Fatma mustafa

Antibiotic sensitivity test

The main purpose of routine in vitro susceptibility testing in the clinical microbiology laboratory is to guide physicians in selecting antimicrobial therapy for treatment of individual patients. The physician utilizes the susceptibility test result along with other available clinical information (e.g. site of infection ,severity of infection, immune status of patient, co-morbidities, etc.) to select the optimal therapeutic agent for that particular patient'. It involves exposing a pure culture of a microorganism to a range of concentrations of an antimicrobial agent and observing the presence or absence of growth after a period of incubation. The results can vary widely depending on the conditions of testing. It is therefore imperative to use standardized methods.

Mueller-Hinton Agar

it is used in antimicrobial susceptibility testing.

Preparation

By following the manufacturer's instructions the media was prepared:

1. Dissolve 30.0g in 1 liter of purified water.
2. Heat in boiling water and agitate frequently until completely dissolved.
3. Autoclave for 15mins at 121° C
4. It was then poured into petri-dishes, about 16-18 ml in each.
5. Allow to solidify for leaving it at room temperature.
6. The shelf life is about one month.
7. It was stored at 2-8° C.

_ **Minimum inhibitory concentration (MIC)** The MIC is the minimum (lowest) concentration of an antibiotic that will inhibit the growth of a bacterial strain. This can be determined by several methods including macro- and micro dilution tests, extended breakpoint sensitivity tests, and e-test strips. Determination of MIC is important in the management of certain infections .

_**Minimum bactericidal concentration (MBC)** The MBC is the lowest concentration of the antibiotic that will kill a bacterial strain. The MBC is less clinically relevant than the MIC, as MBC tests are harder to standardize

Antibiotic sensitivity testing can be carried out by two broad methods, as follows:

a) Disc diffusion tests

b) Dilution tests

Disc diffusion tests

Disc diffusion tests are the most commonly used methods in a laboratory to determine susceptibility of bacteria isolates to antibiotics. In this method, as the name suggests, discs impregnated with known concentrations of antibiotics are placed on agar plate that has been inoculated with a culture of the bacterium to be tested. The plate is incubated at 37°C for 18–24 hours. After diffusion, the concentration of antibiotic usually remains higher near the site of antibiotic disc, but decreases with distance. Susceptibility to the particular antibiotic is determined by measuring the zone of inhibition of bacterial growth around the disc .

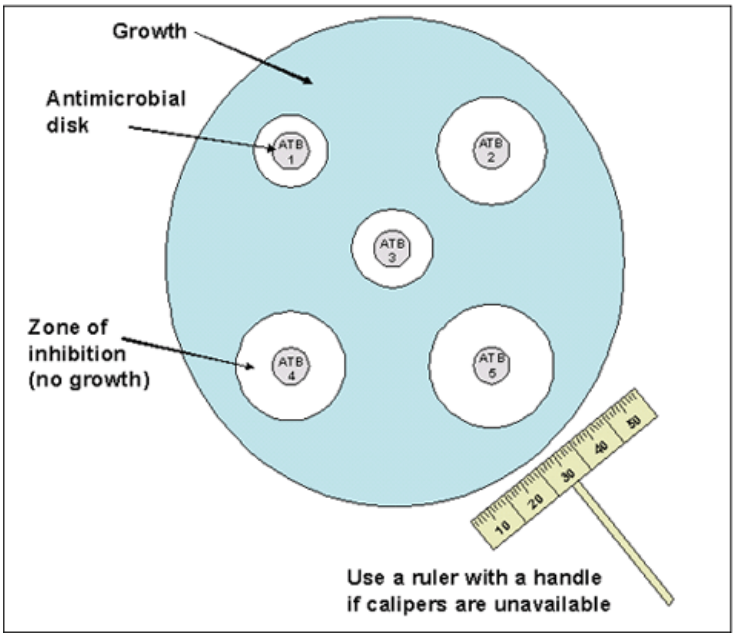
Types of disc diffusion tests

Disc diffusion tests are of the following types:

a) Kirby–Bauer disc diffusion method

b) Stokes disc diffusion method

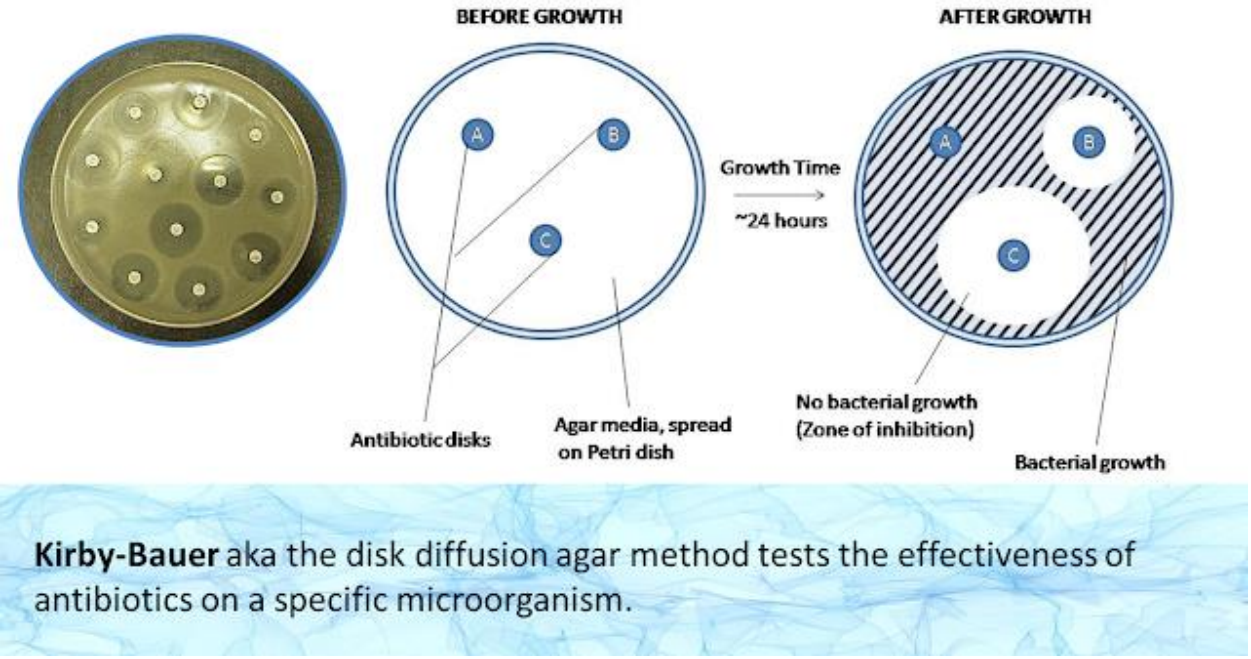
c) Primary disc diffusion test



Kirby–Bauer disc diffusion method

The Kirby-Bauer test, known as the disk-diffusion method, is the most widely used antibiotic susceptibility test in determining what choice of antibiotics should be used when treating an infection. This method relies on the inhibition of bacterial growth measured under standard conditions. For this test, a culture medium, specifically the Mueller-Hinton agar, is uniformly and aseptically inoculated with the test organism and then filter paper discs, which are impregnated with a specific concentration of a particular antibiotic, are placed on the medium. The organism will grow on the agar plate while the antibiotic "works" to inhibit the growth. If the organism is susceptible to a specific antibiotic, there will be no growth around the disc containing the antibiotic. Thus, a "zone of inhibition" can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. The measurement is compared to the criteria set by the Clinical and Laboratory Standards Institute (CLSI). Based on the criteria, the

organism can be classified as being Resistant (R), Intermediate (I) or Susceptible (S).



Kirby-Bauer aka the disk diffusion agar method tests the effectiveness of antibiotics on a specific microorganism.

Dilution test

Following methods are carried out to determine the MIC:

a) Broth dilution method

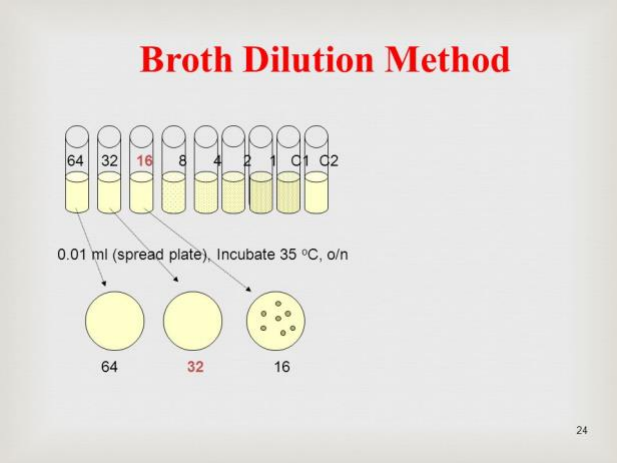
b) Agar dilution method

c) (E-test)

Broth dilution method

The aim of broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (minimal inhibitory concentration, MIC) that, under defined test conditions, inhibits the visible growth of the bacterium being investigated. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents. Agar dilution involves the incorporation of different concentrations of the antimicrobial substance into a nutrient agar medium

followed by the application of a standardized number of cells to the surface of the agar plate. For broth dilution, often determined in 96-well microliter plate format, bacteria are inoculated into a liquid growth medium in the presence of different concentrations of an antimicrobial agent. Growth is assessed after incubation for a defined period of time (16-20 h) and the MIC value is read



Epsilometer test

The E-test has been developed to provide a direct quantification of antimicrobial susceptibility of microorganisms. E-test is a laboratory test used to determine minimum inhibitory concentration (MIC) and whether or not a specific strain of bacterium or fungus is susceptible to the action of a specific antibiotic.

E test is a quantitative technique that is based on combination of concept of both dilution and diffusion principle for susceptibility testing. E test strip is placed on to an inoculated agar plate; there is an immediate release of antibiotics from the plastic carrier surface into the agar surface. After incubation, bacterial growth becomes visible, symmetrical inhibition ellipse along the strip is seen. The MIC value is read from the scale in terms of µg/ml where the ellipse edge intersects the strip.

Lec .Fatma mustafa

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.

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

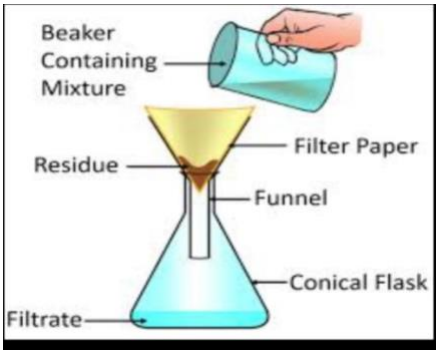
Boiling technique is widely used for sterilization of metallic devices like scalpels, surgical scissors, needles etc. These substances are boiled to high temperatures to kill the disease-causing bacteria.

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 .



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 .

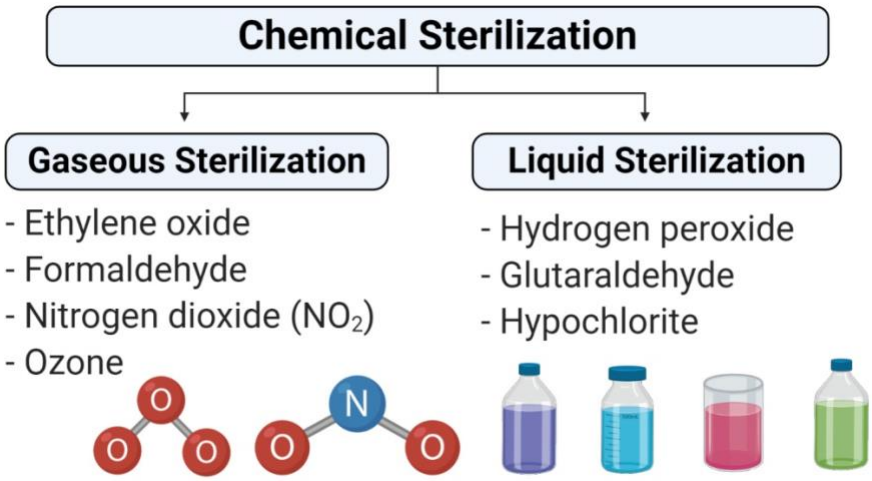
B-Chemical method

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:

- Silver
- Alcoholes
- Hydrogen Peroxide
- Glutaraldehyde and Formaldehyde
- Bleach

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

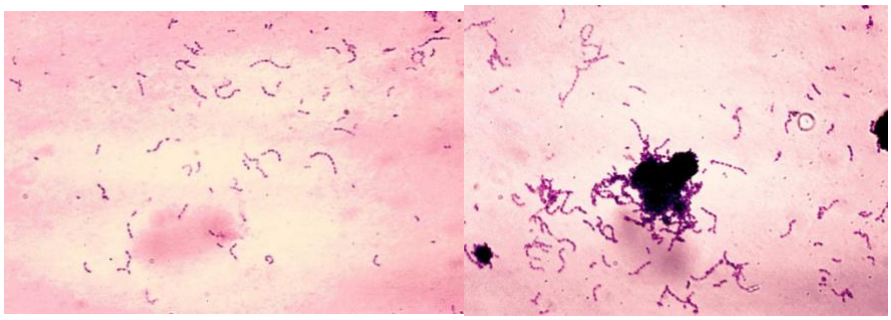

Chemical Sterilization
Gaseous Sterilization
Liquid Sterilization
- Ethylene oxide
- Formaldehyde
- Nitrogen dioxide (NO_2)
- Ozone
- Hydrogen peroxide
- Glutaraldehyde
- Hypochlorite
O
O
O
N
O
O

Streptococci

Fatma mustafa

The streptococci are gram-positive bacteria usually chaine ,catalase negative, oxidase negative

- S pyogenes nonmotile, and nonsporulating; they usually require complex culture media. S pyogenes characteristically is a round a -to-ovoid coccus 0.6-1.0 μm in diameter
- -Facultative Anaerobes
- -Fastidious Growth Requirements
- -Fermentative Metabolism of Carbohydrates:
- Lactic acid, ethanol, acetate endproducts produced; No gas
- -Catalase Negative (2H2O2 ---> O2 + 2H2O)
- Separation of streptococci from staphylococci
- -Oxidase Negative
- -Beta, Alpha, or Gamma Hemolysis on blood agar



Differential Bacterial Growth Media

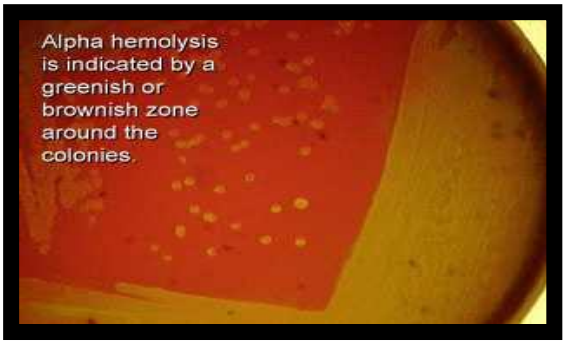
Blood Agar (BAP) differential media. BAP is rich in nutrients and contains blood. It is not selective, and will grow many different types of microbes. Blood agar medium is, however, differential. It will display a color change in the presence of bacteria that can lyse (break down) the red blood cells in the medium. Bacteria that grow on this medium will produce one of three hemolytic patterns

Classification of Streptococci based on Hemolysis

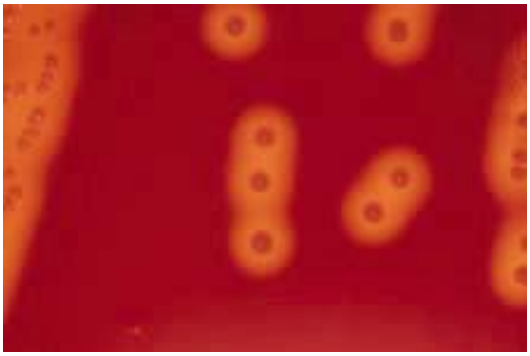
reactions on blood agar

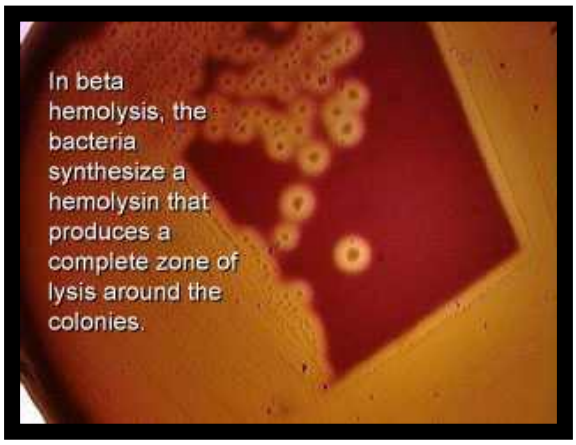
Many streptococci are able to hemolyze red blood cell

(alpha-hemolysis) partial hemolysis (Greenish Discoloration) associated with reduction of red cell hemoglobin. S. pneumoniae are alpha-hemolytic



.(Beta –hemolysis) complete lysis of red cells surrounding the colony appearance as Clear zone Streptococcus pyogenes are beta-hemolytic





gamma-hemolytic Non hemolytic colonies. Enterococcus faecales Most of oral streptococci and enterococci are non- hemolytic.

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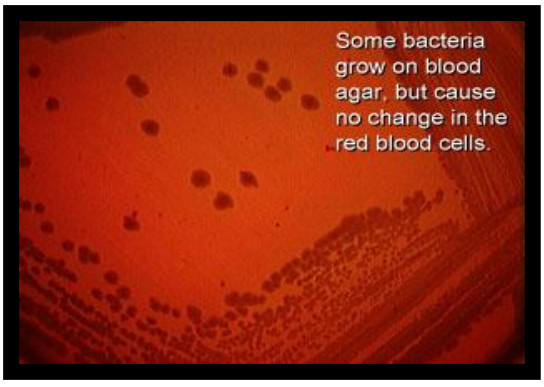
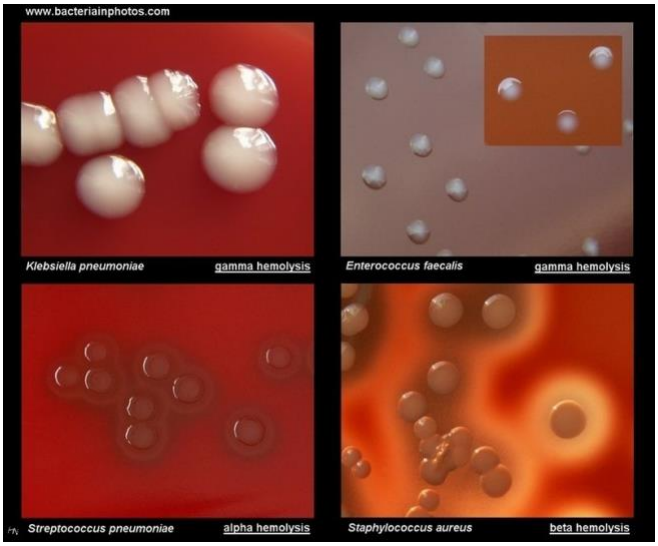




Selective Strep Agar

is designed to inhibit gram-negative bacilli and staphylococci, , and identification of pathogenic streptococci, including beta-hemolytic streptococci and *S. pneumoniae*.

Tryptic Soy Agar is the basal medium for Selective Strep Agar. Organic nitrogen, particularly amino acids and long-chained peptides are supplied by the combination of casein and soy peptones. This combination renders the medium highly nutritious. Osmotic equilibrium is maintained by sodium chloride. Sheep blood (5%) has been added to facilitate growth and to detect hemolytic activity. Selective agents are added to suppress muchoftheoralflora,including, *Micrococcus*, *Haemophilus* and *Neisseria* species. All species of *Streptococcus* will grow on this medium.

Anti Streptolysin-O-Titre(ASOT):

in case of rheumatic fever and streptococcal infection.

Procedure :

*Reagent must have the room temp. before use then mix gently.

1.Put 10 µ of serum in a black slide then put on it 10 µ of the reagent .

2.Mix and rotate the slide by hands in a circle direction for 2 min .

-If precipitation occurs the result + ve. .

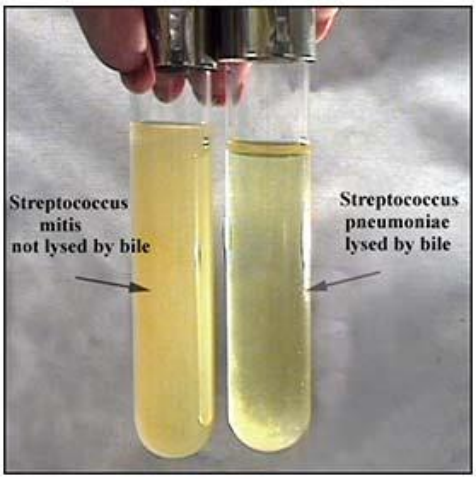
-If no ppt occurs the result is -ve .

Bile Solubility test

- **Principle:**
  - *S. pneumoniae* produce a self-lysing enzyme to inhibit the growth
  - The presence of bile salt accelerate this process
- **Procedure:**
  - Add ten parts (10 ml) of the broth culture of the organism to be tested to one part (1 ml) of 2% Na deoxycholate (bile) into the test tube
  - Negative control is made by adding saline instead of bile to the culture
  - Incubate at 37 C for 15 min
  - Record the result after 15 min

- Results:
  - Positive test appears as clearing in the presence of bile while negative test appears as turbid
  - *S. pneumoniae* soluble in bile whereas *S. viridans* insoluble

Bile Solubility Test



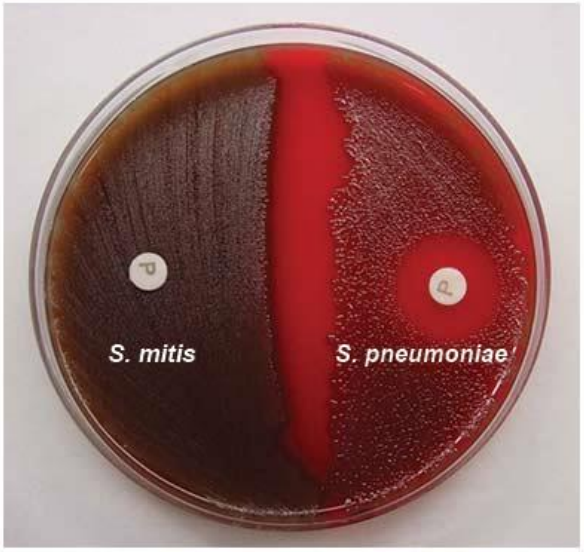
- **Optochin Susceptibility Test**
- Principle:
  - Optochin (OP) test is presumptive test that is used to identify *S. pneumoniae*

. Optochin (ethyl hydrocuprein hydrochloride) is a chemical that is toxic to some bacteria but harmless to others. It is useful in the identification of ***Streptococcus pneumoniae***, the alpha-hemolytic Streptococcus most commonly susceptible to this chemical. This test determines whether the bacterium is either sensitive (susceptible) to optochin or resistant to the chemical.

–

- Procedure:
  - BAP inoculated with organism to be tested
  - OP disk is placed on the center of inoculated BAP
  - After incubation at 37oC for 18 hrs, accurately measure the diameter of the inhibition zone by the ruler
  - ≥14 mm zone of inhibition around the disk is considered as positive and ≤13 mm is considered negative

- *S. pneumoniae* is positive (S) while *S. viridans* is negative (R)



Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

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Bacterial calture methodes

There are many types of bacteria, Isolation and identification of the . bacteria is very important in medicine.A microbiological culture, or microbial culture, is a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions. The first requirement for physically isolating of bacteria is that it can be cultured in the laboratory. This requires knowledge of optimal temperature for growth, optimal oxygen requirements, and optimal nutritional needs.

Purpose of Culturing

- To isolate bacteria in pure cultures.
- To demonstrate their properties.
- To determine sensitivity to antibiotics.
- To estimate viable counts.

_Maintain stock cultures

Culture methods include:

Streak culture

Stab culture

Pour plate method

Liquid culture

Spread plate method

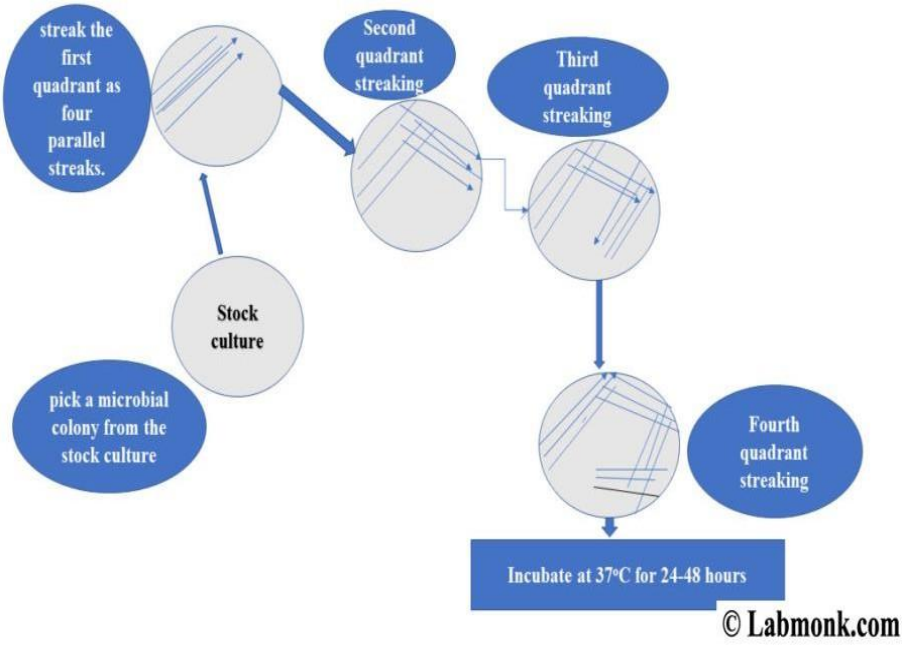
Anaerobic culture methods

Streak Plate method to Purify Single bacteria

This method was used to isolate bacteria and enable you to isolate pure culture

Principle This is essentially a method to dilute the number of organisms, decreasing the density - individual colonies to be isolated from other colonies.

1. 1 Begin with inoculating the first, or primary, quadrant of the agar plate. Use a light touch. Don't penetrate or scrape the agar surface. Cover plate with lid.
2. Flame the loop, cool by touching an uninoculated portion of the surface.
3. Now rotate the plate. Open lid and streak again, remember: you are picking up growth from quadrant one, and using this as your inoculum for quadrant two.
4. Flame loop; rotate plate, and repeat procedure for quadrants three and four.
5. 5- Incubation



Streaking on slant

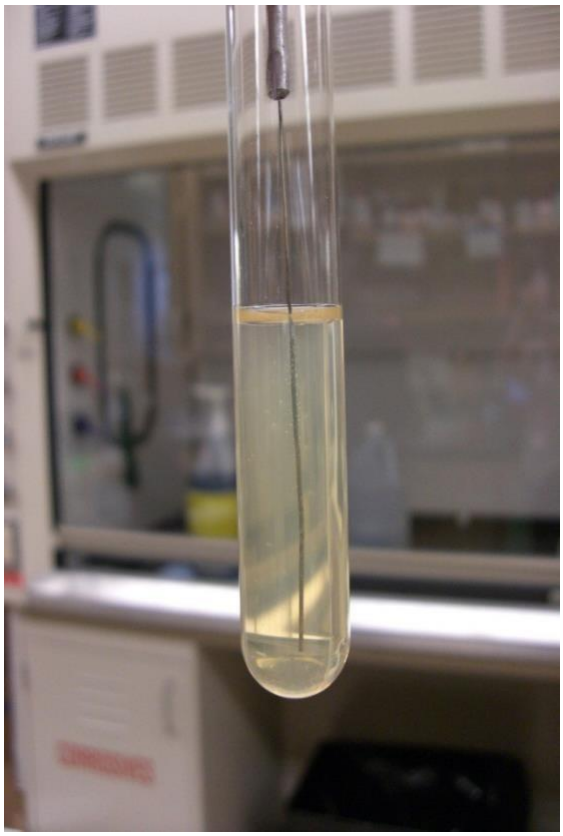
stabbing by Needle

:This method is used to study the ability of bacteria to -

.Grow with the presence of O2 Or not

Motility

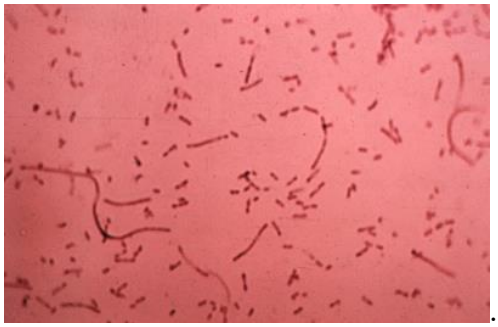
.Production of some chemical compounds during metabolism



Haemophilus influenzae

fatma mustafa

Haemophilus influenzae is aGram-negative coccobacillus, and it's Oxidase and Catalase positive



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Culture

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,

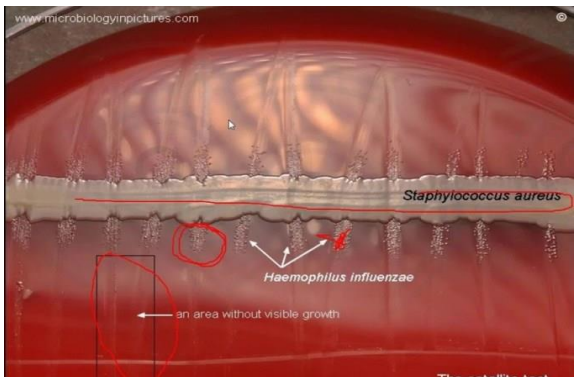
Most strains of Haemophilus spp does not grow on 5% Sheep Blood Agar, which .(contains hemin (factor X) but lacks NAD (factor V)

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

Haemophilus influenzae on Chocolate Agar

e wi

Satellite phenoenon of Haemophilus influencae with Staphylococcus aureus on blood agar



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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

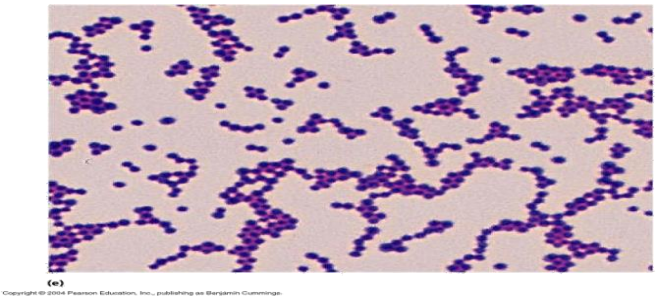
Rinse with water to remove excess stain Blot dry

Observe under Oil Immersion

Gram positive

Shape: Cocci

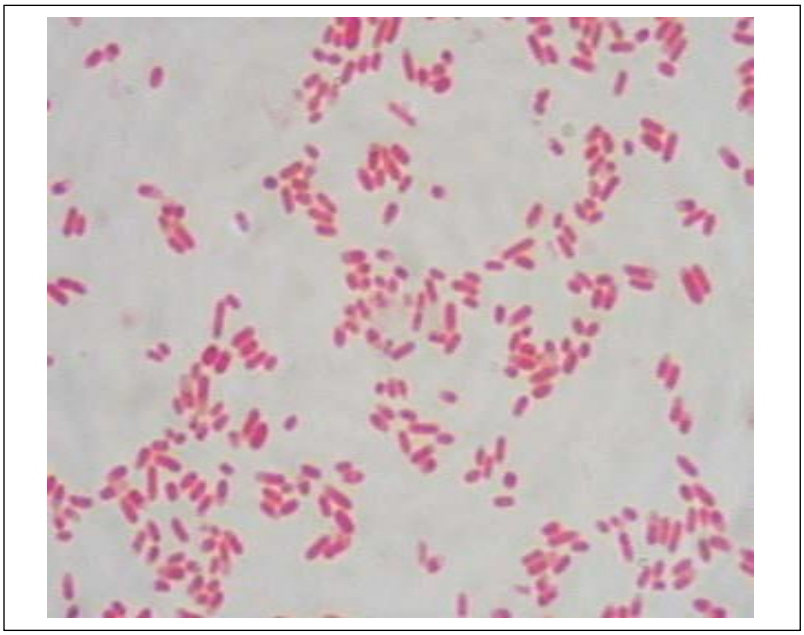
Colour: Violet (Purple)

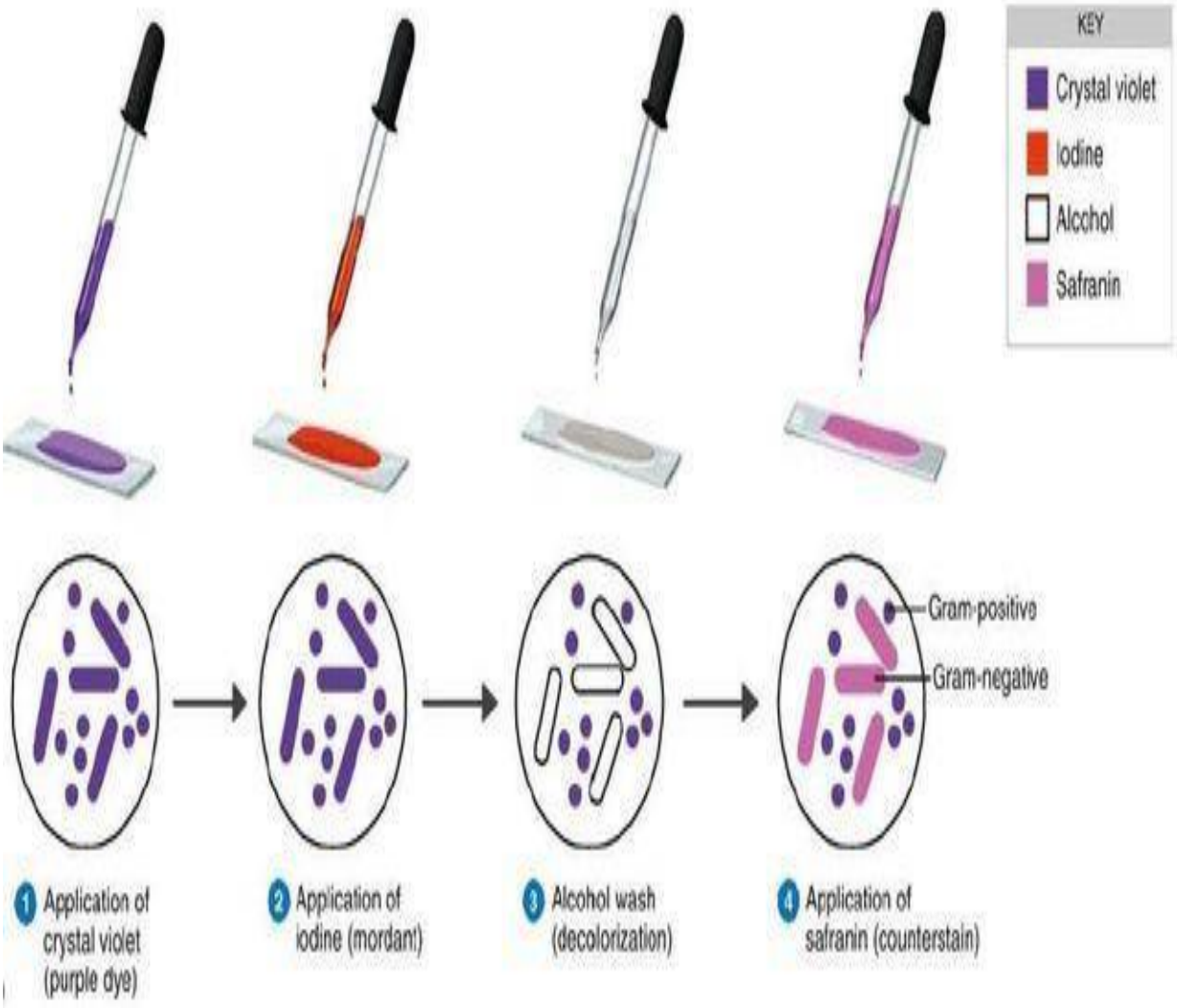


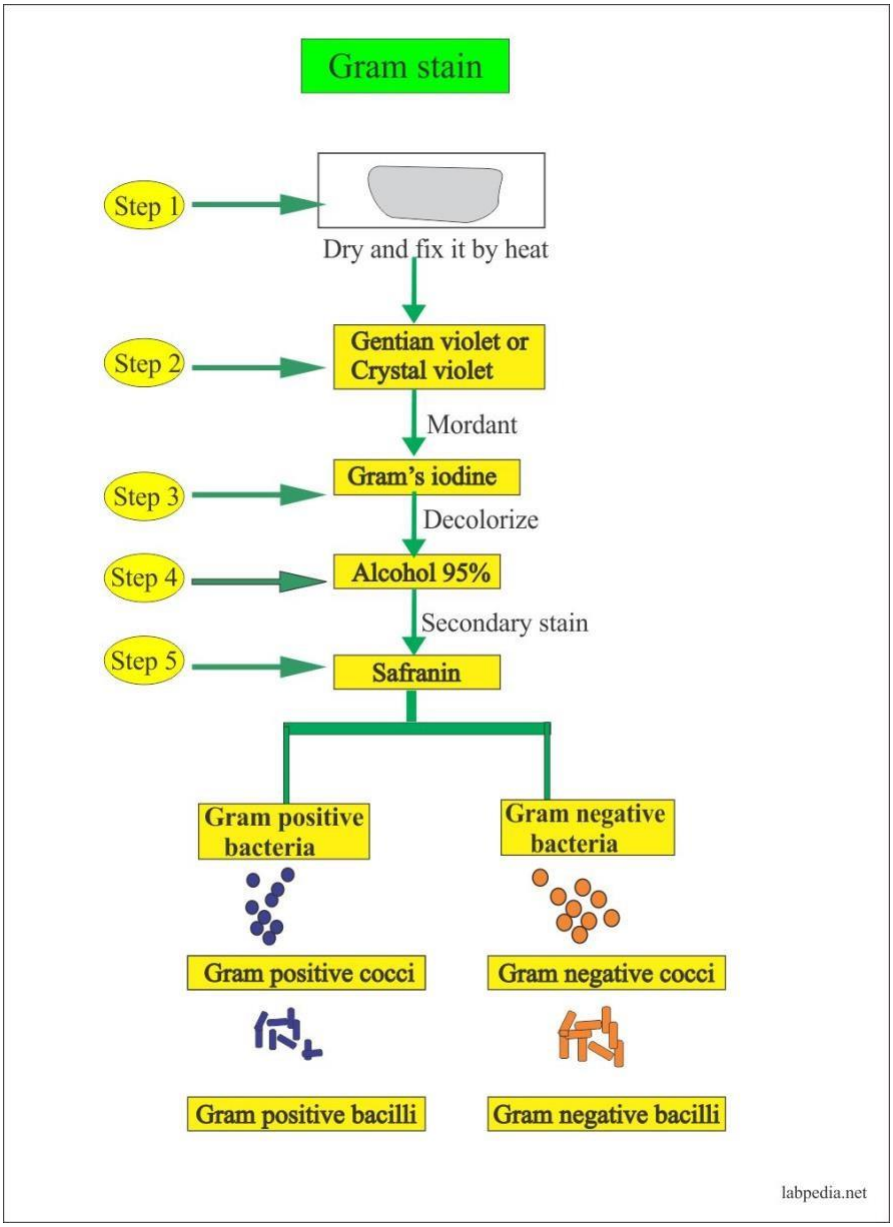
Gram negative

Shape rod

Colour pink




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

Laboratory safety Lab1

All students must read and understand the information in this document with regard to laboratory safety and emergency procedures prior to the first laboratory session. Your personal laboratory safety depends mostly on you. , research and teaching workplaces (labs, shops, etc.) are full of potential hazards that can cause serious injury and or damage to the equipment. Working alone and unsupervised in laboratories is forbidden if you are working with hazardous substances or equipment. With prior approval, at least two people should be present so that one can shut down equipment and call for help in the event of an emergency.

Safety training and/or information should be provided by a faculty member, teaching assistant, lab safety contact, or staff member at the beginning of a new assignment or when a new hazard is introduced into the workplace.

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.

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.

11-If leaving a lab unattended, turn off all ignition sources and lock the doors.

12-Never pipette anything by mouth.

13-Clean up your work area before leaving.

14-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.

10-Do not store food in laboratories.

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.

11-Do not store heavy items above table height. Any overhead storage of supplies on top of cabinets should be limited to lightweight items only. Also, remember that a 36" diameter area around all fire sprinkler heads must be kept clear at all times.

12-Areas containing lasers, biohazards, radioisotopes, and carcinogens should be posted accordingly. However, do not post areas unnecessarily and be sure that the labels are removed when the hazards are no longer present.

13-Be careful when lifting heavy objects. Only shop staff may operate forklifts or cranes.

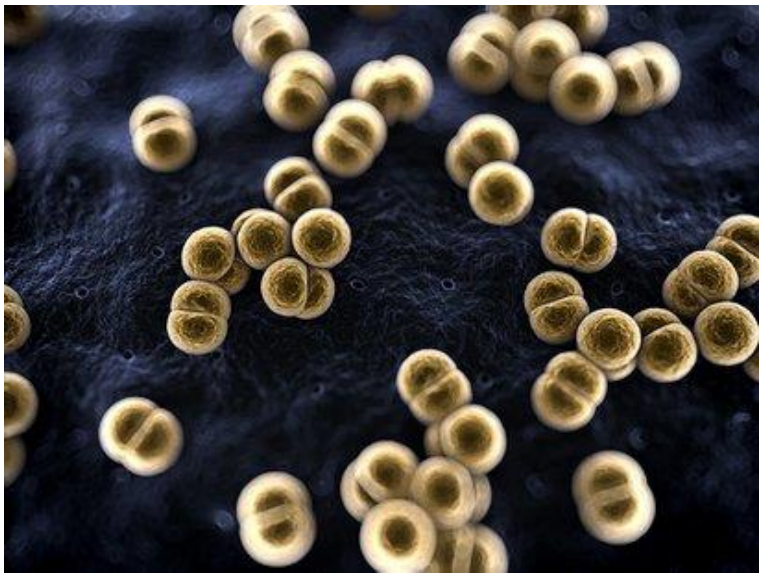
14-Clean your lab bench and equipment, and lock the door before you leave the laboratory.

Fatma mustafa

Genus: Neisseria

Species: meningitidis and gonorrhoeae

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



General characteristics

*** G –ve diplococcic, kidney shape.**

*** Oxidase positive.**

*** Ferment carbohydrates.**

*** Non –hemolytic.**

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

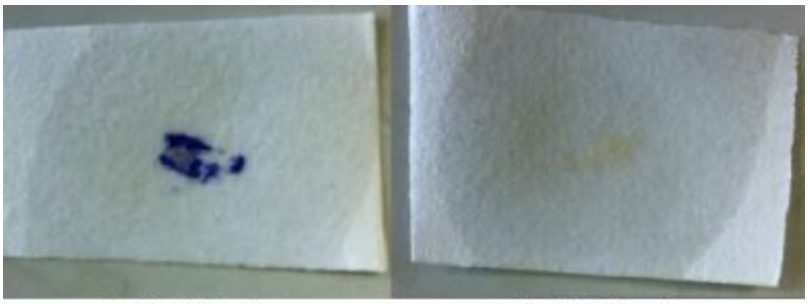
.

Oxidase test

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

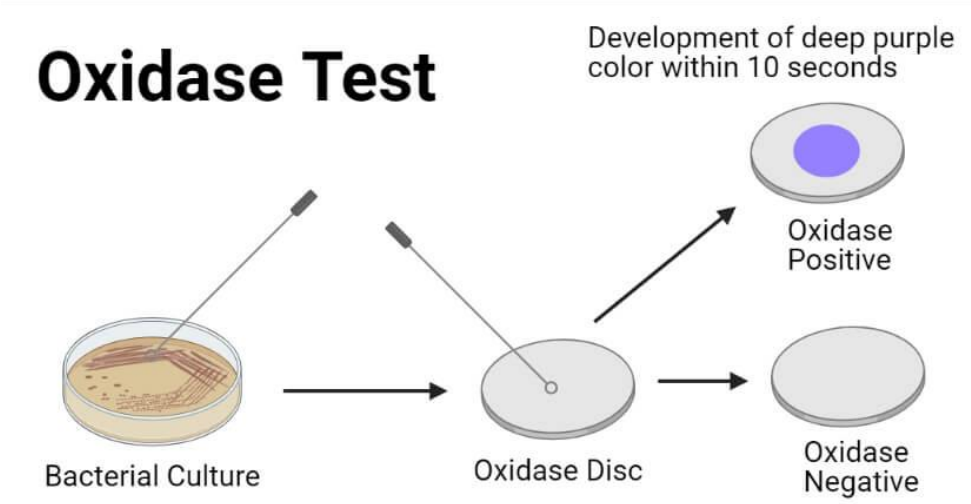
Procedure of Oxidase test:

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

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.

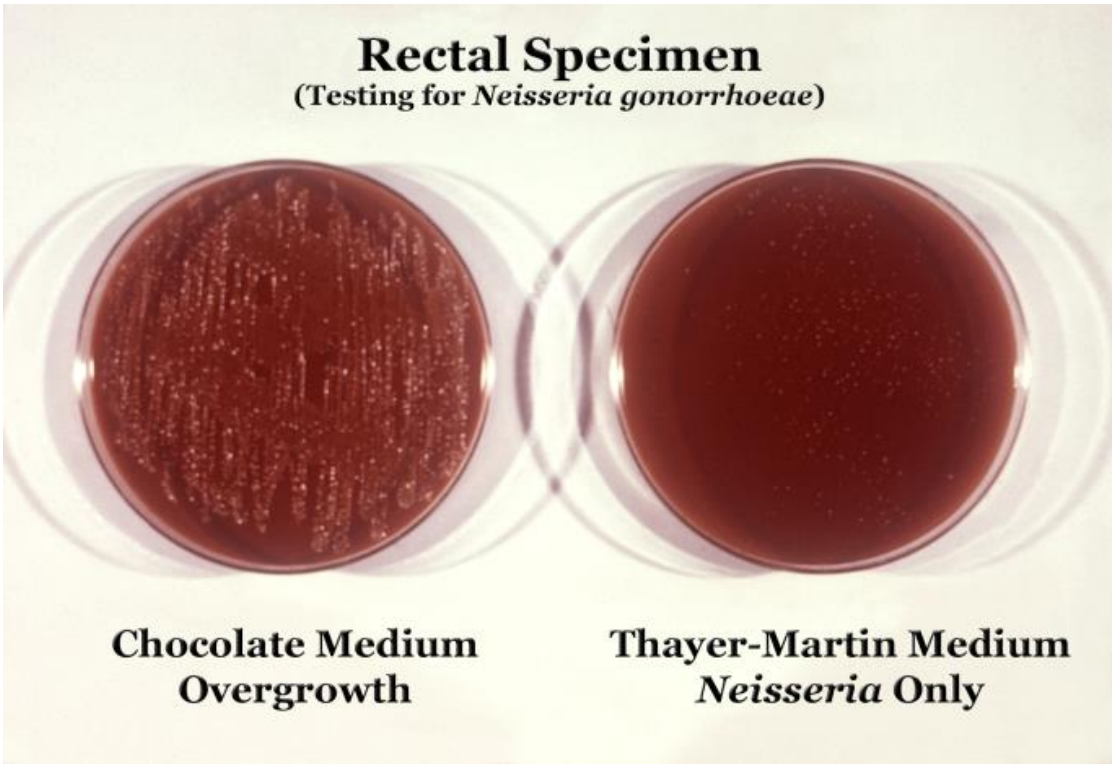
2- Culture; culturing the organism on Thayer-Martin agar

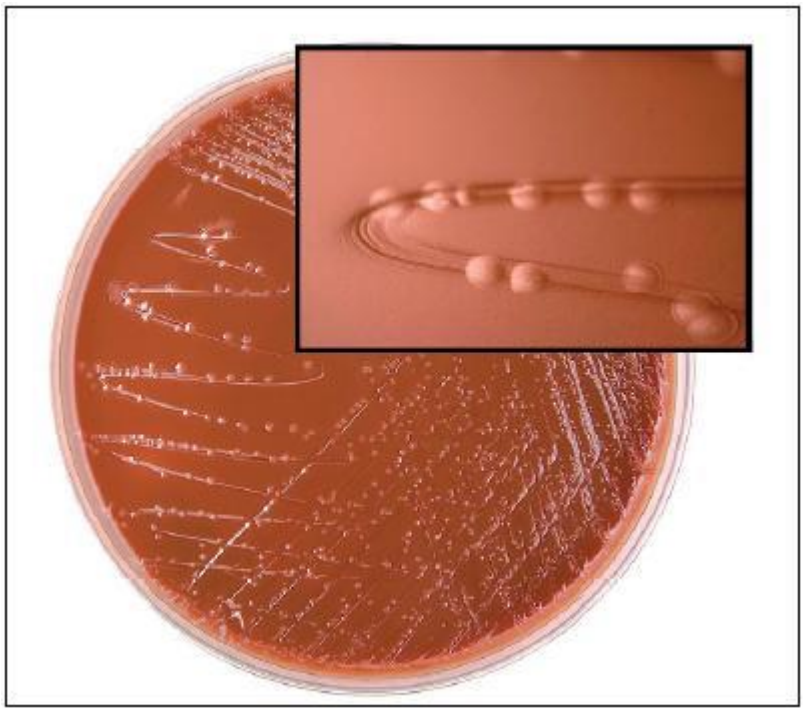
3- Oxidase test

4- Sugar fermentation tests

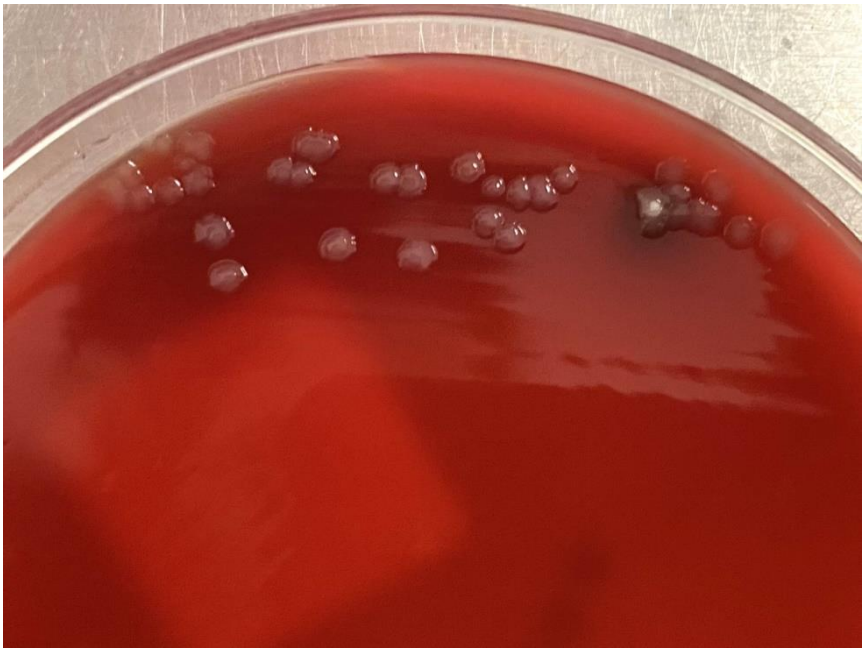
5- Serology


www.microbiologyinpictures.com
©
Neisseria gonorrhoeae


Rectal Specimen
(Testing for *Neisseria gonorrhoeae*)
Chocolate Medium
Overgrowth
Thayer-Martin Medium
Neisseria Only



Neissena gonorrhoeae on Thayer Martin

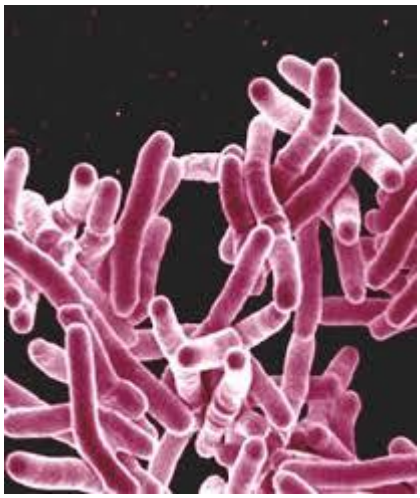


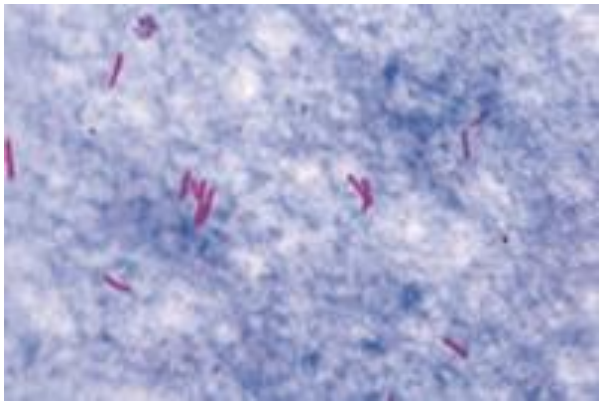
Neisseria meningitidis

Mycobacterium tuberculosis

Fatma mustafa

Mycobacterium tuberculosis is a , acid-fast bacillus that often appears beaded or unstained using Gram stain, ziehl-neelsen stains technique using for staining Mycobacterium tuberculosis is employed for identification of acid fast bacteria.Slender.straight.or slightly culvert bacilli with rounded ends occurring singly or in pair





Media used

Löwenstein–Jensen medium,The most widely used solid media is Lowenstein.._Jensen more commonly known as LJ medium, is a growth medium[specially used for culture of Mycobacterium species, notably Mycobacterium tuberculosis When grown on LJ medium, M. tuberculosis appears as brown, granular colonies . The medium must be incubated for a significant length of time, usually four weeks, due to the slow doubling .time of M. tuberculosis (15–20 hours) compared with other bacteria

..

Fig: Cultural Characteristics of *Mycobacterium tuberculosis*

Tuberculin Skin Testing

TST is one method of determining whether a person is infected with *Mycobacterium tuberculosis*. Reliable administration and reading of the TST requires standardization of procedures, training, supervision, and practice. The TST is performed by injecting 0.1 ml of tuberculin purified protein derivative (PPD) into the inner surface of the forearm. The injection should be made with a tuberculin syringe, with the needle bevel facing upward. The TST is an intradermal injection. When placed correctly, the injection should produce a pale elevation of the skin (a wheal) 6 to 10 mm in diameter.The skin test reaction should be read between 48 and 72 hours after administration by a health care worker trained to read TST results. A patient who does not return within 72 hours will need to be rescheduled for another skin test.

The reaction should be measured in millimeters of the induration (firm swelling). The reader should not measure erythema (redness). The diameter of the indurated area should be measured across the forearm (perpendicular to the long axis).

Polymerase chain reaction (PCR)

The TB-PCR is one of the tests that helps diagnose and confirm an infection of Tuberculosis.

Specimen type: Serum (Blood Sample)

Specimen collection procedure:Venipuncture (Collection of blood from a vein, usually from the arm)

Polymerase chain reaction (PCR) is a method widely used to rapidly make millions to billions of copies (complete copies or partial copies) of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it (or a part of it) to a large enough amount to study in detail. PCR was invented in 1983 by the American biochemist Kary Mullis at Cetus Corporation. It is fundamental to many of the procedures used in genetic testing and research, including analysis of ancient samples of DNA and identification of infectious agents. Using PCR, copies of very small amounts of DNA sequences are exponentially amplified in a series of cycles of temperature changes. PCR is now a common and often indispensable technique used in medical laboratory research for a broad variety of applications including biomedical research and criminal forensics.

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

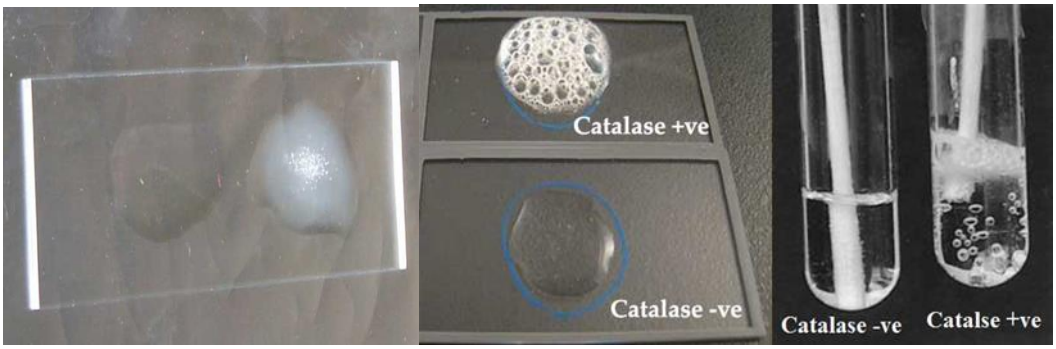
13- Citric Acid Utilization Test

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

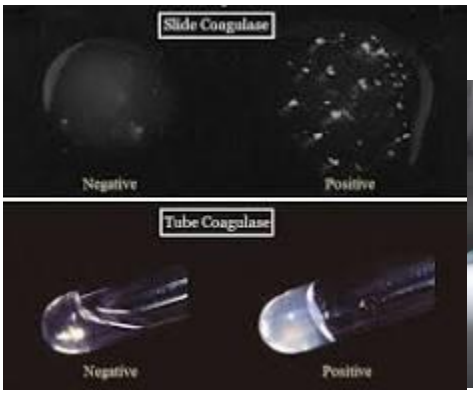
drops of an overnight broth culture of the test organism to l ml of human

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

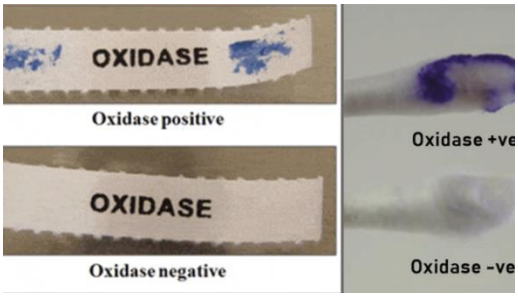
Procedure of Oxidase test:

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

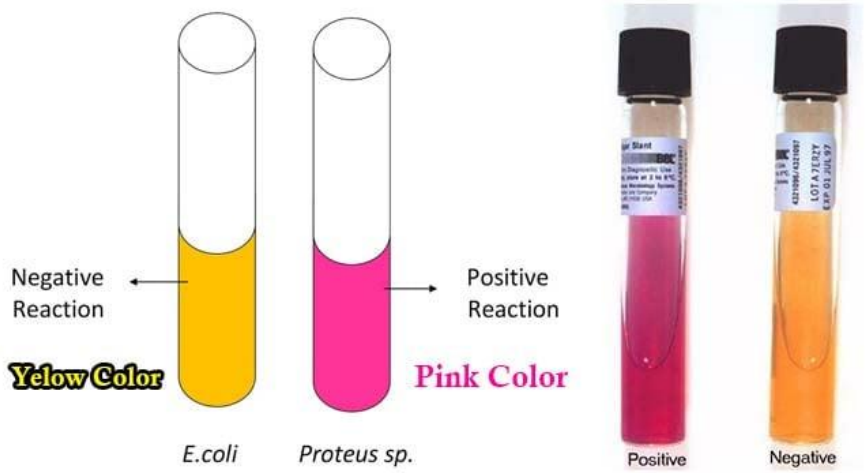
Positive Reaction: Development of a pink

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

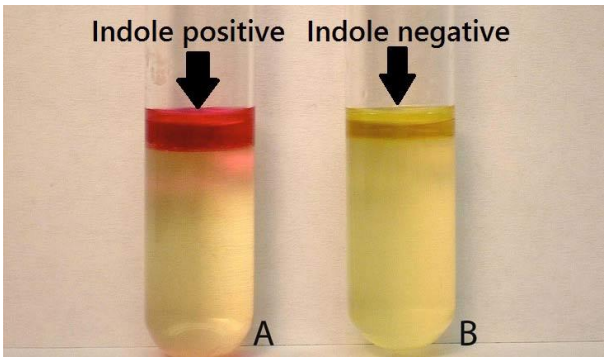
Peptone liquid medium containing tryptophan is inoculated the- tested

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

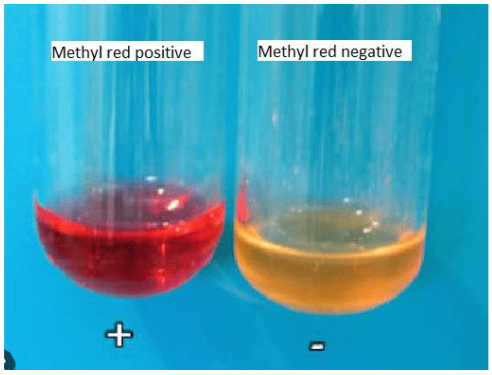


Methyl Red Voges-Proskaur Test (MR-VP test)

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

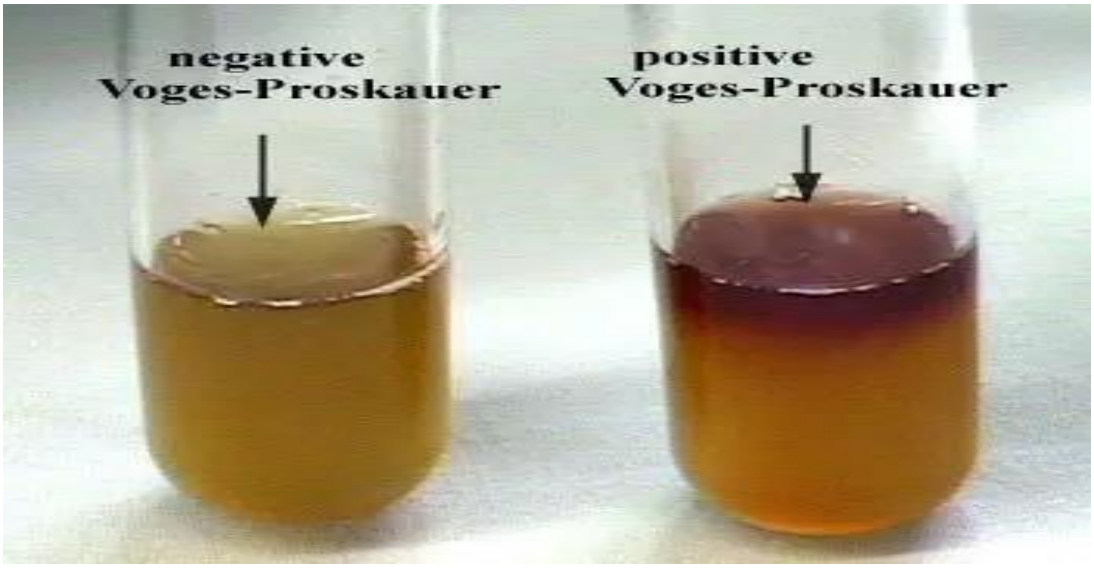
2. Incubate the tubes at 37°C for 24 hours

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

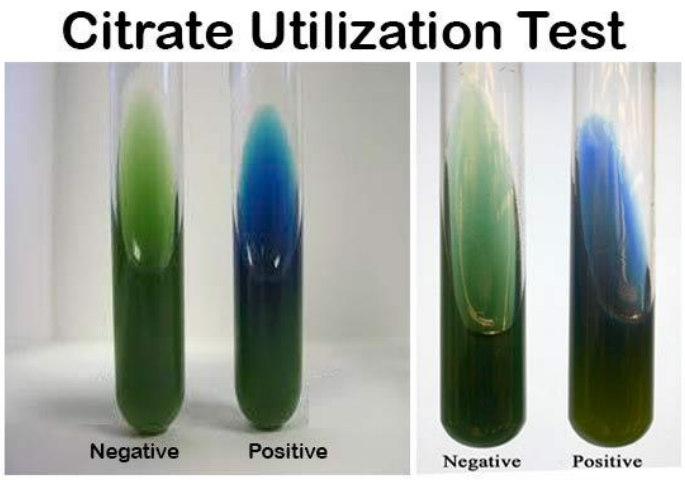


Voges-Proskauer results

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)

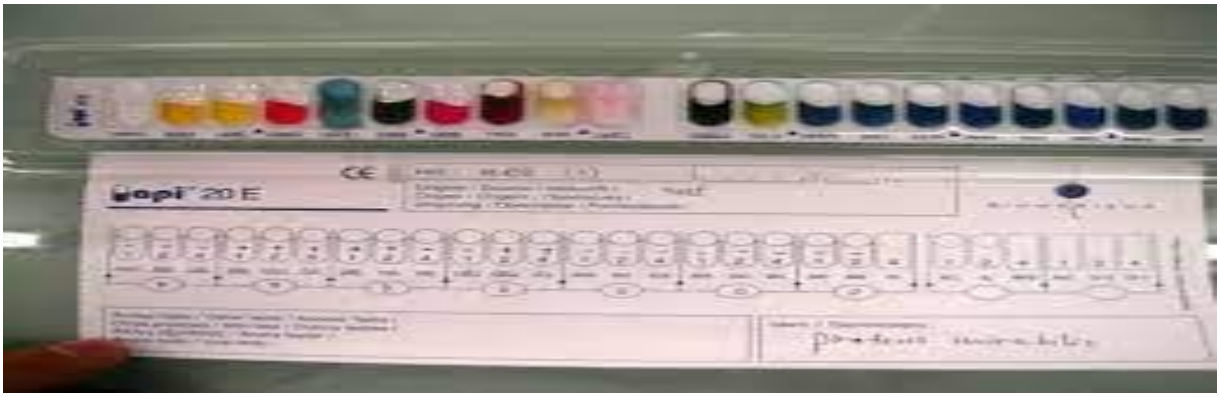


Analytical Profile Index (API):

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

Microbiology Lab **Asst. Lac. *Sura Mustafa***

3rd class / Dentistry

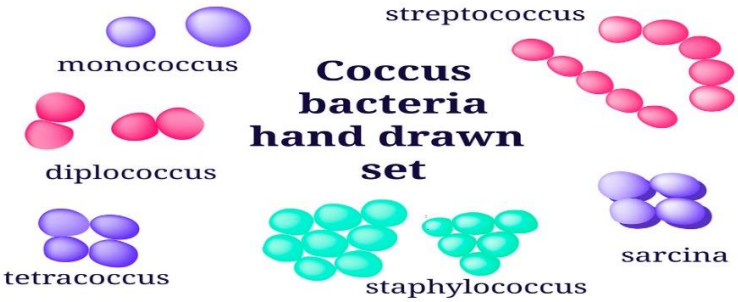
Morphology of bacteria

Bacterial cell shape and arrangement

When viewed under light microscope, the most bacteria appear in various types- like:

1. Cocci -Bacteria having spherical or oval in shape. It appears in multiple **arrangements** like:

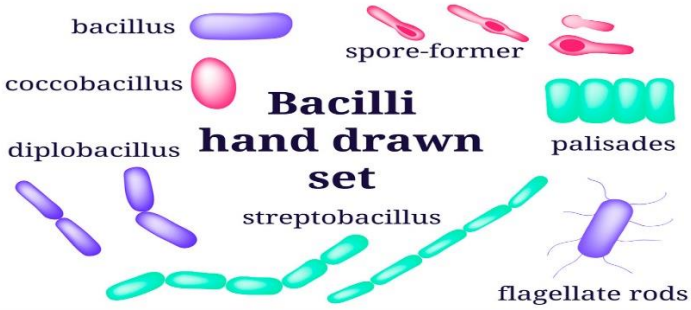
a) **Mono-coccus**- A single cocci cell arranged in a single plane.
b) **Diplococci** – Two cocci cells arranged in a single plane.
c) **Tetra coccus-** Four cocci cells arranged in two different planes
d) **Sarcinal arrangement-** Eight cocci cells arrange in three different planes.
e) **Staphylococcus-cluster** of bacterial cells arranged in different plane.
f) **Streptococcus-** Many bacterial cocci cells are arranged in a linear chain.



2. Bacilli- The bacteria with rod or cylindrical shape. It appears in multiple **arrangements** like:

.

A. Monobacilli- The bacteria with one bacillus cell arranged in a single plane.
B. Coccobacilli-Some bacteria are spherical or slightly elongated known as coccobacilli.
C. Diplobacili- The bacteria with two bacilli cells arranged in a single plane.
D. Bacilli in chain- Many bacilli cells are arranged in a linear chain.
E. Bacilli in cruciform arrangement- Some bacilli cells are arranged in a cruciform.
F. Bacilli in a rosette arrangement.



3. **Spiral shape**-These bacteria are carved, rod or comma shaped with vibriatory motility
   A. Spirilla-Bacteria with a rigid twisted or helical spiral form are known as spirillia.

Morphology of bacteria

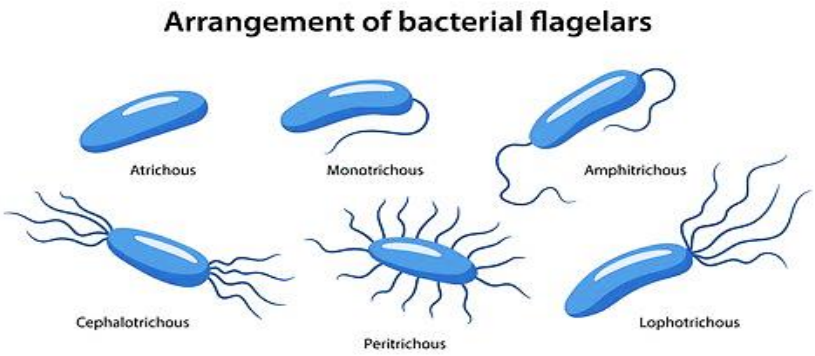
B. Spirochetes - Tightly coiled bacteria with corkscrew shaped, helical, flexible having axial filament.

4. Other shape

A. Polymorphism- Some bacteria may exhibit variation in their shape and size individually, show swollen forms in ageing cultures having high salt concentration.
B. Filamentous
C. Rectangular
D. Star shape

Flagella

Some bacteria have flagella that used for movement, it's a thread like locomotor appendages extending outward from the plasma membrane and the cell wall. It appears in multiple **arrangements** like:



Types of Culture Medium

Culture medium: a gel or liquid that contains nutrients and used to grow microorganisms, it is a balanced mixture of nutrients needed by microorganisms. They are also termed **growth media**. Different cell types are grown in various types of medium. Nutrient broths and agar plates are the most typical growth media for microorganisms. Some bacteria need special media for their growth

Agar: obtained from sea weeds, melts at 98oC and sets at 42 °C. No nutritive value, but acts as solidifying agent only.

Culture media is using for:

1- Development of microorganisms in vitro

2- Studying the characteristics of organisms growing for diagnosis.

3- Isolation and purification of microorganisms for studying them

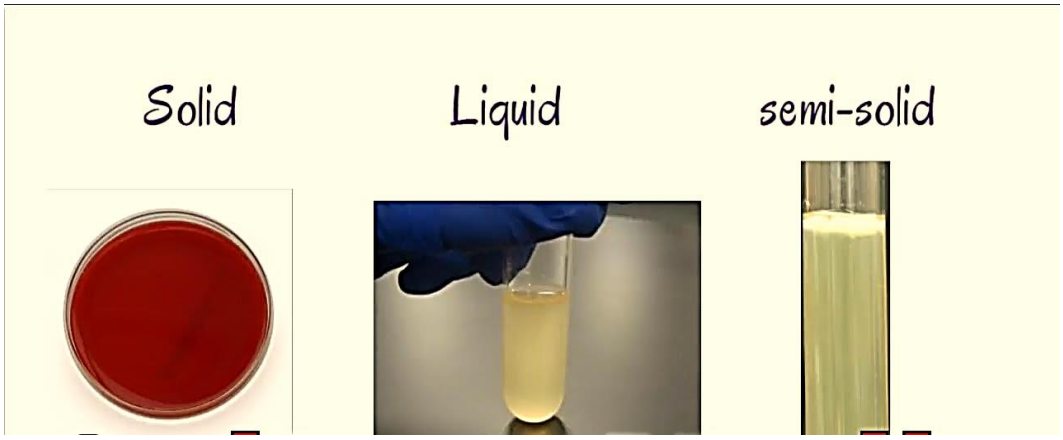
4- Estimation of the number of microorganisms in a sample (food, water, soil...etc)

5- Preservation and maintenance of microorganisms in the laboratory and for long periods.

Classification of Culture media

A- Based on physical state or consistency:

- **Liquid media**: it's a media that do not contain hardeners such as agar and gelatin. Used for inoculum preparation. Eg: Nutrient broth
- **Semi Solid Media**: contains 0.5% agar. Eg: Motility medium
- **Solid Media:** contains 2% to 3% agar. Colony morphology, pigmentation, hemolysis can be appreciated. Isolated pure bacteria can be obtained Eg: Nutrient agar,



Types of Culture Medium

B- Based on Nutritional factors or constituents:

- ✓ **Simple Media** this is the simplest and most common media used in laboratories, Contains basic nutrients for the growth of bacteria. Eg.– peptone water
- ✓ **Complex Media** is contain unknown compound and/or concentration that add for bringing out specific qualities or providing the unique nutrients needed for the bacterium's growth Examples – Nutrient broth which contains meat extract.
- ✓ **Synthetic Media** is a **defined medium**. A defined medium (chemically defined medium) is a medium in which there is no yeast, plant or animal tissue present, and all the chemicals employed are known. These are made from only pure ingredients whose exact composition is known.

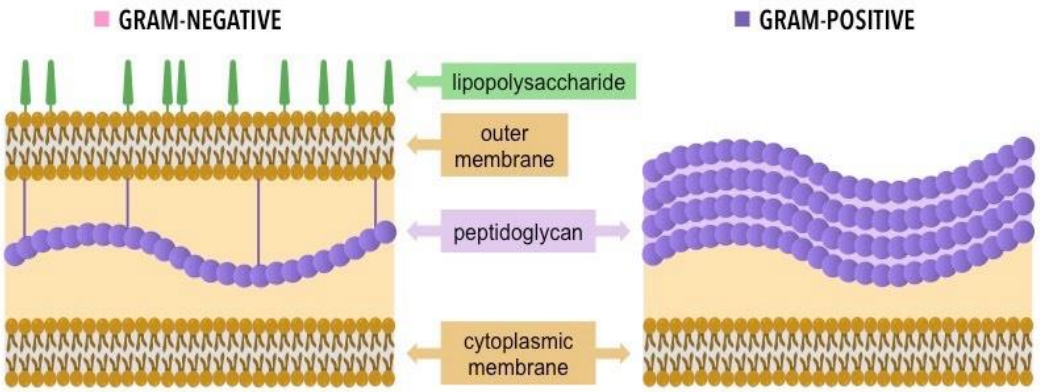
C- Based on use: also called Special media

- ✓ **Enriched media –** For example, a blood agar medium is used for the growth of bacteria like *Streptococcus* which specifically requires blood for proliferation.
- ✓ **Selective media –** contain components that prevent growth many bacterial species except one or same species that make it easier to isolate a specific species. For example, bile salt acts as a selective agent in **BSA** or bile salt agar, it favors the growth of *Vibrio cholerae*.
- ✓ **Differential media –** that aid in identifying the various properties of bacteria. **Peptone agar, lactose, neutral red and sodium cholate** are all ingredients in MacConkey's medium. Here, the colonies made by lactose fermenters are **pink**, but those made by non-lactose fermenters are pale or colorless.
- ✓ **Transport media:** These are employed when might become covered with non-pathogenic germs. Used for transportation of bacteria to laboratories. Example – Stuart's transport medium.
- ✓ **Indicator media –** When media containing an indicator, they tend to change their color. E.e MacConkey's medium & another classic example is the black colonies of *Salmonella typhi* that developed on Wilson and Blair media sulphite-containing.

Gram Staining

Gram staining

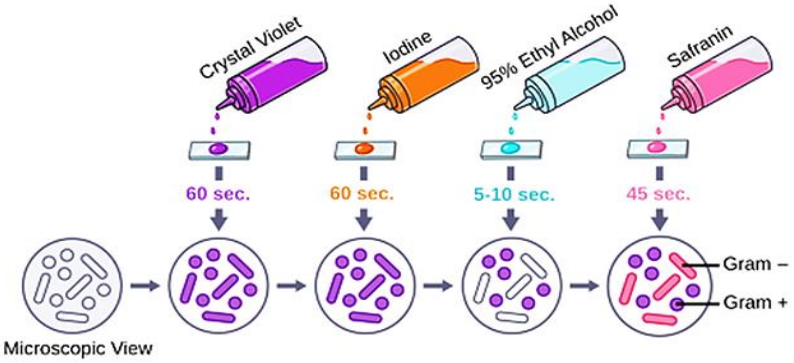
The Gram stain procedure distinguishes between Gram positive and Gram negative bacteria by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall.



Gram Staining Procedure In Short

There are 4 basic steps in Gram Stain Procedure

1. Primary Stain (Crystal violet)
2. Mordant (Lugol's Iodine)
3. Decolorizer (Alcohol)
4. Counter stain (Safranin or Carbol fuchsin)



Gram Staining Procedure step by step:

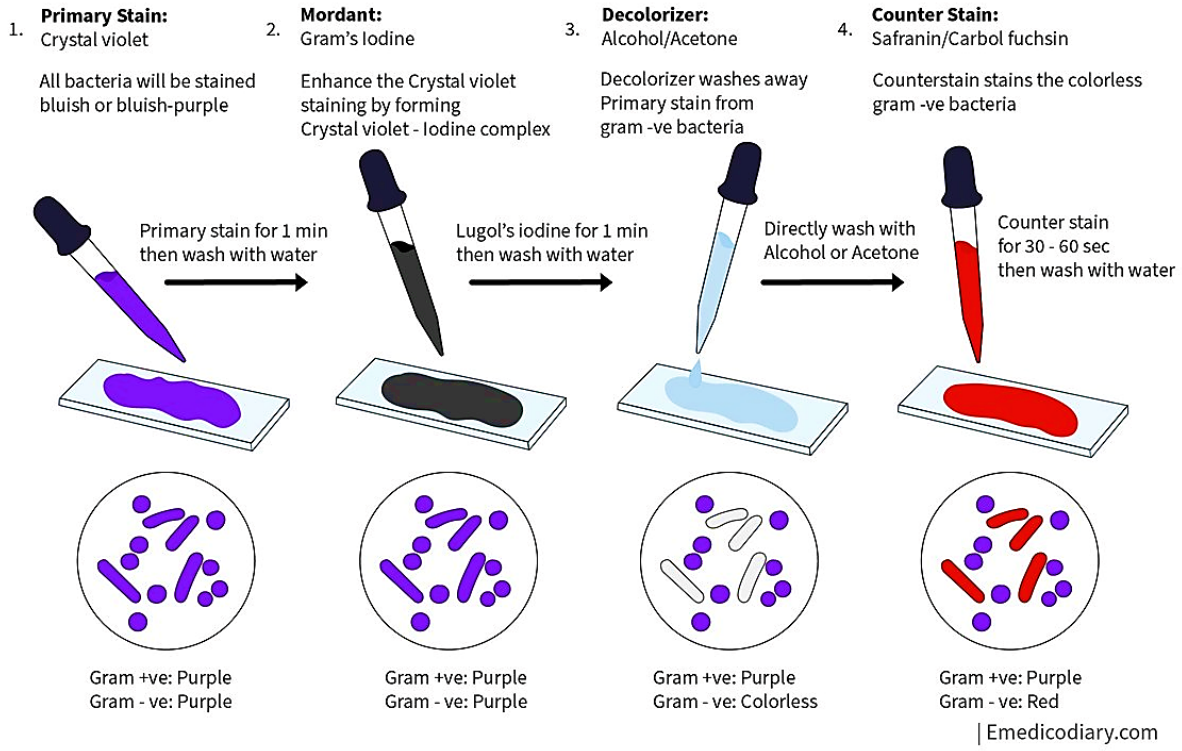
1. Prepare a bacterial smear: Using a sterile metallic loop, transfer a small amount of bacterial culture onto a clean microscope slide. Spread the culture into a thin, even film on the slide.
2. Air dry the smear: Allow the bacterial smear to air dry completely at room temperature. Do not heat-fix the smear.
3. Heat-fix the smear: Pass the slide through the flame of a Bunsen burner in to and from motion 2-3 times to heat-fix the bacterial cells to the slide.

Gram Staining

4. Primary Staining of the smear: Flood the heat-fixed bacterial smear with a Crystal violet stain for 1 minute. Rinse the slide gently with distilled water to remove excess stain.
5. Apply Lugol's iodine: Cover the smear with Gram's iodine solution for 1 minute. This acts as a mordant and helps to fix the crystal violet stain in the bacterial cells.

6. Decolorize the smear: Quickly rinse the slide with 95% ethanol or 70% isopropyl alcohol until no more purple color runs off. This removes the crystal violet stain from some bacteria, making them colorless or "gram-negative". Be careful not to over-decolorize the smear, as this can result in false-negative results.
7. Counterstain the smear: Flood the slide with safranin stain or carbol fuchsin stain for 1 minute. This stains the decolorized bacteria pink or "gram-negative", allowing them to be seen under the microscope.
8. Rinse and dry the smear: Rinse the slide gently with distilled water to remove excess stain, and blot the slide dry with a paper towel.

After completing the above gram staining procedure, observe the stained smear under a microscope using a 100x oil immersion lens. Gram-positive bacteria will appear purple or blue-violet, while gram-negative bacteria will appear pink or red.



Microbiology Lab/ 2 *Fatima Mustafa*
3rd class / Dentistry *Sura Mustafa & Renan Ibrahim*

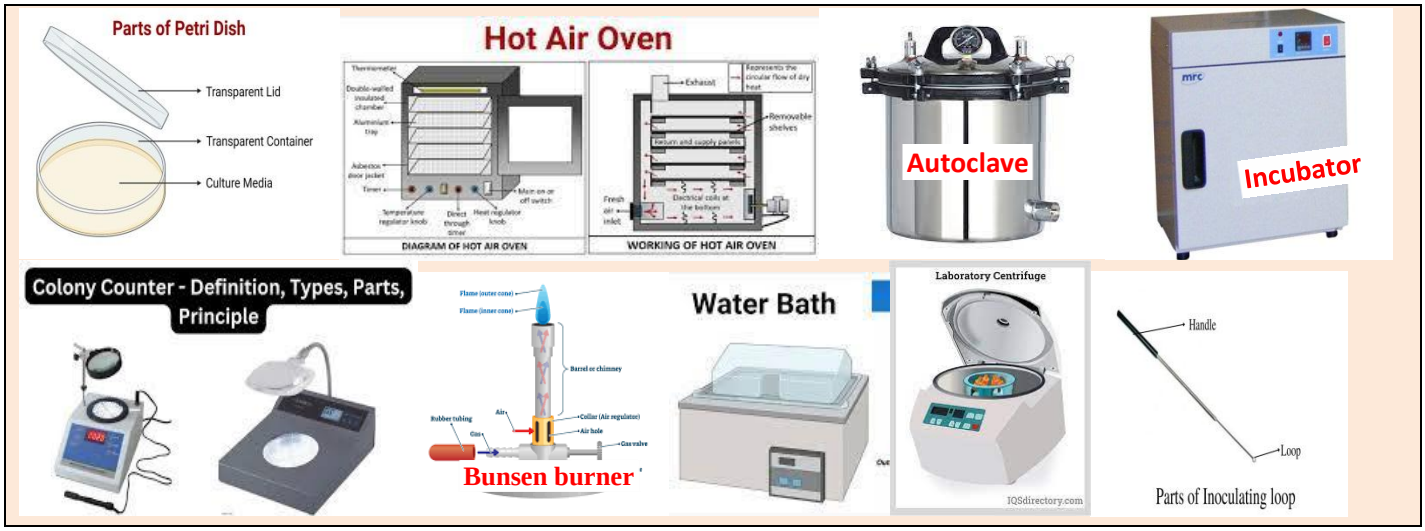
Equipment and Tools in Microbiology Laboratory

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.

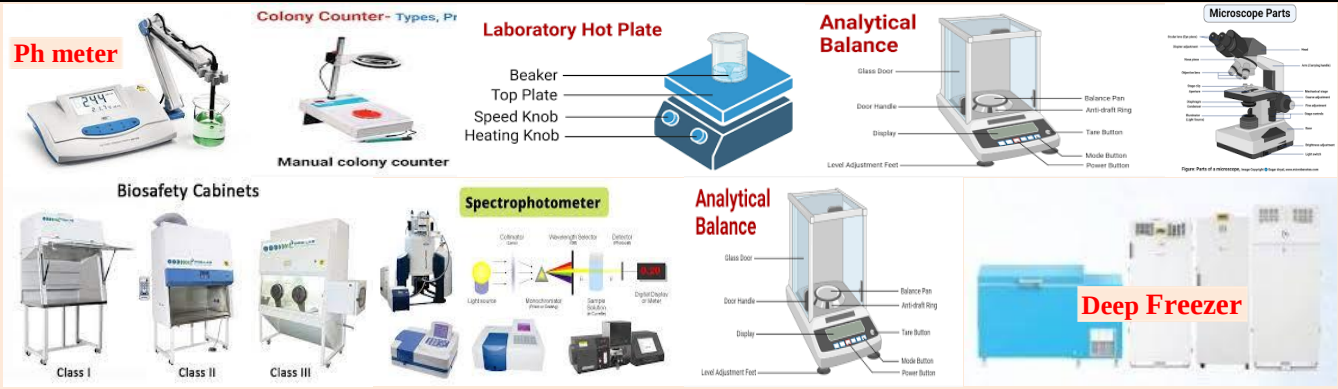
- **Petri dish** : is a flat dish made of plastic or glass with a cover that is used to grow microorganisms
- **Incubator**: is a warm cabinet that you can set it's temperature to a proper for microbes growth. As most of the microbes pathogenic to man grow profusely at body temperature of normal human 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.

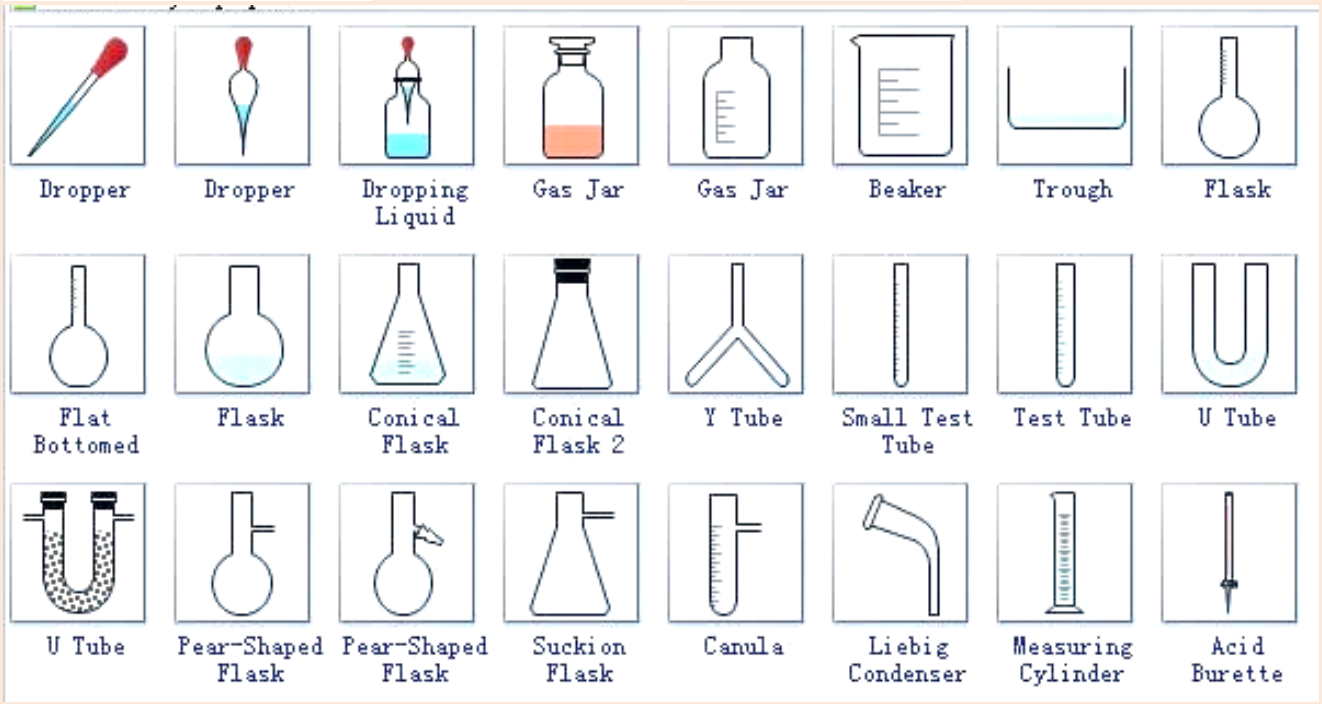
Equipment and Tools in Microbiology Laboratory

- **-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*

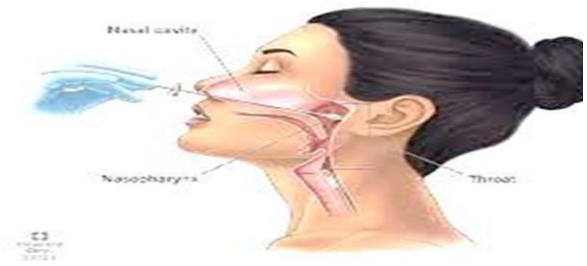
3rd class / Dentistry *Sura Mustafa & Renan Ibrahim*

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

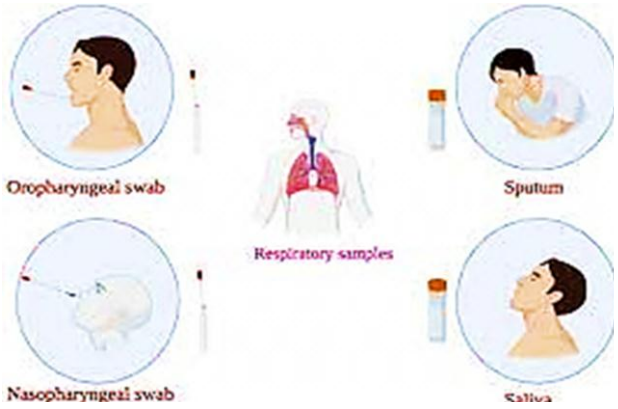
1- **Samples from Upper Respiratory Tract:**



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

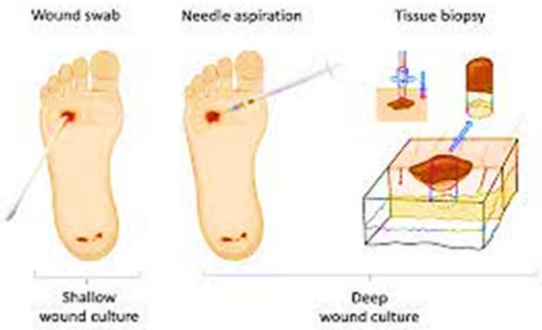
A nasopharyngeal culture is obtained by inserting a thin sterile swab gently through the nose to touch the pharynx; gently rotate and remove. A throat culture is obtained by introducing a sterile swab into the mouth.

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.



Microbiology Lab/3 *Fatima Mustafa*

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

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 **Ass. Teacher**
3rd. class / Dentistry ***Sura Mustafa & Renin Ibrahim***

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

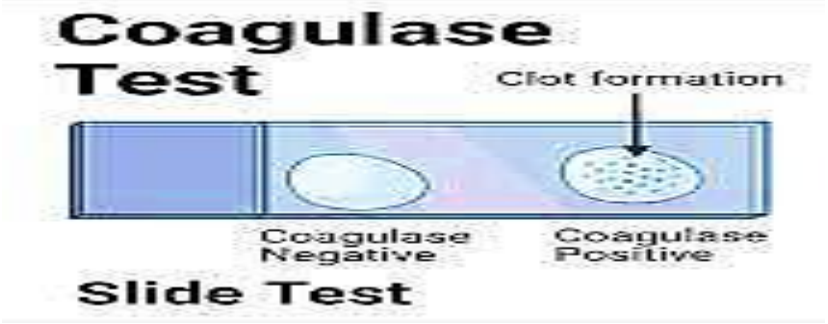
3- Based on Biochemical test

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.

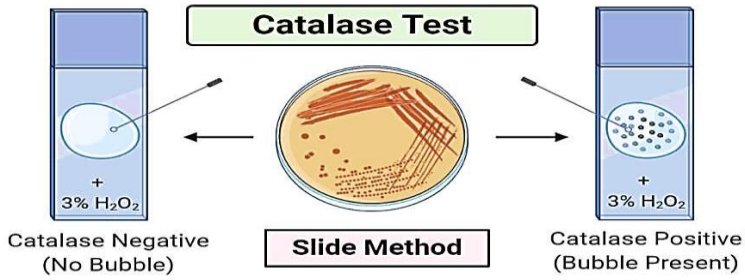


Staphylococci Identification

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

Mycobacterial Lab/6 | **Assistant teacher**
3rd class / Dentistry | ***Sura Mustafa & Renin Ibrahim***

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.

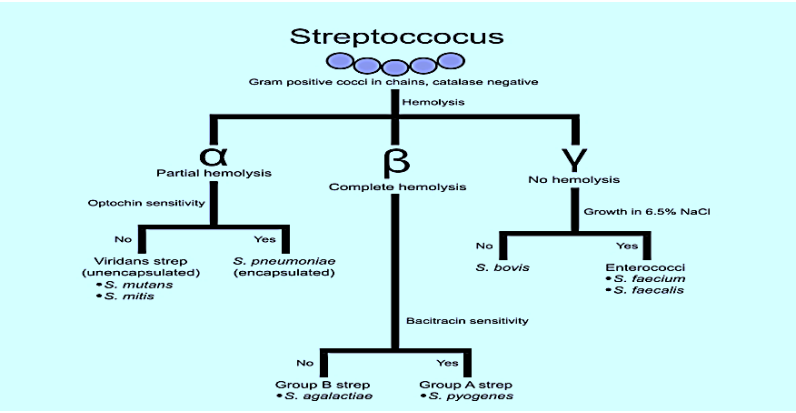
B- ***St. pneumonia:*** Its infection of lung, Otitis media. Isolated from samples such as sputum, blood, wound.

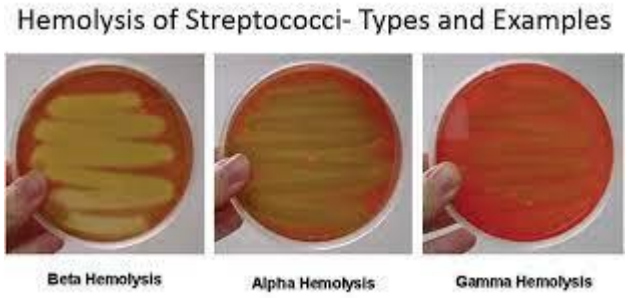
Culture: requires blood or chocolate agar. • Growth improved by 5-10% CO2. Best growth achieved temperature 25 - 40^0C. • Colonies are surrounded by greenish hemolysis, cultured on blood agar with an added optochin disk to show alpha- hemolytic colonies.

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

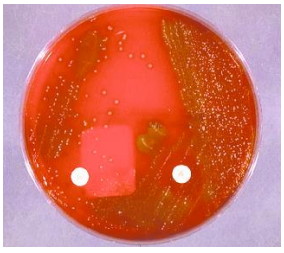
Culture: in MacConkey agar. Colonies are magenta in color and pin point. It can grow in the range of 10 to 45°C and survive at temperatures of 60°C for 30 min. It ferments glucose and does not produce a catalase.

Diagnosis plan for *Streptococci spp*





S. pneumonia



pyogenes

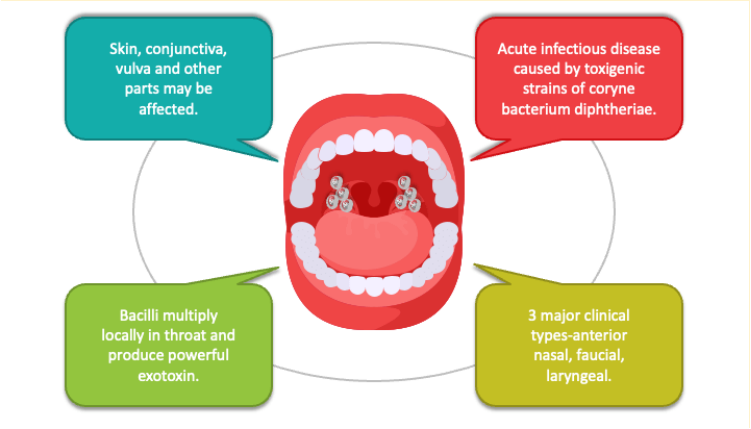
Mycobacterial Lab/7 **Assistant teacher**
3rd class / Dentistry ***Sura Mustafa & Renin Ibrahim***

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.



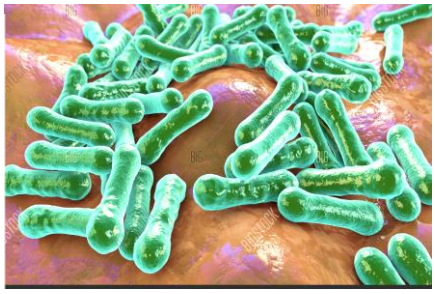
Different methods for laboratory diagnosis

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.

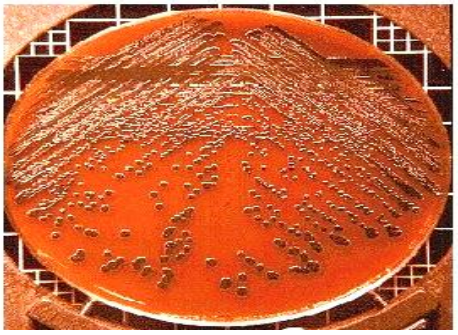
Abert´s stain Procedure

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***

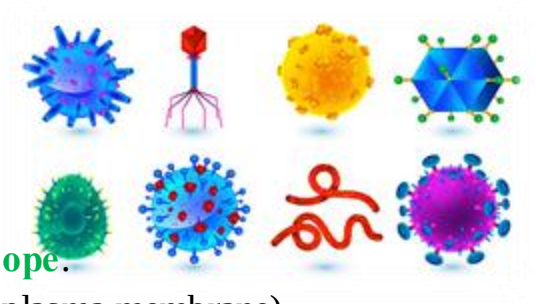
Potassium tellurite is the selective agent that turns the media brown-black as a result from the reduction of potassium tellurite to metallic tellurite. This differentiation is based on the ability of C. diphtheriae to produce black (or brown) colonies, surrounded by a brown/black halo. The dark halo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



Assistant teacher **Virology Lab**
Sura Mustafa **3rd Class /microbiology**

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

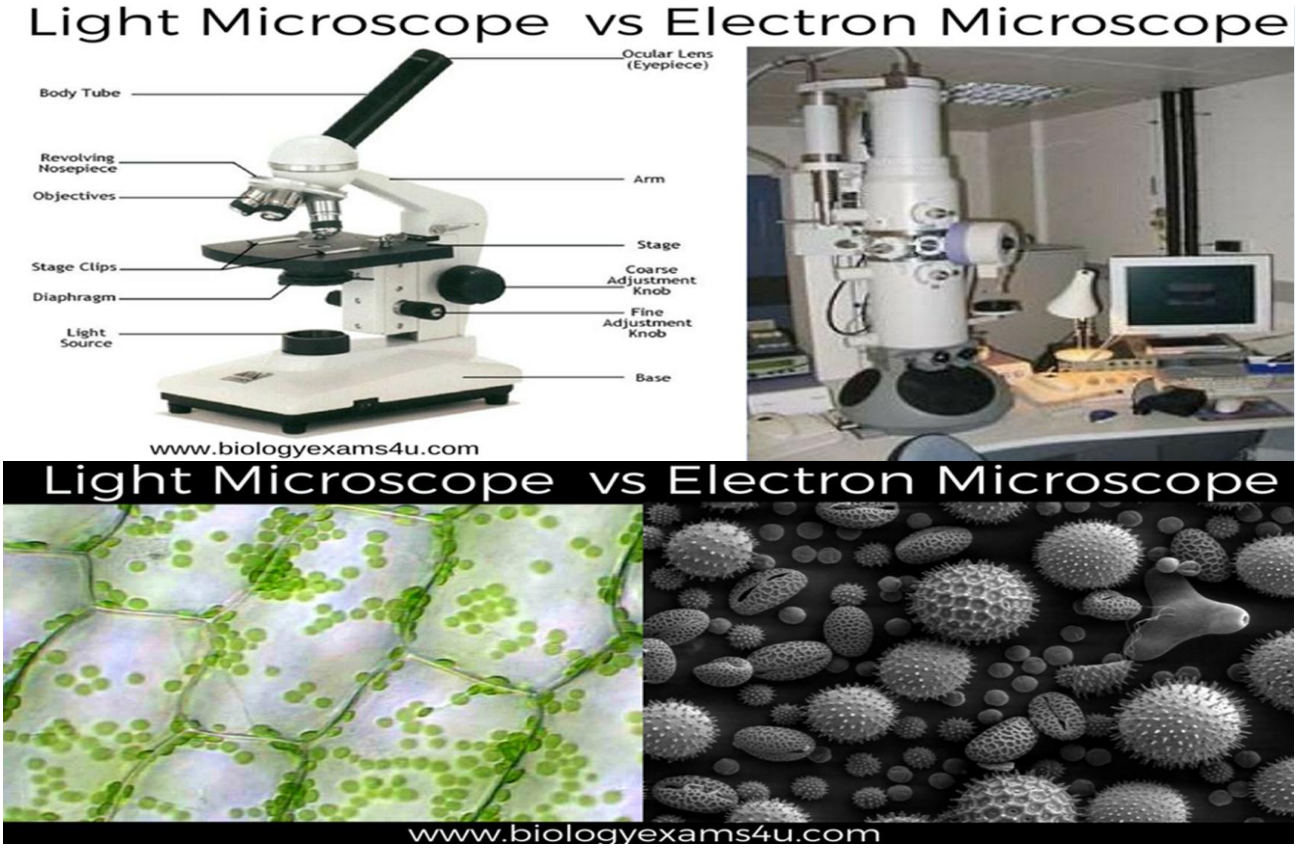
2- Scanning electron microscope (SEM) enlarge 5 to ~ 500,000 times; sharp images of surface features

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).



Microbiology Lab **Asst. Lac. *Sura Mustafa***

3rd class / Dentistry

Microscopy

Microscopy

Microscopes provide the observer with enhanced resolution including contrast (ability to detect different regions of the specimen on the basis of intensity or color) and magnification (ability to make small objects visible)

Microscope Types

1. **Compound Microscope** The image seen with this type of microscope is two dimensional.
2. **Light Microscope** (Optical Microscope)
3. **Electron Microscope**

Part of microscopy

- **Body Tube:** Connects the eyepiece to the objective lenses
- **Revolving Nose Piece**: holds the objectives and can be easily rotated to change viewing power.
- **Scanning Objective**: Lowest magnification and used to view a slide at the microscope lowest power.
- **Low objective power**: Middle magnification and used to View a slide at the microscope lowest power.
- **High objective power**: Highest magnification and used to view a slide at the microscope lowest power.
- **Stage Clips**: Holds the slides in place
- **Diaphragm:** change the intensity of the cone of light. Changes the transparency and contrast.
- **Lamp:** A steady light source
- **Eyepiece**: Lens you look through to view slides.
- **Arm:** Supports the tube and connects it to the base