrifuge.

Electron microscope

Ernst Ruska, a German engineer and academic professor, built the first Electron Microscope in 1933.

- EM is uses a beam of rushing electrons as a source of illumination.
- A special type of microscope having a high resolution of images
- Able to magnify objects in nanometers
- Formed by controlled used of electrons in vacuum captured on a phosphore-scent screen.

Types of Electron Microscopes

There are several different types of electron microscopes, including the:

1- Transmission electron microscope (TEM) enlarge 50 to ~50 million times; the specimen appears flat

2- Scanning electron microscope (SEM) enlarge 5 to ~ 500,000 times; sharp images of surface features

Key elements of a virology laboratory

3- Reflection electron microscope (REM) enlarge 5 to ~50 million times; the specimen appears flat.

Principle of Electron microscope

LIGHT MICROSCOPE	ELECTRON MICROSCOPE
Illuminating source is the Light.	Illuminating source is the beam of electrons.
Specimen preparation takes usually few minutes to hours.	Specimen preparation takes usually takes few days.
Live or Dead specimen may be seen.	Only Dead or Dried specimens are seen.
Condenser, Objective and eye piece lenses are made up of glasses.	All lenses are electromagnetic.
It has low resolving power (0.25µm to 0.3µm).	It has high resolving power (0.001µm), about 250 times higher than light microscope.
It has a magnification of of 500X to 1500X.	It has a magnification of 100,000X to 300,000X.
The object is 5µm or thicker.	The object is 0.1µm or thinner.
Image is Colored.	Image is Black and White.
Vacuum is not required.	Vacuum is essential for its operation.
There is no need of high voltage electricity.	High voltage electric current is required (50,000 Volts and above).