Cell Structure and Function

The cell is the lowest level of structure capable of performing all the activities of life.The first cells were observed and named by Robert Hooke in 1665 from slice of cork.

Human beings are highly organized. A cell is the basic unit of life, and human beingsare multicellular since they are composed of many types of cells. Like cells form tissues, and tissues make up organs.

• All living organisms are made of cells. A cell is a small membrane enclosed structurefilled with an aqueous solution where organelles and other *subcellular* structures are found.

• Cells are of different size and shape.

• The cell's size and shape can be related to its specific function.

CELL THEORY

The cell theory, developed in the mid-nineteenth century, provided scientists with a clearer insight of the study of life. The cell theory involves the following three aspects:

1. Every living organism is composed of one or more cells.

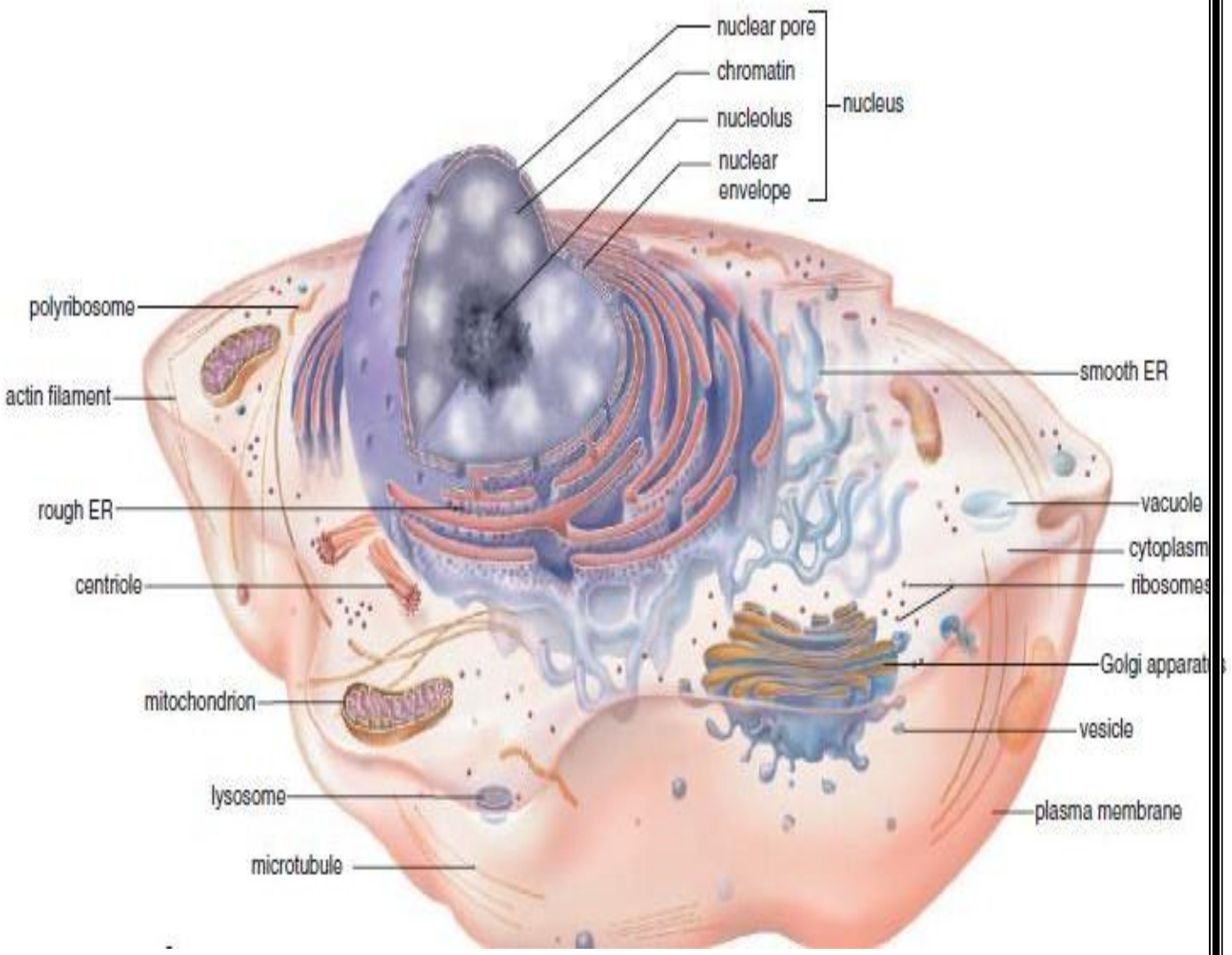
2. Cells are the smallest units that have the properties of life.

3. The continuity of life has a cellular basis.

Eukaryotic cell structure

The human cell has a central nucleus and an outer plasma membrane. Various organelles are found within the cytoplasm, the portion of the cell between the nucleusand the plasma membrane.

Plasma membrane, Nucleus, Nucleolus, Ribosome, Endoplasmic reticulum {RoughER, Smooth ER}, Golgi apparatus, Mitochondria, Lysosomes, Vacuoles, Vesicles, Cilia and flagella, and Centriole



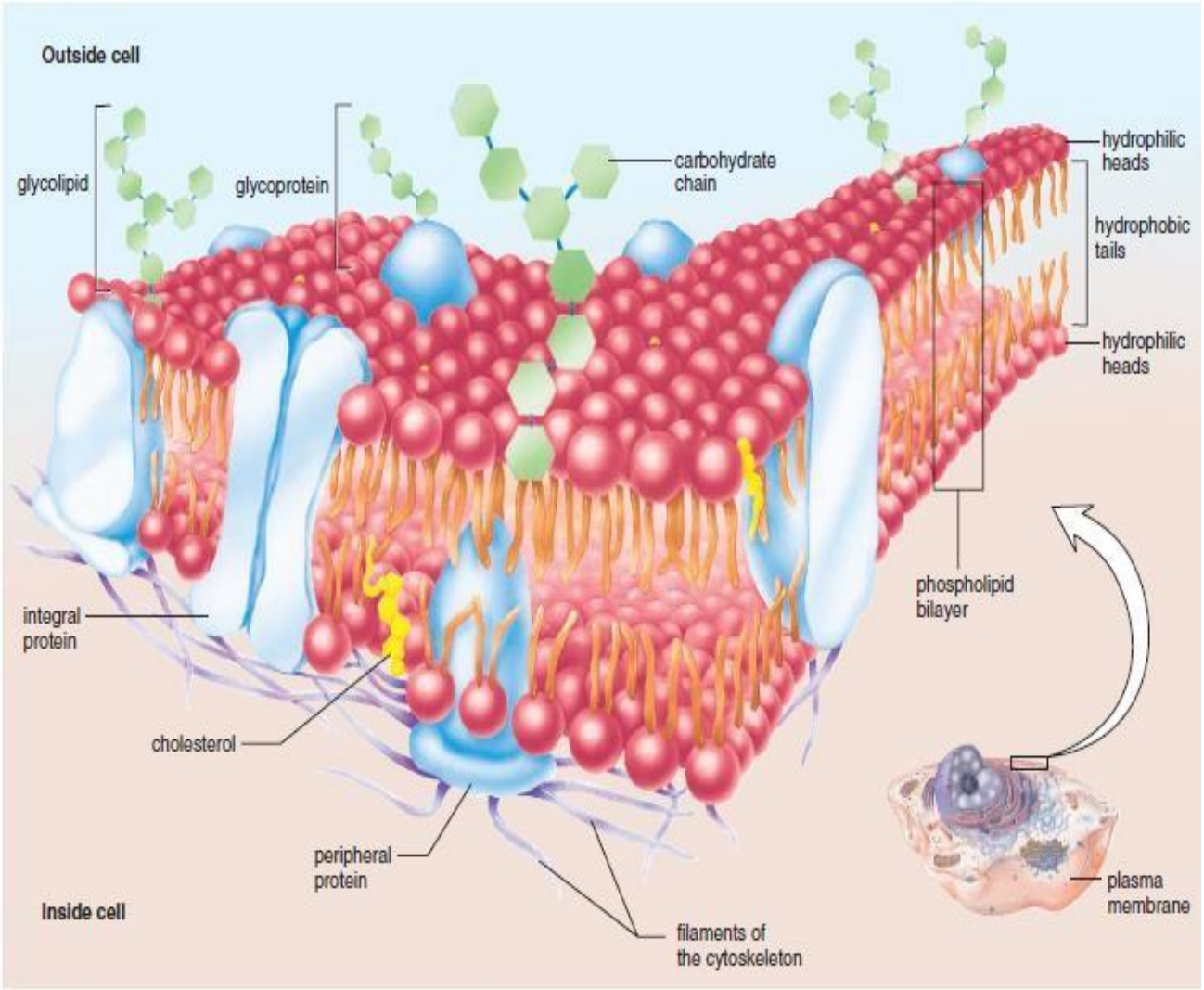
Eukaryotic cell

Table 3.1 Structures in Animal Cells

Name	Composition	Function
Plasma membrane	Phospholipid bilayer with embedded proteins	Selective passage of molecules into and out of cell
Nucleus	Nuclear envelope surrounding nucleoplasm, chromatin, and nucleolus	Storage of genetic information
Nucleolus	Concentrated area of chromatin, RNA, and proteins	Ribosomal formation
Ribosome	Protein and RNA in two subunits	Protein synthesis
Endoplasmic reticulum (ER)	Membranous saccules and canals	Synthesis and/or modification of proteins and other substances, and transport by vesicle formation
Rough ER	Studded with ribosomes	Protein synthesis
Smooth ER	Having no ribosomes	Various; lipid synthesis in some cells
Golgi apparatus	Stack of membranous saccules	Processing, packaging, and distributing molecules
Vacuole and vesicle	Membranous sacs	Storage and transport of substances
Lysosome	Membranous vesicle containing digestive enzymes	Intracellular digestion
Mitochondrion	Inner membrane (cristae) within outer membrane	Cellular respiration

Plasma Membrane Functions

The plasma membrane keeps a cell intact. It allows only certain molecules and ions to enter and exit the cytoplasm freely; therefore, the plasma membrane is said to be **selectively permeable.** Small molecules that are lipid soluble, such as oxygen and carbon dioxide, can pass through the membrane easily. Certain other small molecules, like water, are not lipid soluble, but they still freely cross the membrane. Still other molecules and ions require the use of a carrier to enter a cell.



Plasma membrane

CELLULAR TRANSPORT MECHANISMS

Mechanism	Definition	Example in the Body
Diffusion	Movement of molecules from an area of greater concentration to an area of lesser concentration.	Exchange of gases in the lungs or body tissues.
Osmosis	The diffusion of water.	Absorption of water by the small intestine or kidneys.
Facilitated diffusion	Carrier and transporter enzymes move molecules across cell membranes.	Intake of glucose by most cells.
Active transport	Movement of molecules from an area of lesser concentration to an area of greater concentration (requires ATP).	Absorption of amino acids and glucose from food by the cells of the small intestine. Sodium and potassium pumps in muscle and nerve cells.
Filtration	Movement of water and dissolved substances from an area of higher pressure to an area of lower pressure (blood pressure).	Formation of tissue fluid; the first step in the formation of urine.
Phagocytosis	A moving cell engulfs something.	White blood cells engulf bacteria.
Pinocytosis	A stationary cell engulfs something.	Cells of the kidney tubules reabsorb small proteins.

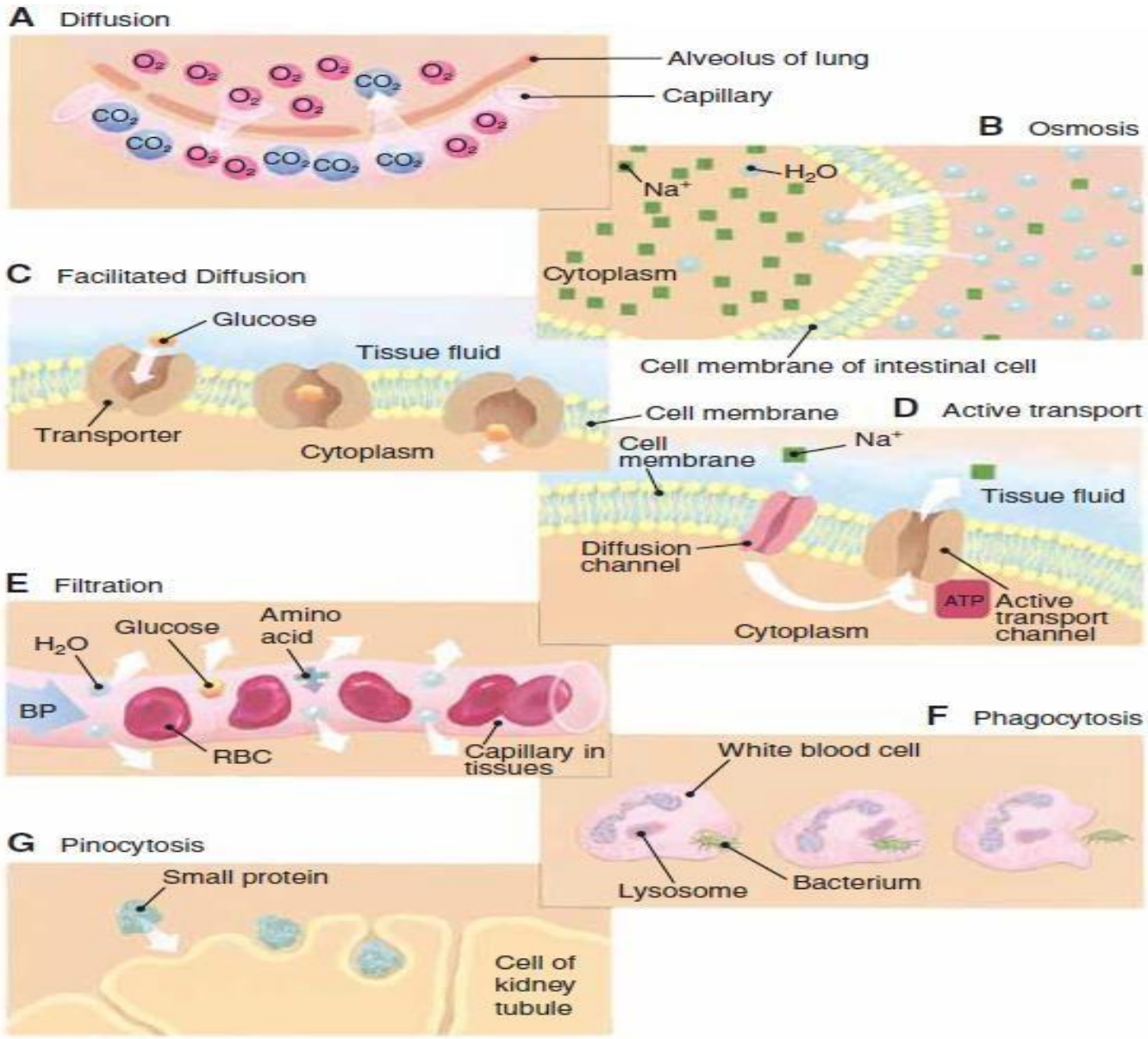


Figure 3–3. Cellular transport mechanisms. (**A**) Diffusion in an alveolus in the lung. (**B**) Osmosis in the small intestine. (**C**) Facilitated diffusion in a muscle cell. (**D**) Active transport in a muscle cell. (**E**) Filtration in a capillary. (**F**) Phagocytosis by a white blood cell. (**G**) Pinocytosis by a cell of the kidney tubules. See text for description.

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Diagnostic bacteriology

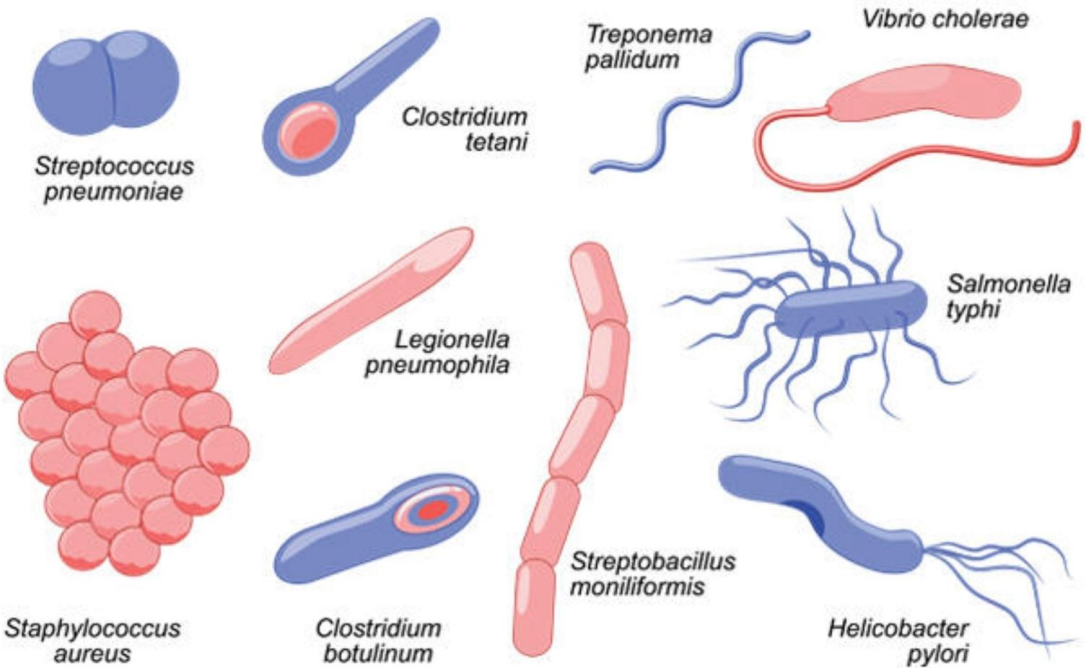
Stage : 4 st

BACTERIA

Bacteria are microbes with a cell structure simpler than that of many other organisms. Their control centre, containing the genetic information, is contained in a single loop of DNA. Some bacteria have an extra circle of genetic material called a plasmid rather than a nucleus. The plasmid often contains genes that give the bacterium some advantage over other bacteria. For example it may contain a gene that makes the bacterium resistant to a certain antibiotic.

Bacteria are classified into five groups according to their basic shapes: spherical (cocci), rod (bacilli), spiral (spirilla), comma (vibrios) or corkscrew (spirochaetes). They can exist as single cells, in pairs, chains or clusters.

Bacterial shapes



Bacteria are found in every habitat on Earth: soil, rock, oceans and even arctic snow. Some live in or on other organisms including plants and animals including humans. There are approximately 10 times as many bacterial cells as human cells in the human body. A lot of these bacterial cells are found lining the digestive system. Some bacteria live in the soil or on dead plant matter where they play an important role in the cycling of nutrients. Some types cause food spoilage and crop damage but others are incredibly useful in the production of fermented foods such as yoghurt and soy sauce. Relatively few bacteria are parasites or pathogens that cause disease in animals and plants.

Main Difference - Gram Positive

vs Gram Negative Bacteria

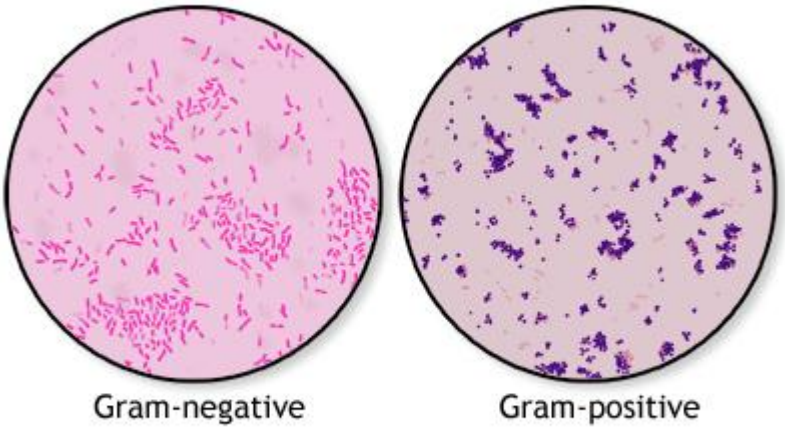
- Gram positive and gram negative bacteria are the two types of bacteria, differentiated by the gram staining technique. Gram staining was developed by Cristian Gram in 1884.
- The stain used during the technique is crystal violet. Gram positive bacteria are more susceptible to antibiotics due to the lack of an outer membrane. Since gram negative bacteria contain an outer membrane, they are less susceptible to antibiotics.Therefore, gram negative bacteria are more pathogenic compared to gram positive bacteria.
- The main difference between gram positive and gram negative bacteria is that gram positive bacteria contain a thick peptidoglycan cell wall along with teichoic acid, allowing the bacteria to stain in purple during gram staining whereas gram negative bacteria contain a thin peptidoglycan cell wall with no teichoic acid, allowing the cell wall to stain in pink during counter staining.
- **example of gram negative bacteria**

  Salmonella, Shigella, and other Enterobacteriaceae,

  Pseudomonas,Moraxella, Helicobacte

- **examples of Gram positive bacteria**

  staphylococci (Staphylococcus aureus, S. epidermidis,)streptococci (Streptococcus pyogenes, S. pneumoniae, etc.) enterococci.



ADAM.

Gram staining:

In conclusion, the gram – positive bacteria appear violet, while gram – negative bacteria are red in color.

Therefore, it is possible to differentiate between bacteria of the same morphology.

Furthermore, it can be used to determine the relative number and morphology of bacteria in smear.

Gram staining method:

1- Prepare the bacterial smear.

2- Flood the slide with crystal violet leave to all for 1-2 min. Wash with tap water.

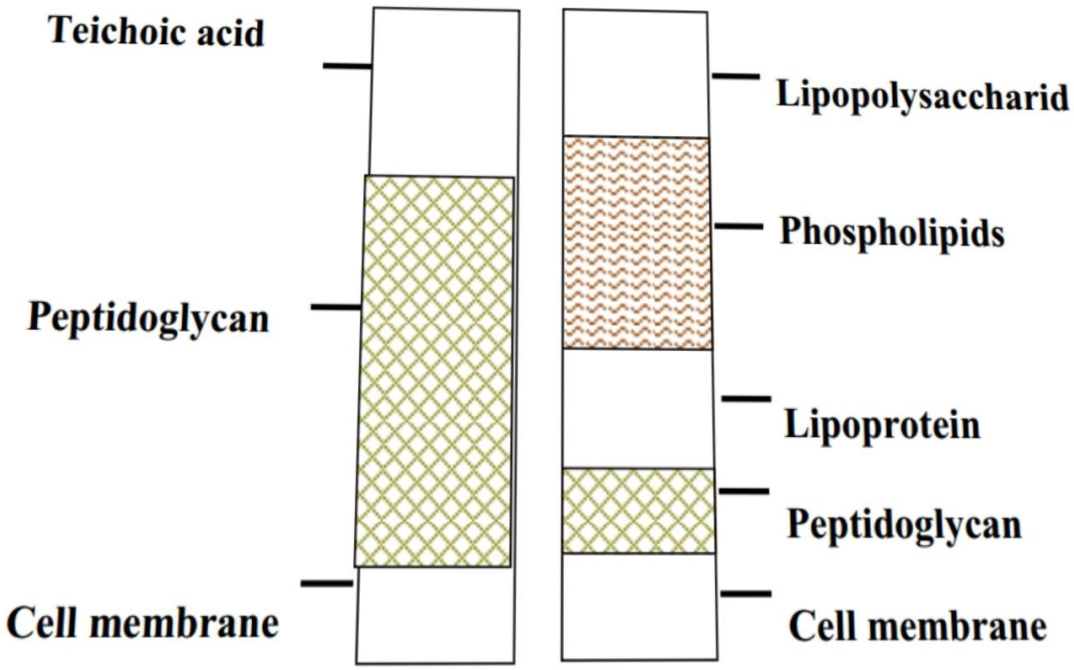
3- Apply gram's iodine (lugol's iodine) . Leave to all one minute. Wash with tap water.

4- Apply 95% ethyl alcohol (a decolorizer). Leave to all for 20-30 seconds. Wash with tap water.

5- Apply diluted carbol – fuchsine (the counter stain) leave to all for 1 min. wash with tap water.

6- Blot, dry in air and examine with oil immersion lens.

Cell wall permeability theory: it suggests that in gram +ve bacteria when you add the crystal violet immediately the pores in the cell wall will be locked while in gram -ve it remain opened. Why? Because of the chemical composition of cell wall are different between gram +ve and gram -ve .



Culture Media

Culture or growth media: an artificial media contains basic requirements needed for micro organisms' growth. Used for recognition and

identification of microorganisms. Then the media which dissolved in the flask must be sterilized by heat (i.e. autoclave) are contained either in test tubes , plates (Petri-dishes).

A. Types of culture media:

Culture media can be divided according to:

A) Physical state (consistency) of media to:

1. Liquid (fluid) media: used for primary cultivation. Its disadvantage is the inability to obtain colony morphology, e.g., nutrient broth, peptone water, brain heart infusion broth.

2. Solid media: the concentration of agar is 1.5-2% used for identification of colony morphology e.g. nutrient agar, MacConkey's agar, Blood agar …etc.

Blood agar if heated slowlyfor about 80C° it will yields **Chocolate agar**.

3. Semisolid media: used for cultivation of spirochetes and to study motility such as (SIM) contain 0.4 -0.8% of agar.

B) Uses of media:

-**Simple media**: this media contains only common ingredients used for cultivation of non-fastidious microorganism. Such as nutrient agar and nutrient broth.

- **Special purpose media**: these media contain common ingredients plus addition another type material There are seven types of this media include:

- **Enriched media**: simple media enriched with appropriate substances, e.g. blood 5-10%, glucose 1-2% serum fluid 10%.
E.g. Blood agar and Chocolate agar.
- **Selective media**: containing inhibitory substance (e.g. bile salts, antibiotic, dyes … etc.) which favor the growth of the concerned microorganism and inhibit the growth of the others. E.g. MacConkey agar and Mannitol salt agar.
- **Differential media**: certain bacterial species produce characteristic growth then can easily recognize or can produce certain effects in the medium. e.g. MacConkey agar which regarde as both selective (because bile salt inhibit gram +ve bacteria and let gram -ve bacteria to gro and differential (because it contains lactose which could differential between gram-ve bacte as two groups lactose fermentor and non fermentor .
- **Enrichment media**: this media allow the growth and Enrichment for one type of bacteria (fastidious one) and inhibits other (by competition on food) e.g. selenite f – broth.
- **Transport media**: certain microorganism is weak and dies rapidly between the times of taken of the specimens and examination so it needs a special media for transport. E.g. Stuart media.
- **Indicator media**: this is use the visual change in the color of an indicator due to

microorganism metabolism as a diagnosis feature e.g. sugar media.

• **Sensitivity media**: a special media used to tested antibiotic sensitivity for given microorganism e.g. Muller Hinton media.

Enriched media: such as blood agar and chocolate agar which ore supplement with blood, one routinely used to culture pathogenic bacteria from clinical samples.

Blood agar: contains a basal medium and 5-10% sheep, horse or rabbit blood. Blood agar is also useful as differential agar according to blood cell hemolytic.

There are 3 types of hemolysis:

1. Beta hemolysis (β- hemolysis): complete lysis or destruction of red blood cells resulting in clear area around colonies. This haemolysis is displayed by Streptococcus pyogenes .

2. Alpha hemolysis (α- hemolysis): partial lysis of RBC, resulting in greenish discoloration around colonies this type is demonstrated by Streptococcus pneumonia.

3. Gamma – haemolysis (γ-haemolysis): not lyse RBCs this type is typical of Enterococcus faecalis .

Preparation of culture media

1. Count how many petri dishes or tube you need.
2. Each petri dish need 15-20 ml o medium.
3. Multiply the number of petri with the number of ml you pour it into petri, ex. You need 10 petri dishes:

10 × 15 (20) = 150 ml the volume you need to prepare

4. Then read the number of gram dissolve 28 gram in 1 letter on culture media bottle. ex. For nutrient agar preparation dissolve 28 gram in 1 letter (1000) ml.
5. Calculate the number of gram from this formula

$$\frac{\text{Gram}}{\text{ml}} = \frac{\text{Gram}}{\text{ml}}$$

$$\frac{28}{1000} = \frac{x}{150}$$

$$X = 28*150$$

1000

X= 4.2 gram

6. Weight 4.2 gram by using electronic balance, the dissolved it in 150 ml distilled water, on ho plate and mixing it until homogenate solution formed.
7. Sterilized it by using autoclave for 15 min. at 121 C.
8. After sterilization, cool it and pour into petri dishes.

Sterilization is a process in which all living microorganisms, including spore, are destroyed. The organism may be killed with steam, dry heat, or incineration. If we say an article is sterile, we understand that it is completely free of all living microorganisms.

Disinfection is a process in which vegetative, non sporing microorganisms are destroyed. Agents that caus disinfection are called **disinfectants** or **germicides.** Such agents are used only on inanimate objects because they are toxic to human and animal tissues.

Sepsis is defined as the growth (multiplication) of microorganisms in tissues of the body.

Asepsis refers to any procedure that prevents the entrance of infectious agents into sterile tissues, thus preventing infection.

Antiseptics are chemical agents (often dilute disinfectants) that can be safely applied externally to human tissues to destroy or inhibit vegetative bacteria.

Methods of sterilization	substances sterilized
Dry heat	
Flaming	**inoculating wires, loops**
Hot air oven	**glassware, oils, powder, paraffin**
Incineration	**biohazardous material- used gloves, needles, etc.**

Moist heat	
Pasteurization	serum, milk
Boiling	needles, glass syringes
Tantalization	sugar solution
Autoclave	heat stable media such as nutrient agar
Filtration	heat labile media, serum, antibiotics, etc

Antiseptics	Disinfectants
70% ethyle alcohole	Lysol
Chlorhexidine	Zephiran
Iodine	Glutaraldehyde
Dettol	Sodium hypochlorite

Cultivation of Bacteria

-**Colony**: A macroscopically visible growth of microorganisms on solid culture medium.

-**Culture**: Any growth, population or cultivation of microorganism.

-**Subculture**: the streaking of isolated colonies of differential media to obtain pure culture.

-**Stock culture**: species or strains of mo. (pure culture), known a maintained in the laboratory for various test and study.

Methods of cultivation:

A - Cultivation of bacteria in fluid media (broth).

- Transfer of a bacterial colony on a plate culture (solid medium in Petri-dish) to a broth (fluid medium in a tube).

1- Take up the inoculating loop by the hand and hold it as you would a pencil, loop down, hold the wire in the flame of the Bunsen burner until it glows red, remove loop from flame and hold it quietly a few moments until cool. Do not put it down or touch it to anything.

2- Hold the sterile , cooling loop in one hand and with the other hand turn the plate .culture with ottom part of the dish up , so that you can clearly see isolated colonies growing on the surface of the

plated agar when we remove the cover partly .

3- With sterile cool loop scrap a small surface area of bacteria colony gently and remove a loop full of culture, with draw the loop and replace the cover of the Petri – dish and put the dish on the table.

4-still holding the loop like pencil but more horizontally in your right hand , use the little finger of the loop hand to remove the closure (cottonplug slip or screw cap) of he culture tube (broth tube) , keep your little finger curled around this closure when it is free do not place it on the table .

5-passing the neck of the open tube rapidly through the Bunsen flame 2-3times (don't over – heat, if it glass, it could crack, if it is plastic it could melt) this flaming sterilize the air in and immediately around the month of the tube.

-insert the loop into the open tube and inoculate a sterile broth with the charged loop gently rubs the loop against the wall of the tube.

7-with draw the loop slowly, being careful not to touch it to the month of the tube and do not touch it to anything, replace the tube closure and put the tube back in the rack .

8-Now carefully flam the loop until it glows red (Be sure all the wire is sterilized), when the wire has cooled the loop can be placed on the bench top.

9-lable the tube you have just inoculated with name of the provided organisms and the date.

10-incubate the tube at 37c for 24 hour.

B- Cultivation of bacteria on solid media.

1-) streaking method:

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

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

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

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

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

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

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

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

8-Incubate the plate (inverted) at 37C for 24 hours.

2-) Streaking and stabbing on slant solid media.

1- Flaming the loop with straight wire.

2-By the sterile cooling loop take a small part of provided culture.

3- Use the other hand to pick up a tube of sterile slant media and remove the tube closure with the little finger of the loop hand.

4-flam the neck of the tube inserts the charged loop into bottom of the tube and make stab down then withdraw the loop and streak the slant surface of the media.

5-with draw the loop out tube closure and return the tube to the neck.

6-flame until the loop wire glows red and when the wire has cooled put the loop down.

7- Label the tube with name of the provided organism and the date.

8- Incubate the slant tube at 37C for 24-48 hr.

C-) Cultivation of semi – solid media:

Semi – solid media cultivated by stabbing technique:

1- Flaming the straight wire of the loop.

2- Take by cooled loop a small part of provided culture to inoculate it in a tube of semi- solid medium as SIM or gelatin medium making a single stab down the center of the tube to about half the depth of the medium.

3- Incubate at 37c for 24 hr.

Cultural characteristics:

To study the macroscopic characteristics of pure culture on a solid medium, the following points have to be considered in describing a colony:

-**Size:** measured in mm.

-**Shape**: spindle, circular, filamentous irregular.

-**Elevation**: flat, raised, convex, umbonate .

-**Margin**: entire, lobate .

-**Consistency**: dry, mucoid .

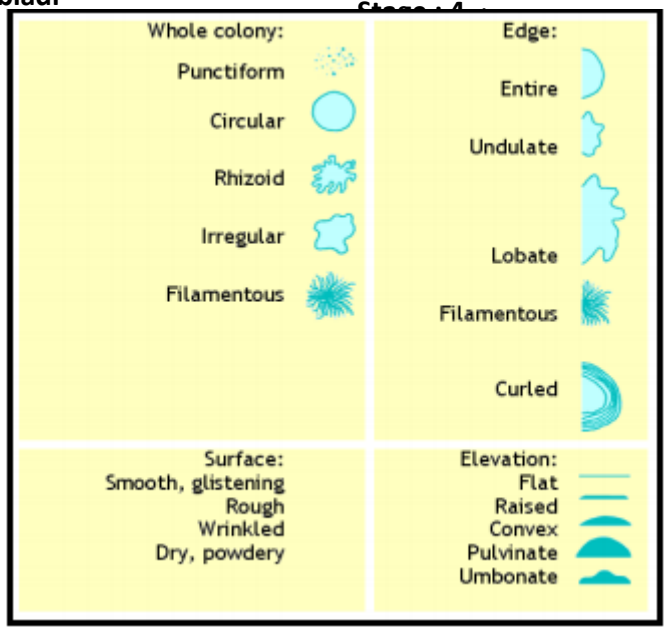
-**Surface texture**: smooth, rough.

-**Color or pigmentation**: yellow, green.

-**Optical density**: opaque, transparent, translucent.

-**odour**: bad , musty .

-Changes in the inoculated media**(hemolysis)** .



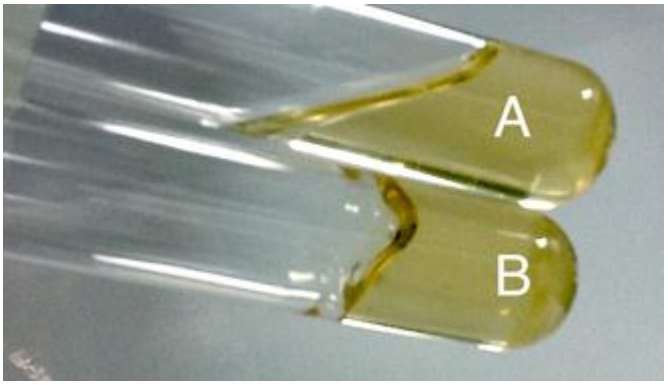
Biochemical test

Gelatin hydrolysis (gelatinase test) :

- Medium used: Nutrient broth + 12-15% gelatin.
- Inoculation: by stabbing, and incubation at 37c ̊for 24-48hours.
- Result: liquefaction of gelatin after exposure of the inoculated medium to refrigeration temperature, i.e. the semi solid medium becomes liquefied in positive cases due to the action of gelatinase enzyme produced by the bacteria.

Geletine→ **geletinase** → polypeptides→ **polypeptidase** →Amino acids.

E.g. *E. coli* is negative, *staphylococcus aureus* is positive.



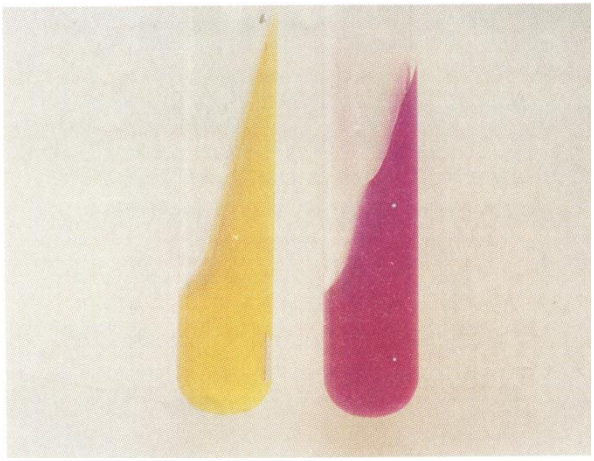
Production of ammonia from urea (Urease test)

- Medium: urea broth or urea agar containing phenol red indicator
- Inoculation: as for broth media, incubation for 24 - 48 hr. at 37c.̊

Result: change of color yellow to pink in +ve cases due to the hydrolysis of urea to ammonia by the action of urease enzyme produced by the bacteria and the medium because alkaline affecting PH of the indicator urea urease → 2NH3(Ammonia) + CO2+ H2O

In – ve cases the color of the medium not change.

e.g. *E. coli* is ureases –ve , *proteous spp* + ve



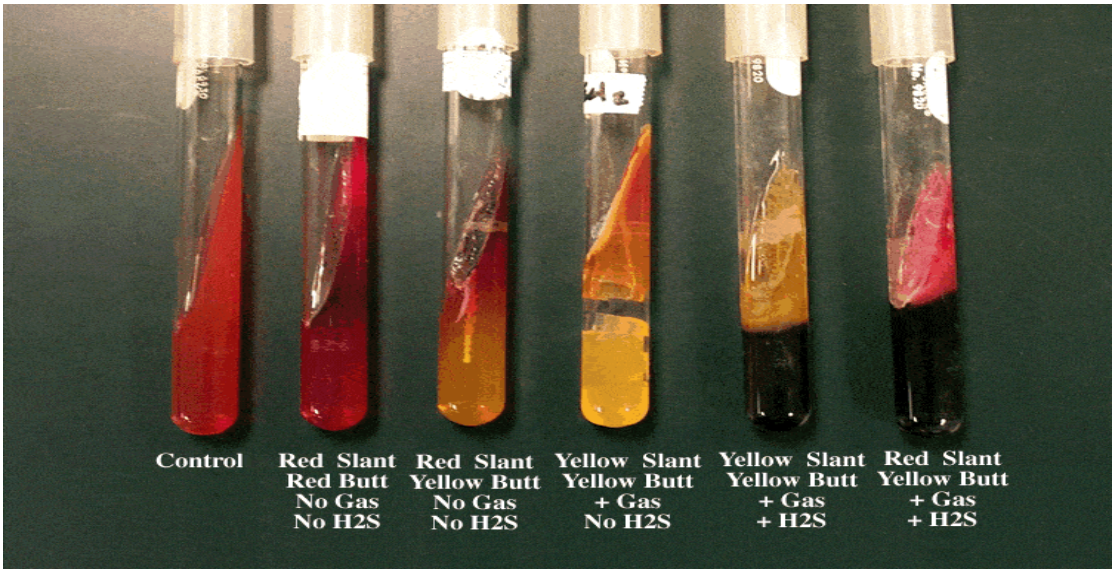
Triple sugar iron (TSI) test.

- Aim: to demonstrate fermentation of sugars and production of H2s and gas.
- Medium: consist of 0.1% glucose, 0.1% lactose, 1% sucrose, phenol red indicator, peptone, ammonium thiosulfate and ferrous sulfate, made as slant and button.
- Inoculation: by stabbing the bottom followed by streaking of the slant incubation overnight at 37c ̊.
- Result: read the result as slant / button.

1-Acid (A)/Acid (A) or Yellow (Y)/Yellow (Y) with or without gas (CO2 and H2S) due to fermentation of glucose with lactose (or sucrose).
e.g . *E. coli.*
2-Alkaline (K)/ Acid (A) or pink (p)/Yellow (A) with or without gas (CO2 and H2) due to fermentation of glucose only. e.g. *Shigella sounnei* .
3-Alkaline k/Acid (A) or pink (P/Y)+H2O , and sometimes CO2 and H2 due to fermentation of glucose only and a black precipitate formation from H2S .e.g *Salmonella paratyphi* .
4-Alkaline (K)/Alkaline (K) (P/P) here no sugar is fermented only peptones *Pseudomonas aeruginosa*.

Mechanism: pink color of the slant results from utilization of peptones by the organism and release of NH3 yielding an alkaline pH which affects the indicator (phenol red).
Yellow colour of the medium or bottom only result from the change in the color of indicator (phenol red) due to acid formation from sugar fermentation.

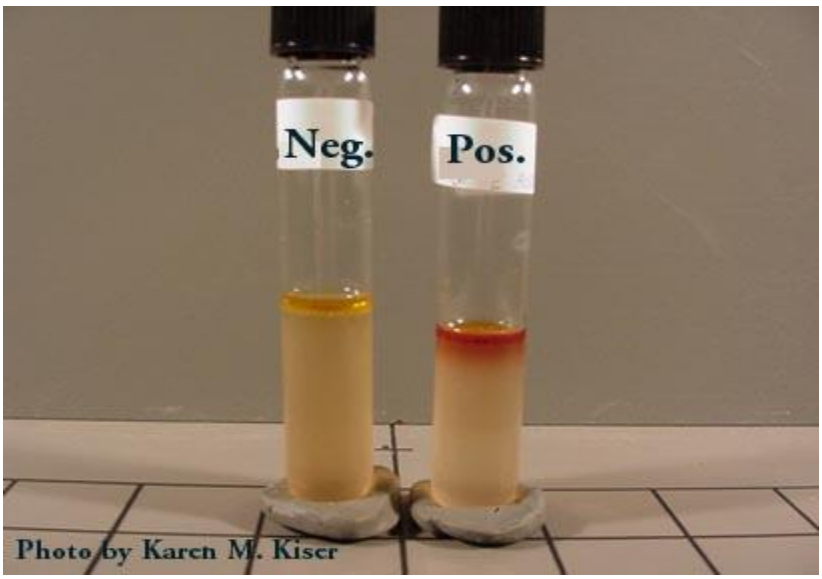
Black colour develops due to production of H2S ppt. From ammonium thiosulfate + iron salt (ferrous sulfate).



Indole production from tryptophan:

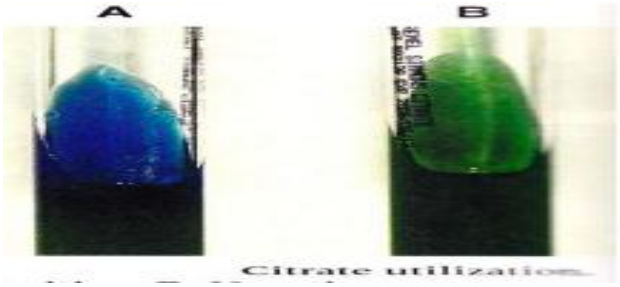
- Medium: nutrient broth containing peptone which is rich in tryptophan.
- Inoculation: as for broth cultures.
- Indicator: add kovac's reagent.
- Result: a deep red colored ring develops in the presence of indole and this is a positive result.Tryptophan→ indole + pyruvic acid + ammonia

In – ve result the ring stays yellow.e.g. *E. coli* is indole +ve , *Salmonella spp* and *Klebseilla* – ve



Citrate utilization test:

- Medium: Simon's citrate agar containing Bromothymol blue indicator
- Inoculation: streaking of the slant after stabbing of the bottom incubation at 37c° for 24 - 48 hr.
- Result: utilization of citrate as the source of carbon for energy and growth of the organism.Growth of organism on the citrate agar result in an alkaline reaction which makes the bromothymol blue change from green to blue color in positive cases, in negative cases there is no change in color of the medium and no growth of the organism. e.g *E. coli* is– ve Salmonella spp + ve .



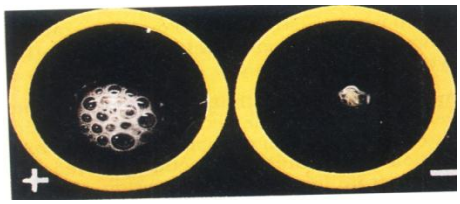
Catalase test:

Aim: to detect the production of catalase enzyme by the microorganism

growing aerobicall.

Reagent (indicator): 3% hydrogen peroxide (H2O2.(Result: a loopful of bacteria growth is emulsified with a loopful of H2O2 on clean slide, the production of Effervescence or foam causal by liberation of free O2 as gas bubbles indicates the presence of catalase.

2H2O2 → catalase → 2H2O + O2 E.g. *Staphylococcus spp* + ve



Streptococcus spp – ve .

Oxidase test:

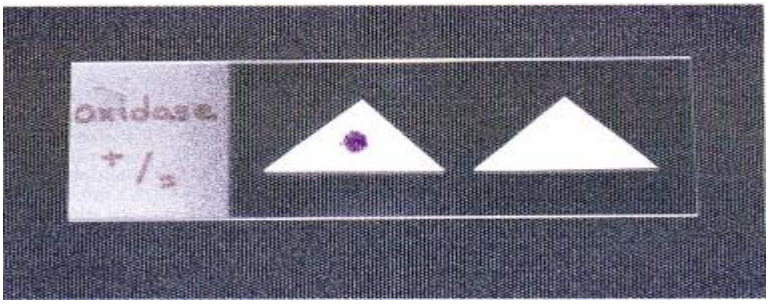
Aim: to detect production of oxidase enzyme by bacteria.

Reagent: 1% a queous solution of tetramethyl – p- phenylene diamine– HCL.

Result: few drops of the reagent are added to a filter paper and using wooden stick or glass rod, some of the bacterial growth is transferred to the impregnated filter paper; a purple coloration is produced within 5 -10 sec indicating the presence of oxidase enzyme. Delayed + ve result may appear with in 10-60 sec. but more than that (or a colorless result) is – ve.

E.g. *Staphylococcus spp*. And Enterobacteriacae spp. and *E.coli* – ve.

Streptococcus spp , *Vibrio* and *pseudomonas spp* + ve .

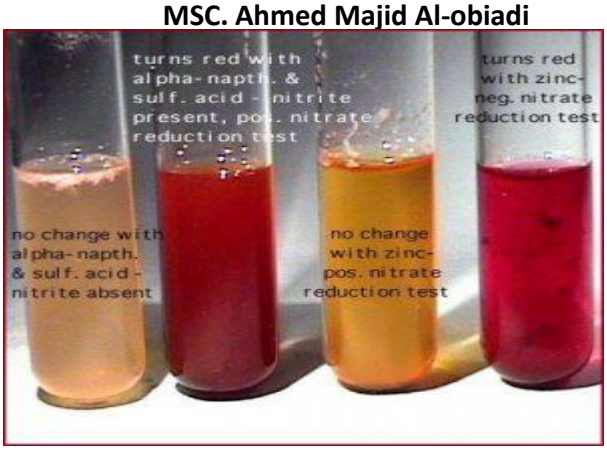


Nitrate reduction test:

- Medium: nitrate peptone water.
- Inoculation: as for broth medium incubation at 37c ̊for 18-24hr.
- Indicator (reagent): add α - naphthylamin and sulfanilic acid .
- result: red color develops within few minutes in + ve cases (NO3 reduced to NO2), if no red color develops, small quantity of zinc dust are added the development of a red colour indicates – ve result because the reduction occur by Zink dust and not by bacteria, but if no red colour develops (grey or brown colour) this indicate that the bacteria is reduce the NO3 to NO2 to NH3 to N2 and the result considered + ve.

e.g. *E. coli* is nitrate + ve ,

Clostridium botulinum – ve .



Methyl red / Voges proskauer (MR/ VP) test.

Medium: glucose phosphate broth.

Inoculation: as for broth media incubation at 37c ̊ for 2-7 days.

Reagent (indicator): methyl red added for MR test.

α-naphthol and 40% KOH or NaOH added for VP test.

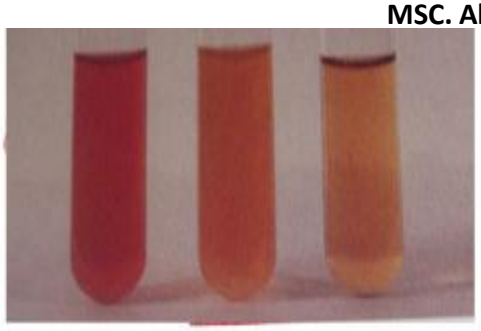
Result: the inoculated broth is divided into 2 portions, in the MR part, a red color indicates acidic pH (4.5 or less) and positive result, while yellow color is negative. In + ve cases, glucose is fermented and end products of fermentation are acids. e.g. lactic acid and butyric acid.

In the VP part development of a red color within 5 minute, constitutes a + ve reaction due to formation of Acetyl methyl carbinol (Aceton)(from glucose fermentation. Yellow color appearance is – ve.

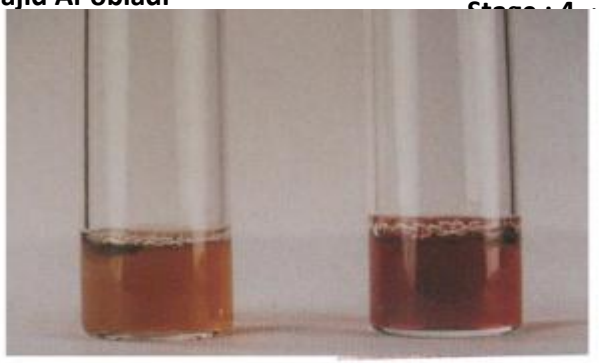
e.g. *E. coli* is MR+ / VP –

Klebsiella spp is MR -/VP +

Note: some organisms may have both tests – ve, but very rare and not find organisms with both tests + ve.



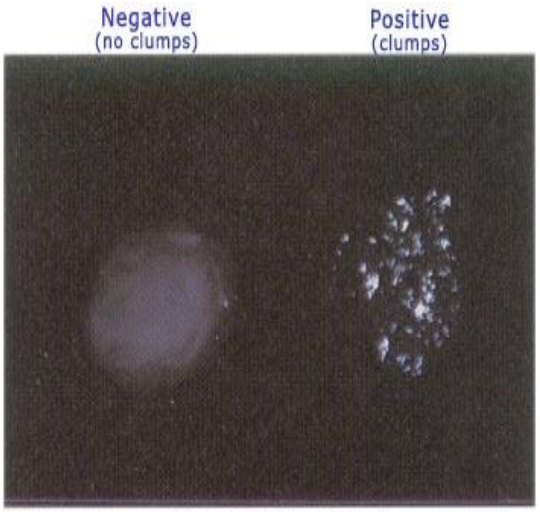
Methyl red test results in MR-VP medium tubes. The inoculated tubes received five drops of methyl red after incubation. Left: *Escherichia coli* (methyl-red-positive); center: *Enterobacter aerogenes* (methyl-red-negative); right: uninoculated



Results of the Voges-Proskauer test in MR-VP tubes. Tubes have received six drops of Barritt's Reagent A and two drops of Barritt's Reagent B. *Escherichia coli* (left) is VP negative, and *Enterobacter aerogenes* (right) is VP positive.

Coagulase Test :

Zero point one ml of 18 hrs. culture broth was added to 0.1 ml of human plasma without dilution and incubated at 37 ºC for 4 hrs. the clotting hourly noticed. The appearance of the clotting indicates a positive result



Slide Coagulase Test

Blood stream Infections

Invasion of the bloodstream by microorganisms constitutes one of the most serious situations in infectious disease. Microorganisms present in the circulating blood—whether continuously, intermittently, or transiently—are a threat to every organ in the body. The suffix emia is derived from the Greek word meaning "blood" and refers to the presence of a substance in the blood; bacteremia refers to the presence of bacteria in the blood, fungemia refers to the presence of fungi in the bloodstream, and septicemia indicates bacteria are present in the

blood, producing an infection and reproducing within the bloodstream. Microbial invasion of the bloodstream resulting from any organism can have serious immediate consequences, including shock, multiple organ failure, disseminated intravascular coagulation (DIC), and death. Approximately 200,000 cases of bacteremia and fungemia occur annually, with mortality rates ranging from 20% to 50%. Timely detection and identification of blood-borne pathogens are two of the most important functions of the microbiology laboratory. Pathogens of all four major groups of microbes—bacteria, fungi, viruses, and parasites—may be found circulating in blood during the course of many diseases. Positive blood cultures may help provide a clinical diagnosis, as well as a specific etiologic diagnosis.

GENERAL CONSIDERATIONS

The successful recovery of microorganisms from blood by the laboratory depends on many, often complex, factors: the type of bacteremia, the specimen collection method, the blood volume, the number and timing of blood cultures, the interpretation of results, and the type of patient population being served by the laboratory. All of these parameters must be considered in the development of the blood culture protocol within the laboratory in order to maximize the detection and recovery of microorganisms and ensure quality patient care.

ETIOLOGY

As previously mentioned, all major groups of microbes can be present in the bloodstream during the course of many diseases.

- Bacteria

The organisms most commonly isolated from blood are gram-positive cocci, including coagulase-negative staphylococci, Staphylococcus aureus, and Enterococcus spp., and other organisms likely to be inhabitants of the hospital environment that colonize the skin, oropharynx, and gastrointestinal tract of patients. Some of the most common, clinically significant bacteria isolated from blood cultures. In general, the number of fungi and coagulase-negative staphylococci Of importance, the laboratory isolation of certain bacterial species from blood can indicate the presence of an underlying, occult, or undiagnosed neoplasm. Alterations in local conditions at the site of the neoplasm allowing bacteria to proliferate and seed the bloodstream have been suggested as a potential mechanism for the association between bacteremia and cancer. Another possible mechanism is reduced killing of bacterial cells by the host phagocytes. Organisms associated with neoplastic disease include *Clostridium septicum* and other uncommonly isolated *clostridial species*, *Streptococcus galldyticus*, and *Campylobacter* spp.

Fungi

Fungemia (the presence of fungi in blood) is usually a serious condition, occurring primarily in immunosuppressed patients and in those with serious or terminal illness.

Candida albicans is by far the most common species, but Malassezia furfur can often be isolated in patients, particularly neonates, receiving lipid-supplemented parenteral nutrition. Candida spp. account for approximately 8% to 10% of all nosocomial bloodstream infections.

Except for Histoplasma, which multiply in leukocytes (white blood cells), fungi do not invade blood cells, but their presence in the blood usually indicates a focus of infection elsewhere in the body. Fungi in the bloodstream can disseminate (be carried) to all organs of the host, where they may grow, invade normal tissue, and produce toxic products. Fungi gain entrance to the circulatory system via loss of integrity of the gastrointestinal or other mucosa; through damaged skin; from primary sites of infection, such as the lung or other organs; or by means of intravascular catheters. Systemic fungal infections begin as pneumonia and may disseminate from the lungs, which serve as the portal of entry. Arthroconidia of Coccidioides immitis and microconidia of Histoplasma capsulatum and Blastomyces dermatitidis are ingested by alveolar macrophages in the lung.

These macrophages carry the fungi to nearby lymph nodes, usually the hilar odes. The fungi multiply within the node tissue and ultimately are released into the circulating blood, from which they are capable of seeding other organs or are destroyed by the body's defenses. Molds are particularly insensitive to host defenses such as antibody and phagocytic cells because of their large size and their sterol containing cell wall structure.

Parasites

Eukaryotic parasites may be found transiently in the bloodstream as they migrate to other tissues or organs. Their presence, however, cannot be considered consistent with a state of good health. For example, tachyzoites

of the parasite Toxoplasma gondii may be found in circulating blood. They invade cells within lymph nodes and other organs, including the lungs, liver, heart, brain, and eyes. The resulting cellular destruction accounts for the

manifestations of toxoplasmosis. Also, microfilariae are seen in peripheral blood during infection with *Dipetalonema, Mansonella, Loa loa, Wuchereria, or Brugia*. Malarial parasites invade host erythrocytes and hepatic parenchymal cells. The significant anemia and subsequent tissue hypoxia (reduction in oxygen levels) may result from destruction of red blood cells by the parasite.

Vascular trapping of normal erythrocytes by the infected red blood cells, which are less flexible and tend to clog small capillaries, is a major cause of morbidity. The host's immunologic response is to remove the parasites and

damaged red blood cells; the immune response may also have deleterious effects. Parasites in the bloodstream are usually detected by direct visualization. Those parasites for which traditional diagnosis is dependent on observation of the organism in peripheral blood smears include Plasmodium, Trypanosoma, and Babesia. Patients with malaria or filariasis may display a periodicity in their episodes of fever that allows the physician to time the collection of blood for microscopic examination intended for optimal detection. Rapid

serological methods and molecular methods are currently used to detect malaria, babesiosis, and trypanosomiasis.

Viruses

Although many viruses do circulate in the peripheral blood at some stage of disease, the primary pathology relates to infection of the target organ or cells. Those viruses that preferentially infect blood cells are Epstein-Barr virus (invades lymphocytes), cytomegalovirus (invades monocytes, polymorphonuclear cells, and lymphocytes), and human immunodeficiency virus (HIV) (involves only certain T lymphocytes and perhaps macrophages) and other human retroviruses that attack lymphocytes. The pathogenesis of viral diseases of the blood is the same as that for viral diseases of any organ; by diverting the cellular machinery to create new viral components or by other means, the virus may prevent the host cell from performing its normal function. The cell may be destroyed or damaged by viral replication, and immunologic responses of the host may also contribute to the pathogenesis.

TYPES OF BACTEREMIA

Bacteremia may be transient, continuous, or intermittent. Most people have experienced transient bacteremia; teething infants and people having dental procedures have had oral flora gain entry to the bloodstream through breaks in the gums. Other conditions in which bacteria are only transiently present in the bloodstream include manipulation of infected tissues, devices or instrumentation inserted through contaminated mucosal surfaces, and surgery involving nonsterile sites. These circumstances may also lead to significant septicemia, although normally the bacteria are cleared from the blood by scavenging leukocytes, resulting in no infection. Septicemia can occur when the bacteria multiply more rapidly than the immune system is capable of killing and removing the organism.

In septic shock, bacterial endocarditis, and other endovascular infections, organisms are released into the bloodstream at a fairly constant rate (continuous bacteremia). Also, during the early stages of specific infections, including typhoid fever, brucellosis, and leptospirosis, bacteria are continuously present in the bloodstream. In most other infections, such as in patients with undrained abscesses, bacteria can be found intermittently in the bloodstream. Of note, the causative agents of meningitis, pneumonia, pyogenic arthritis, and osteomyelitis are often recovered from blood during the early course of these diseases. In the case of transient seeding of the blood from a sequestered focus of infection, such as an abscess, bacteria are released into the blood approximately 45 minutes before a febrile episode.

The symptoms of septicemia are fever, chills, and malaise; these are caused by the presence of the invading microorganism and the toxins produced by these microorganisms. The older the patient is, the greater the risk and the rate of mortality as a result of septicemia.

TYPES OF BLOODSTREAM INFECTIONS

The two major categories of bloodstream infections are intravascular (those that originate within the cardiovascular system) and extravascular (those that result from bacteria entering the blood circulation through the lymphatic system from another site of infection). Of note, other organisms, such as fungi, may also cause intravascular or extravascular infections. However, because bacteria account for the majority of significant vascular infections, these types of bloodstream infections are discussed in more detail. Factors contributing to the initiation of bloodstream infections are immunosuppressive agents, widespread use of broad-spectrum antibiotics that suppress the normal flora and allow the emergence of resistant strains of bacteria, invasive procedures allowing bacteria access to the interior of the host, more extensive surgical procedures, and prolonged survival of debilitated and seriously ill patients Intravascular Infections.

Intravascular infections include infective endocarditis, mycotic aneurysm, suppurative thrombophlebitis, and intravenous (IV), catheter-associated bacteremia. Because these infections are within the vascular system, organisms are present in the bloodstream at a fairly constant rate (i.e., a continuous bacteremia). These infections in the cardiovascular system are extremely serious and considered life threatening.

- Infective Endocarditis. The development of infective endocarditis (infection of the endocardium most commonly caused by bacteria) is believed to involve several independent events. Cardiac abnormalities, such as congenital valvular diseases that lead to turbulence in blood flow or direct trauma from IV catheters, can damage cardiac endothelium. This damage to the endothelial surface results in the deposition of platelets and fibrin.

If bacteria transiently gain access to the bloodstream (this can occur after an innocuous procedure such as brushing the teeth) after alteration of the capillary endothelial cells, the organisms may stick to and then colonize the damaged cardiac endothelial cell surface. After colonization, the surface will rapidly be covered with a protective layer of fibrin and platelets. This protective environment is favorable to further bacterial multiplication.

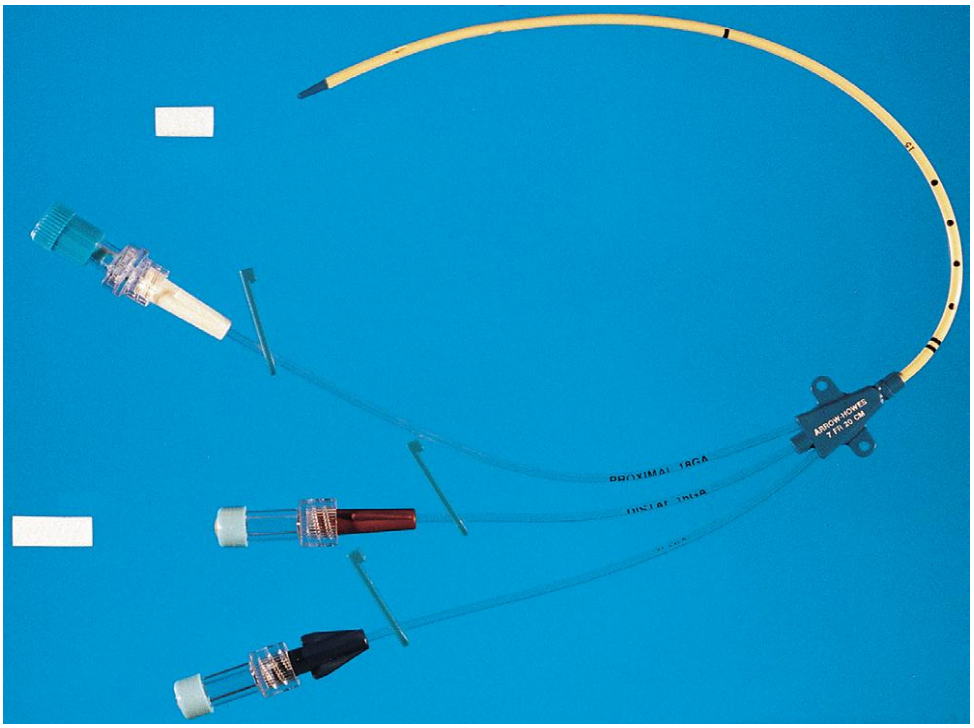
This web of platelets, fibrin, inflammatory cells, and entrapped organisms is called a vegetation . The resulting vegetations ultimately seed bacteria into the blood at a slow but constant rate. The primary causes of infective endocarditis are the viridans streptococci, comprising several species.These organisms are normal inhabitants of the oral cavity, often gaining entrance to the bloodstream as a result of gingivitis, periodontitis, or dental manipulation. Heart valves, especially those previously damaged, present convenient surfaces for attachment of these bacteria. Streptococcus sanguis and Streptococcus mutans are frequently isolated in streptococcal endocarditis. Gram-negative bacilli, known as the AACEK group,

Aggregatibacter aphrophilus, Actinobacillus actinomycetemcomitans Cardiobacterium hominis, Eikenella corrodens, and Kingella kingae, can also be associated with endocarditis.

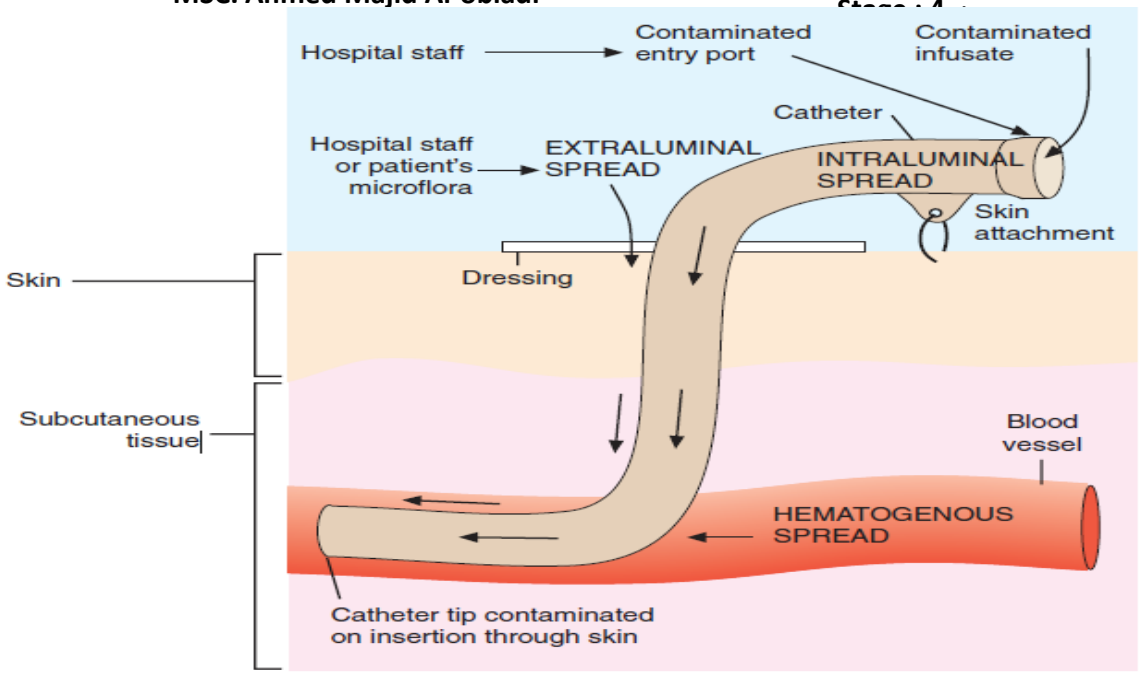
With the ever-increasing use of IV catheters, arterial lines, and vascular prostheses, organisms considered normal or hospital-acquired inhabitants of the human skin are able to gain access to the bloodstream and attach to various surfaces, including heart valves and vascular endothelium. It has been estimated that more than 200,000 nosocomial infections (bloodstream) occur annually in the United States in adults and children. The majority of these infections are caused by the use of intravascular catheters. Staphylococcus epidermidis and other coagulase-negative staphylococci have been increasingly implicated as the cause of infection associated with intravascular catheters. S. epidermidis is the most common etiologic agent identified in prosthetic valve endocarditis, with S. aureus being the second most common. S. aureus is an important cause of septicemia without endocarditis and is found in association with other foci, such as abscesses, wound infections, and pneumonia, as well as sepsis related to indwelling intravascular catheters. Mycotic Aneurysm and Suppurative Thrombophlebitis. Two other intravascular infections, mycotic aneurysms and suppurative thrombophlebitis, result from damage to the endothelial cells lining blood vessels. With respect to mycotic aneurysm, an infection causes inflammatory damage and weakening of an arterial wall; this weakening causes a bulging of the arterial wall (i.e., aneurysm) that can eventually rupture. The etiologic agents are similar to those that cause endocarditis. Suppurative thrombophlebitis is an inflammation of a vein wall. The pathogenesis of this intravascular infection involves an alteration in the vein's endothelial lining followed by clot formation. The site is then seeded with organisms, thereby establishing a primary site of infection. Suppurative thrombophlebitis represents a frequent complication of hospitalized patients caused by the increasing use of IV catheters.

- Intravenous Catheter–Associated Bacteremia. IV catheters are an integral part of the care for many hospitalizedpatients. More than 3 million central venous catheters are used annually in the United States. For example, central venous catheters are used to administer fluids, blood products, medications, antibiotics, and nutrition, and for hemodynamic monitoring. A shortterm, triple-lumen (channel opening within a tube) central venous catheter is shown in Figure 68-2. Unfortunately, a major consequence of these medical devices is colonization of the catheter by either bacteria or fungi, which can lead to catheter infection and serious bloodstream infection. This consequence is a major nosocomial source of illness and even death.IV catheter–associated bacteremia (or fungemia) is believed to occur primarily by two routes (Figure 68-3). The first route involves the movement of organisms from the catheter entry site through the patient's skin and down the external surface of the catheter to the

catheter tip within the bloodstream. After arriving at the tip, the organisms multiply and may cause a bacteremia. The second way that IV catheter–associated bacteremia may occur is by migration of organisms along the inside of the catheter (the lumen) to the catheter tip. The catheter's hub, where tubing connects into the IV catheter, is considered the site at which organisms gain access to the patient's bloodstream through the catheter lumen. The most common etiologic agents for IV catheter–associated bloodstream infections, regardless of the route of infection, are organisms found on the skin.Certain strains of S. epidermidis appear to be uniquely suited for causing catheterrelated infections because of their ability to produce a biofilm or "slime" that consists of complex sugars (polysaccharides) believed to help the organism adhere to the catheter's surface. The initial attachment of S. epidermidis to the catheter's polystyrene surface is related to a cell surface protein. Once attached, the organism



triple-lumen central venous catheter. The ends from which the catheter is accessed are usually referred to as the hubs. After the catheter is inserted, the tip resides within the bloodstream



Possible routes by which microorganisms gain access to the bloodstream to cause intravenous catheter–associated bacteremias.

proliferates, subsequently forming a biofilm. Uncommon routes of IV catheter–tip infection include contaminated fluids or blood-borne seeding from another infection site.

Extravascular Infections

Except for intravascular infections, bacteria usually enter the circulation through the lymphatic system. Most cases of clinically significant bacteremia are a result of extravascular infection. When organisms multiply at a local site of infection such as the lung, they are drained by the lymphatics and reach the bloodstream. In most individuals, organisms in the bloodstream are effectively and rapidly removed by the reticuloendothelial system in the liver, spleen, and bone marrow and by circulating phagocytic cells. Depending on the extent of immunologic control of the infection, the organism may be circulated more widely, thereby causing a bacteremia or fungemia.The most common portals of entry for bacteremia are the genitourinary tract (25%), respiratory tract (20%), abscesses (10%), surgical wound infections (5%), biliary tract (5%), miscellaneous sites (10%), and uncertain sites (25%). For the most part, the probability of bacteremia occurring from an extravascular site depends on the site of infection, its severity, and the organism. For example, any organism producing meningitis is likely to produce bacteremia at the same time. Of importance, certain organisms causing extravascular infections commonly invade the bloodstream; some of these organisms are listed in Table 68-1. In addition to these organisms, a large number of other bacteria and fungi that cause extravascular infections are also capable of invading the bloodstream. Whether these organisms invade the bloodstream depends on the host's ability to control the infection and the organism's pathogenic potential. Some of the organisms associated with potential bloodstream infections from a localized site include members of the family Enterobacteriaceae,

Streptococcus pneumoniae, Staphylococcus aureus, Neisseria gonorrhoeae, anaerobic cocci, Bacteroides, Clostridium, beta-hemolytic streptococci, and Pseudomonas. These are only some of the organisms frequently isolated from blood. Almost every known bacterial species and many fungal species have been implicated in extravascular bloodstream

TABLE 68-1 Organisms Commonly Associated with Bloodstream Invasion from Extravascular Sites of Infection

Organism	Extravascular Site of Infection
Anaerobic organisms	Wound, soft tissue
Brucella spp.	Reticuloendothelial system
Candida albicans	Genitourinary tract
Chlamydia pneumoniae	Respiratory
Clostridium spp.	Wound, soft tissue
Coagulase negative staphylococci	Wound, soft tissue
Enterobacteriaceae (*E.coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Enterococcus* spp.)	Genitourinary tract infections, central nervous system
Haemophilus influenzae	Meninges (CNS), epiglotitis, periorbital region, respiratory
Legionella spp.	Respiratory
Listeria monocytogenes	Meninges (CNS)
Neisseria meningitidis	Meninges (CNS)
Pseudomonas aeruginosa	Wound, soft tissue, central nervous system
Salmonella enterica typhi	Small intestine, regional lymph nodes of the intestine, reticuloendothelial system
Streptococcus penumoniae	Meninges (CNS), respiratory
Streptococcus pyogenes	Wound, soft tissue
Staphylococcus aureus	Wound, soft tissue, meninges (CNS)

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DETECTION OF BACTEREMIA

Mortality rates associated with bloodstream infection range from 20% to 50%. Because bacteremia frequently provides evidence of a life-threatening infection, the prompt detection and recovery of microorganisms from blood is of paramount importance. To detect bloodstream infections, a patient's blood must be obtained by aseptic venipuncture and then incubated in culture media. Bacterial growth can be detected using techniques ranging from manual to totally automated methods. Once growth is detected, the organism is isolated, identified, and if considered pathogenic or treatment is necessary for the patient, the organism is then tested for susceptibility to various antimicrobial agents.

SPECIMEN COLLECTION

Preparation of the Site Because blood culture media have been developed as enrichment broths to encourage the multiplication of as few as a single organism, these media will enhance growth of contaminating organisms, including a normal inhabitant of human skin. Therefore, careful skin preparation before collecting the blood sample is of paramount

importance to reduce the risk of introducing contaminants into blood culture media. The vein from which the blood is to be drawn must be chosen before the skin is disinfected. If a patient has an existing IV line, the blood should be drawn below the existing line; blood drawn above the line will be diluted with fluid being infused. It is less desirable to draw blood through a vascular shunt or catheter, because these prosthetic devices are difficult to decontaminate

Completely.

Antisepsis. Once a vein is selected, the skin site is defatted (fat removal) with 70% isopropyl alcohol and an antiseptic is applied to kill surface and subsurface bacteria. Regardless of the antiseptic used, it is critical to follow the manufacturer's recommendation for the length of time the antiseptic is allowed to remain on the skin. Available data indicate that iodine tincture (iodine in alcohol) and chlorhexidine are equivalent for skin preparation before drawing blood cultures. The steps Specimen Volume Adults. For many years, it has been recognized that most bacteremias in adults have a low number of colonyforming units (CFU) per milliliter (mL) of blood. For example, in several studies, fewer than 30 CFU per mL of blood were commonly found in patients with clinically significant bacteremia. Therefore, a sufficient sample volume is critical for the successful detection of bacteremia.

There is a direct relationship between the volume of blood and an increased probability that the laboratory will isolate the infecting the organism. Therefore, collection of two sets of cultures using10 to 20 mL of blood per culture is strongly recommended for adults. To illustrate, Cockerill and colleagues reported that in patients without infective endocarditis, volumes of 20 mL increased the yield, identification of the organism, by 30% compared with 10-mL volumes. Unfortunately, a study confirmed that it is common practice to under inoculate blood culture bottles; findings from this study suggested that the yield increases by 3.2% for each milliliter of blood cultured.

Children. It is not safe to take large samples of blood from children, particularly infants. The optimal volume of blood required for successful identification of organisms from infants and children has not been clearly delineated. Similar to adults, this patient population has low level (small numbers of organisms) bacteremia. In light of low-level bacteremia in infants and children and based on the premise that it is safe to obtain as much as 4% to 4.5% of a patient's known total blood volume for culture and the relationship between blood volume and patient weight, Baron and colleagues have determined recommendations for blood volumes for cultures from infants and children . For infants and small children, only 1 to 5 mL of blood should be drawn for bacterial culture. Blood culture bottles are available designed specifically for the pediatric patient. Because blood specimens from septic children may yield fewer than 5 CFU/mL of the organism, quantities less than 1 mL may not be adequate to detect pathogens. Nevertheless, smaller volumes

should still be cultured because high levels of bacteremia (more than 1000 CFU/mL of blood) are detected in some infants.

- Number of Blood Cultures

Because periodicity of microorganisms in the bloodstream may be characteristic for some diseases, continuousm for some and random in others, patterns of bacteremia must be considered in establishing standards for the timing and number of blood cultures. If the volume of blood is adequate, usually two or three blood cultures are sufficient to achieve the optimum blood culture sensitivity. In patients with endocarditis who have not received antibiotics, a single blood culture is positive in 90% to 95% of the cases, whereas a second blood culture establishes the diagnosis in at least 98% of patients, depending on the study. For patients who have received prior antibiotic therapy, three separate blood collections of 16 to 20 mL each, and an additional blood culture or two taken on the second day, if necessary, detects most etiologic agents of endocarditis. This presumes use of a culture system adequate for growth of the organism involved, which often entails extending the incubation period. Similarly, for patients without infective endocarditis, 65.1% are detected in the first culture, 80% by the first two cultures, and 95.7% were detected in the first three blood cultures.

- Timing of Collection

The timing of cultures is not as important as other factors in patients with intravascular infections because organisms are released into the bloodstream at a fairly constant rate. Because the timing of intermittent bacteremia is unpredictable, it is generally accepted that two or three blood cultures be spaced an hour apart. However, a study found no significant difference in the yield between multiple blood cultures obtained simultaneously or those obtained at intervals. The authors concluded that the overall volume of blood cultured was more critical to increasing organism yield than timing. When a patient's condition requires therapy to be initiated as rapidly as possible, little time is available to collect multiple blood culture samples over a timed interval. An acceptable compromise is to collect 40 mL of blood at one time, 20 mL from each of two separate venipuncture sites, using two separate needles and syringes before the patient is given antimicrobial therapy. Regardless, blood should be transported immediately to the laboratory and placed into the incubator or instrument as soon as possible. With blood culture instrumentation, a delay beyond 2 hours can delay the detection of positive cultures.

Miscellaneous Matters

Anticoagulation. Blood drawn for culture must not bem allowed to clot. If acteria become entrapped within a clot, their presence may go undetected. Thus, blood drawn for culture may be either inoculated directly into the blood culture broth media or into a sterile blood

collection tube containing an anticoagulant for transport to the laboratory for subsequent inoculation. Heparin, ethylenediaminetetraacetic acid (EDTA), and citrate inhibit numerous organisms and are not recommended for use. Sodium polyanethol sulfonate (SPS, Liquoid) in concentrations of 0.025% to 0.03% is the best anticoagulant available for blood cultures. As a result, the most commonly used preparation in blood culture media today is 0.025% to 0.05% SPS. In addition to its anticoagulant properties, SPS is also anticomplementary and antiphagocytic, and interferes with the activity of some antimicrobial agents, notably aminoglycosides. SPS, however, may inhibit the growth of a few microorganisms, such as some strains of Neisseria spp., Gardnerella vaginalis, Streptobacillus moniliformis, and all strains of Peptostreptococcus anaerobius. Because of the inhibitory effect of SPS on some organisms in conjunction with the necessity for an additional step to transfer the blood to the ultimate culture bottles that increases the risk of exposure to blood-borne pathogens as well as contamination, using collection tubes instead of direct inoculation into culture bottles may compromise organism recovery. For these reasons, the use of intermediate collection tubes is discouraged. Although the addition of 1.2% gelatin has been shown to counteract decreases. Dilution. In addition to the volume of blood collected and type of medium chosen, the dilution factor for the blood in the medium must be considered. To conserve space and materials, it is desirable to combine the largest feasible amount of blood from the patient (usually 10 mL) with the smallest amount of medium that will still encourage the growth of bacteria and dilute out or inactivate the antibacterial components of the blood. Traditionally, a 1 : 10 ratio of blood to medium was required for successful bacterial growth; however, several new commercial media containing resins or other additives have demonstrated enhanced recover with as low as a 1 : 5 ratio. For this purpose, a 1 : 5 ratio of blood to unmodified medium has been found to be adequate in conventional blood cultures. All commercial blood culture systems (discussed later in this chapter) specify the appropriate dilution.

Blood Culture Media. The diversity of bacteria recovered from blood requires an equally diverse and large number of media to enhance the growth of these bacteria. Basic blood culture media contain a nutrient broth and an anticoagulant. Several different broth formulations are commercially available. Most blood culture bottles available commercially contain trypticase soy broth, brain-heart infusion broth, supplemented peptone, or thioglycolate broth. More specialized broth bases include Columbia or Brucella broth.

TYPES OF BLOOD CULTURE BOTTLE

The addition of penicillinase to blood culture media for inactivation of penicillin has been largely superseded in recent years by the availability of a resin-containing medium that inactivates most antibiotics nonselectively by adsorbing them to the surface of the resin particles. Resin-containing media may enhance isolation of staphylococci, particularly when patients are receiving bacteriostatic drugs. The BACTEC system (Becton Dickinson Microbiology Systems, Sparks, Maryland) offers

everal resin-containing media. In addition to resincontaining media, BacT/ALERT has a blood ulture bottle with supplemented brain heart infusion (BHI) broth containing activated charcoal articles that significantly increase the yield of microorganisms over standard blood culture media. n addition, resins or charcoal may be added to commercial media to absorb and inactivate ntimicrobial agents within the patient's blood. Care should be exercised when interpreting gram tains from resin- and charcoal-containing bottles. The additives may be confused with gram-ositive organisms. In general, each blood culture set includes a blood culture bottle designated for erobic recovery and one for anaerobic recovery of bacteria. Because of the decline in the late 1990s n the proportion of positive blood cultures yielding anaerobic bacteria coupled with the increasing ressure for laboratories to be cost effective, some investigators have recommended laboratories iscard this routine practice of processing all blood samples aerobically and anaerobically. It has been proposed that anaerobic cultures should be selectively. **CULTURE TECHNIQUES**

pecial blood culture broth systems are available for the isolation of mycobacteria. The systems are seful in detecting disseminated infections caused by Mycobacterium tuberculosis and non-uberculosis mycobacteria. Conventional Blood Cultures Incubation Conditions. The atmosphere in ommercially prepared blood culture bottles is usually at a low oxidationreduction potential, ermitting the growth of most facultative and some anaerobic organisms. To encourage the growth f obligate (strict) aerobes, such as yeast and Pseudomonas aeruginosa, transient venting of the ottles with a sterile, cotton-plugged needle may be necessary. Constant agitation of the bottles uring the first 24 hours of incubation also enhances the growth of most aerobic bacteria. Self-Contained Subculture System A modification of the biphasic blood culture medium is the BD Septi-Chek system (Becton Dickinson Microbiology Systems, Sparks, Maryland) (Figure 68-4) consisting

f a conventional blood culture broth bottle with an attached chamber containing a slide coated with gar or several types of agars. Special media for isolation of fungi and mycobacteria are also vailable. To subculture, the entire broth contents are allowed to contact the agar surface by nverting the bottle, a simple procedure that does not require opening the bottle or using needles. The arge volume of broth subcultured and the enclosed method provide faster detection for many rganisms than is possible with conventional systems. The Septi Chek system appears to enhance he recovery of Streptococcus pneumoniae, but such biphasic systems do not efficiently recover anaerobic isolates.

Lysis Centrifugation

he Isolator (Alere, Waltham, MA) is a lysis centrifugation system commercially available. The solator consists of a stoppered tube containing saponin to lyse blood cells and SPS as an nticoagulant . After centrifugation, the supernatant is discarded, the sediment containing the athogen is vigorously vortexed, and the entire sediment is plated to solid agar. Benefits of this ystem include rapid and improved recovery of filamentous fungi, the presence of actual colonies for irect identification and susceptibility testing after initial incubation, the ability to quantify the

olony-forming units present in the blood, rapid detection of polymicrobial bacteremia, dispensing vith the need for a separate media for initial culture setup based on clinical impression (e.g., direct lating onto media supportive of Legionella spp. or Mycobacterium spp.), and potential enhanced ecovery of intracellular microorganisms caused by lysis of host cells. Possible limitations of the ystem seem to be a relatively high rate of plate contamination and a decreased ability to detect ertain bacteria, such as Streptococcus pneumoniae, Listeria monocytogenes, Haemophilus nfluenzae, and anaerobic bacteria, compared with conventional systems. If a mixed infection is suspected, an additional blood culture collection tube should be inoculated simultaneously.

- Instrument-Based Systems

Conventional blood culture techniques are labor intensive and time consuming. During these times f cost constraints in health care and a corresponding requirement for clinically relevant care, the evelopment of improved instrumentation for blood cultures was needed. Instruments are capable of apid and accurate detection of organisms in blood specimens. By using newer instrumentation, laboratories processing a large volume of blood cultures can also provide results cost effectively.

BACTEC Systems. Many laboratories use the BACTEC system (Becton Dickinson Microbiology ystems, Sparks, Maryland), which measures the production of carbon dioxide (CO_2) by netabolizing organisms. Blood or sterile body fluid for routine culture is inoculated into bottles ontaining appropriate substrates. The first BACTEC systems were semiautomated. Vials, ontaining 14C-labeled substrates (glucose, amino acids, and alcohols) were incubated and often gitated on a rotary shaker. At predetermined time intervals thereafter, the bottles were placed into he monitoring module, where they were automatically moved to a detector. The detector inserted wo needles through a rubber septum seal at the top of each bottle and withdrew the accumulated gas bove the liquid medium and replaced it with fresh gas of the same mixture (aerobic or anaerobic). Any amount of radiolabeled CO_2, the final end product of metabolism of the 14C-labeled substrates above a preset baseline level), was considered to be suspicious for microbial growth. Microbiologists retrieved suspicious bottles and worked them up (performed subcultured and dentification procedures) for possible microbial growth. Subsequent modifications further utomated the incubation and measuring device, and detection was accomplished by nonradioactive neans. The BACTEC blood culture systems are fully automated with the incubator, shaker, and etector all in one instrument. These fully automated blood culture systems use fluorescence to neasure CO_2 released by organisms; a gas-permeable fluorescent sensor is on the bottom of each ial (Figure). As CO_2 diffuses into the sensor and dissolves in water present in the sensor matrix, ydrogen (H^+) ions are generated. These H^+ ions cause a decrease in pH, which, in turn, increases he fluorescent output of the sensor. There is continuous monitoring of each bottle and detection is xternal to the bottle. Of importance, the noninvasion of the blood culture bottle eliminates the potential for cross-contamination of cultures.

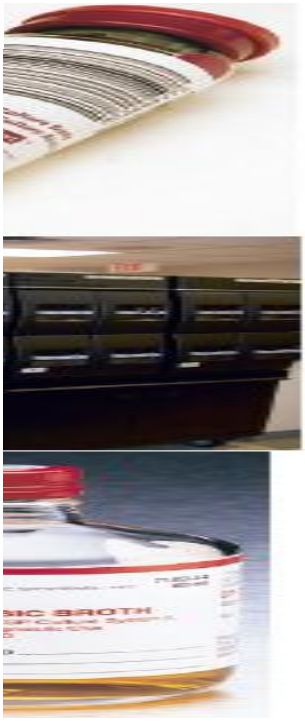
BacT/ALERT Microbial Detection System. Other laboratories use the BacT/Alert System (bioMérieux, Durham, North Carolina), which measures CO2-derived pH changes with a colorimetric sensor in the bottom of each bottle . The sensor is separated from the broth medium by a membrane permeable to CO2. As organisms grow, they release CO2, which diffuses across the membrane and is dissolved in water present in the matrix of the sensor. As CO2 is dissolved, free hydrogen ions are generated. These free hydrogen ions cause a color change in the sensor (blue to light green to yellow as the pH decreases); a sensor in the instrument reads this color change.

Versa TREK System. The Versa TREK system (Thermo Scientific, TREK Diagnostics, Cleveland, Ohio) utilizes a unique agitation system during blood culture inoculation. The aerobic media bottles each contain a small magnetic stir bar enhancing oxygenation during incubation. Like the other systems, this is also a continuously monitoring instrument. Table 68-3 summarizes characteristics of some blood culture instruments that are available at the time of printing of the text.

Techniques to Detect IV Catheter–Associated Infections

The insertion of an IV catheter during hospitalization is common practice. Infection, either locally at the catheter insertion site or sepsis, caused by bacteremia, is one of the most common complications of catheter placement. Because the skin of all patients is colonized with microorganisms that are also common pathogens in catheters, techniques used to diagnose catheter-related infections attempt to quantitate bacterial growth. Diagnosis of an IV catheter–related bacteremia (or fungemia) is difficult, because there are often no signs of infection at the catheter insertion site and the typical signs and symptoms of sepsis can overlap with other clinical manifestations; even the finding of a positive blood culture does not identify the catheter as the source. To date, various methods, such as semiquantitative cultures, Gram stains of the skin entry site, and culture of IV catheter tips following catheter removal. The terminal end of the IV catheter is removed and rolled several times across a blood agar plate. The tip is then removed from the agar plate and placed in enrichment broth. Both the plate and enrichment broth are incubated at 37° C for 18 to 24 hours. Following inoculation, the blood agar plates are examined, and any isolates are identified according to the laboratory protocol. The enrichment broth may be subcultured to blood agar and anaerobic media for further analysis and potential detection of intraluminal colonization. Many methods involve some type of quantitation in an attempt to differentiate colonization of the catheter from probable infection. Two major approaches to the diagnosis of catheter-related infection (CRI) in which the catheter remains in place are based on the premise that a greater number of organisms will be present in the intravascular catheter compared to the number found in blood specimens obtained from distant peripheral veins. The first approach, differential quantitative cultures, involves drawing two blood cultures—one from a peripheral site and the other from the suspected infected line.

Quantitative cultures are processed for each specimen by inoculating the same volume of blood to tandard microbiology media and colonies counted the following day. A colony count ratio greater han 4 to 10 : 1 between the central venous blood and a peripheral blood specimen indicates a robable CRI with a sensitivity of 78% to 94% and a specificity of 99% to 100%. The second pproach involves the comparison of the differential time to positivity of blood specimens obtained from a peripheral and



Diagnosis of staphylococcus ssp.

1. Culture:

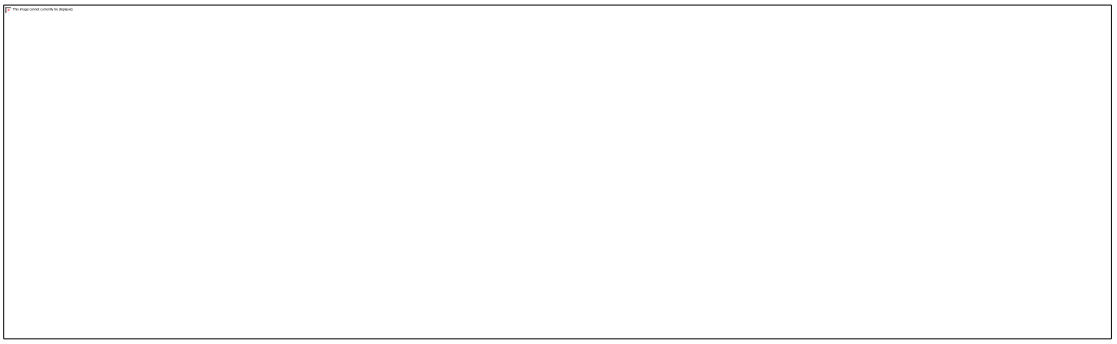
On Manitol Salt Agar:

Mannitol Salt Agar (MSA) contains the carbohydrate mannitol, 7.5% sodium chloride (NaCl), and the pH indicator **phenol red**.

Phenol red is yellow below pH 6.8, red at pH 7.4 to 8.4, and pink at pH 8.4 and above.

Mannitol provides the substrate for fermentation and makes the medium differential. Sodium chloride makes the medium selective because its concentration is high enough to dehydrate and kill most bacteria.

ost staphylococci are able to grow on MSA, but do not ferment the mannitol, so the growth appears pink or red and the medium remains unchanged. Staphylococcus aureusferments the mannitol, which produces acids and lowers the pH of the medium. The result is formation of bright yellow colonies usually surrounded by a yellow halo**.**



On blood agar:

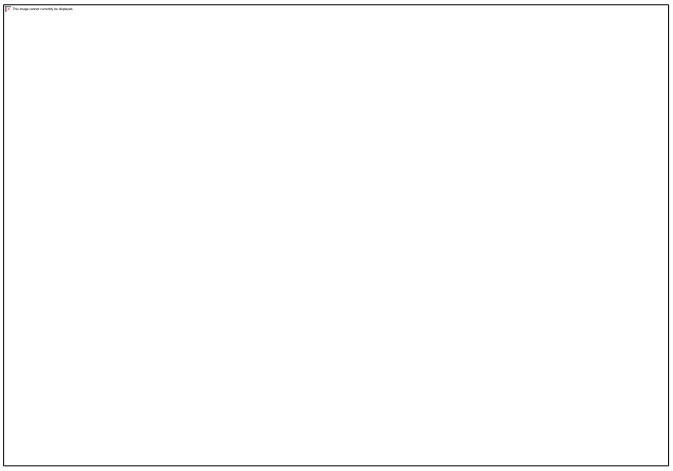
The three major types of hemolysis are α-hemolysis, β-hemolysis, and gama-hemolysis.

β-hemolysis, the completedestruction of RBCs and hemoglobin, results in a clearing of the medium around the colonies.

α-hemolysis is the partial destruction of RBCs and produces a greenish discoloration of the agar around the colonies.

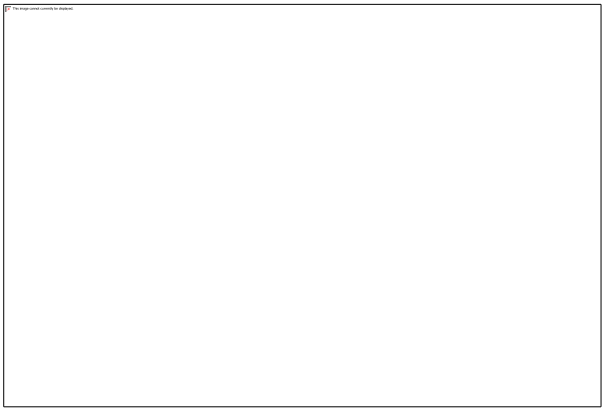
gama-hemolysis is nonhemolysis and appears as simple growth with no changeto the medium

Staphylococcus aureus on blood agar with 5% defibrinated sheep blood. Individual colonies on agar are round, convex, and 1-4 mm in diameter with a sharp border. On blood agar plates, colonies of *Staphylococcus aureus* are frequently surrounded by zones of **clear beta-hemolysis**.

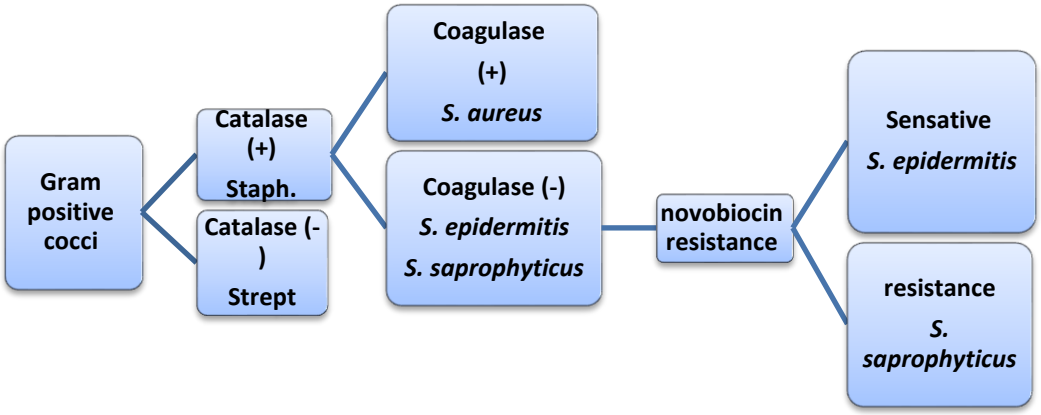


2. Microscopic appearance

Gram-positive Cocci in grape-like clusters

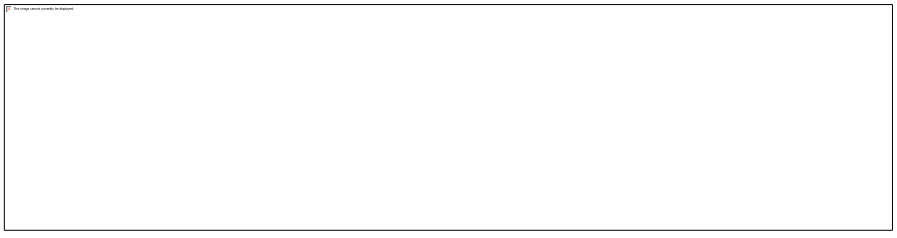


3. Biochemical tests



Catalase:

Bacteria that produce catalase can be detected easily using typical store-grade hydrogen peroxide. When hydrogen peroxide is added to a catalase-positive culture, oxygen gas bubbles form immediately and If no bubbles appear, the organism is catalase-negative. This test can be performed on a microscope slide or by adding hydrogen peroxide directly to the bacterial growth.



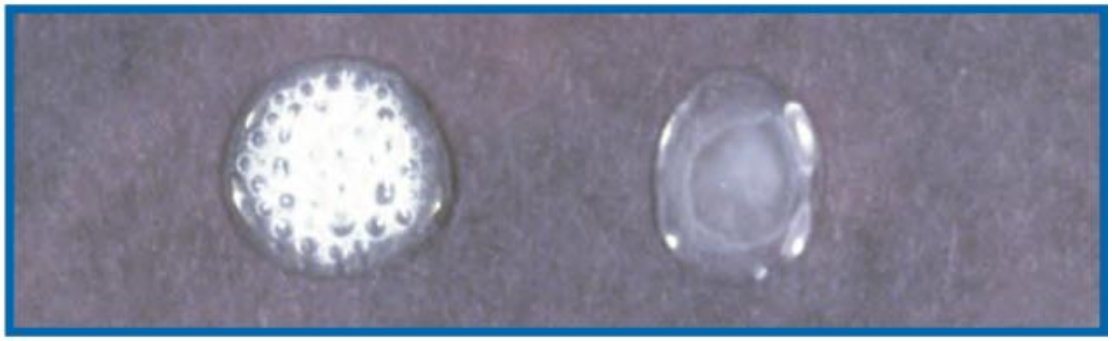


TABLE OF RESULTS		
Result	**Interpretation**	**Symbol**
Bubbles	Catalase is present	+
No bubbles	Catalase is absent	–

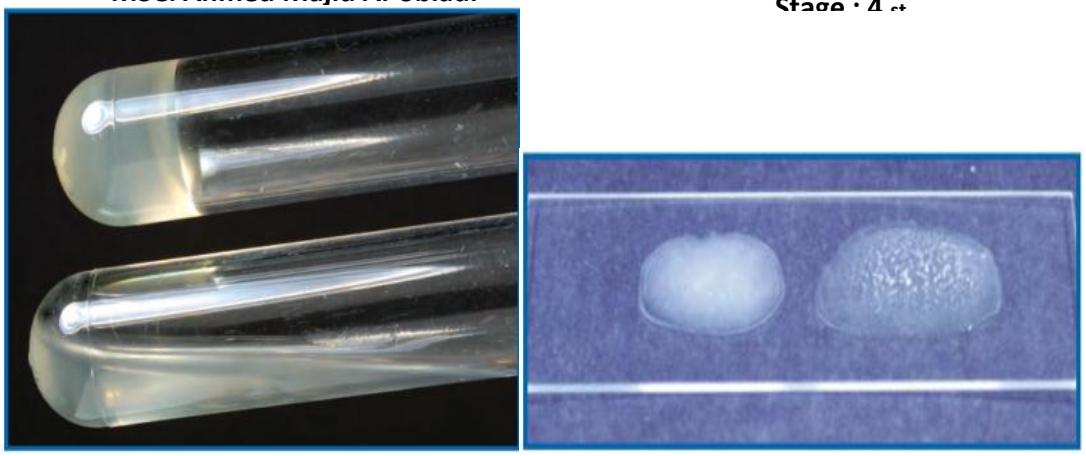
Coagulase Tests

Coagulase enzymes occur in two forms—**bound coagulase** and **free coagulase**. Bound coagulase, also called **clumping factor**, is attached to the bacterial cell wall and reacts directly with fibrinogen in plasma. The fibrinogen then precipitates, causing the cells to clump together in a visible mass. Free coagulase is an extra cellular enzyme (released from the cell) that reacts with a plasma component called coagulase-reacting factor (CRF). The resulting reaction is similar to the conversion of prothrombin and fibrinogen in the normal clotting mechanism

Two forms of the Coagulase Test have been devised to detect the enzymes: the **Tube Test** and the **Slide Test**. The Tube Test detects the presence of either bound or free coagulase, and the Slide Test detects only bound coagulase.

The Tube Test is performed by adding the test organism to plasma in a test tube. Coagulation of theplasma (including any thickening or formation of fibrin threads) within 24 hours indicates a positive reaction. The plasma typically is examined for clotting (without shaking) periodically for about 4 hours

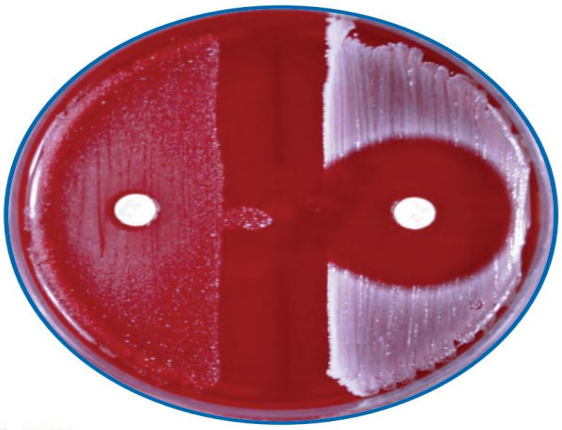
In the Slide Test, bacteria are transferred to a slidecontaining a small amount of plasma. Agglutination ofthe cells on the slide within 1 to 2 minutes indicates thepresence of bound coagulase.



Novobiocin resistance

On blood agar spread the bacteria to be testing then place the novobiocin disk, incubate for 24 h

If the bacteria are sensitive to novobiocin this indicate that Staph is *S. epidermitis*, if the bacteria are resistance this means its *S. saprophyticus*



5-79 Novobiocin Disk Test ✦ A novobiocin-resistant (R) organism is on the left; a susceptible (S) organism is on the right.

Gelatin Hydrolysis Test

Gelatin is a protein derived from collagen a component of vertebrate connective tissue. Gelatinases comprise a family of extracellular enzymes produced and secreted by some microorganisms to hydrolyze gelatin.

The presence of gelatinases can be detected using Nutrient Gelatin, a simple test medium composed of gelatin, peptone, and beef extract.

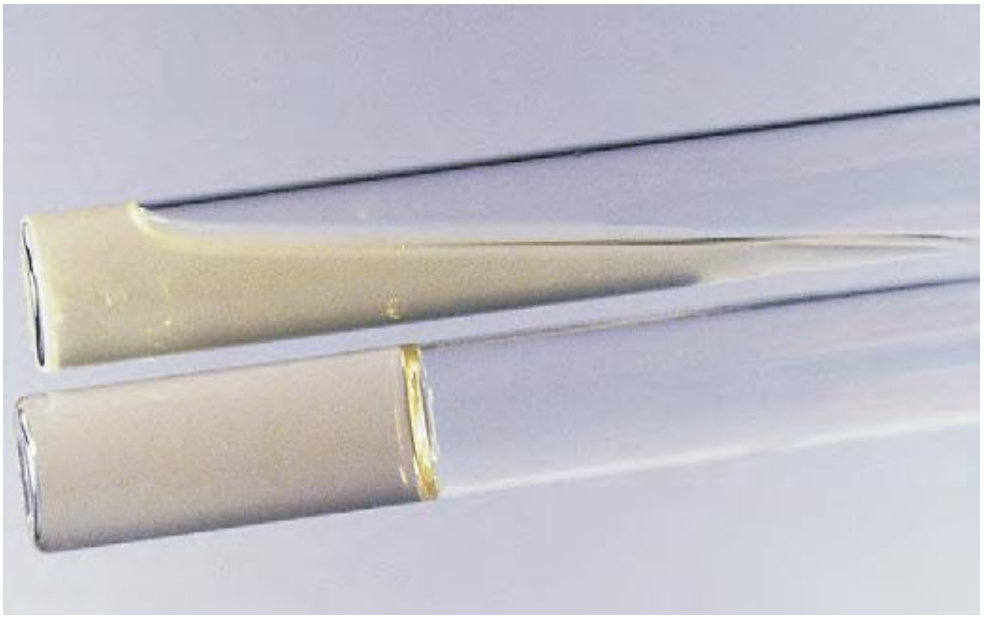
When a tube of Nutrient Gelatin is stab-inoculated with a gelatinase positive organism, secreted gelatinase will liquefy the medium.

Gelatinase-negative organisms do not secrete the enzyme and do not liquefy the medium.

A 7-day incubation period is usually sufficient to see liquefaction of the medium. However, gelatinase activity is very slow in some organisms.

A slight disadvantage of Nutrient Gelatin is that it melts at 28°C (82°F). Therefore, inoculated stabs are typically incubated at 25°C along with an uninoculated control to verify that any liquefaction is not temperature related.

This test is used to determine the ability of a microbe to produce gelatinases. *Staphylococcus aureus*, which is gelatinase-positive, can be differentiated from *S. epidermidis*.

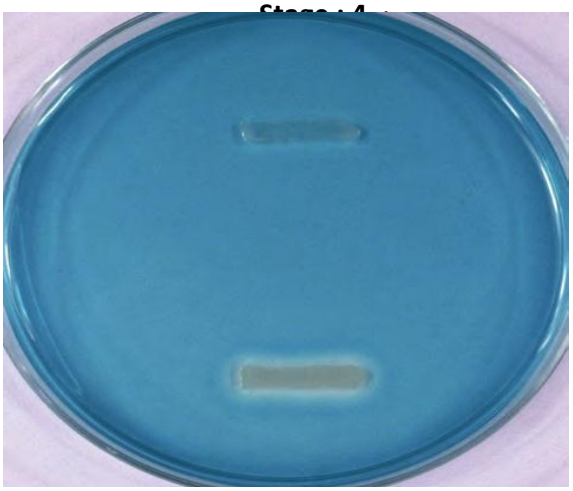


DNA Hydrolysis Test

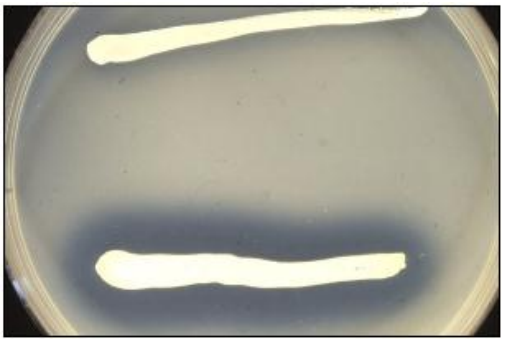
An enzyme that catalyzes the depolymerization of DNA into small fragments is called a deoxyribo nuclease, or DNase. Ability to produce this enzyme can be determined by culturing and observing an organism on a DNase Test Agar plate.

DNase Test Agar contains an emulsion of DNA, peptides as a nutrient source, and methyl green dye. The dye and polymerized DNA form a complex that gives the agar a blue-green color. Bacterial colonies that secrete DNase will hydrolyze DNA in the medium into smaller fragments unbound from the methyl green dye. This results in clearing around the growth

DNase Test Agar is used to distinguish Serratia species from Enterobacter species, *Moraxella catarrhalis* from Neisseria species, and *Staphylococcus aureus* (+) from other Staphylococcus species.



Some type of DNase agar require addition of diluted hydrochloric acid (HCl) is poured onto the plate and there will be a clear zone close to the colonies or the streak appear in positive test.



ONPG Test – (o-nitrophenyl-β-D galactoside)

this test is used to demonstrate the presence or absence of the enzyme B-galactosidase using the substrate Ortho-nitrophenyl-D-galactopyranoside in order to differentiate Lactose-fermenting from non lactose-fermenting organisms.

Procedure :

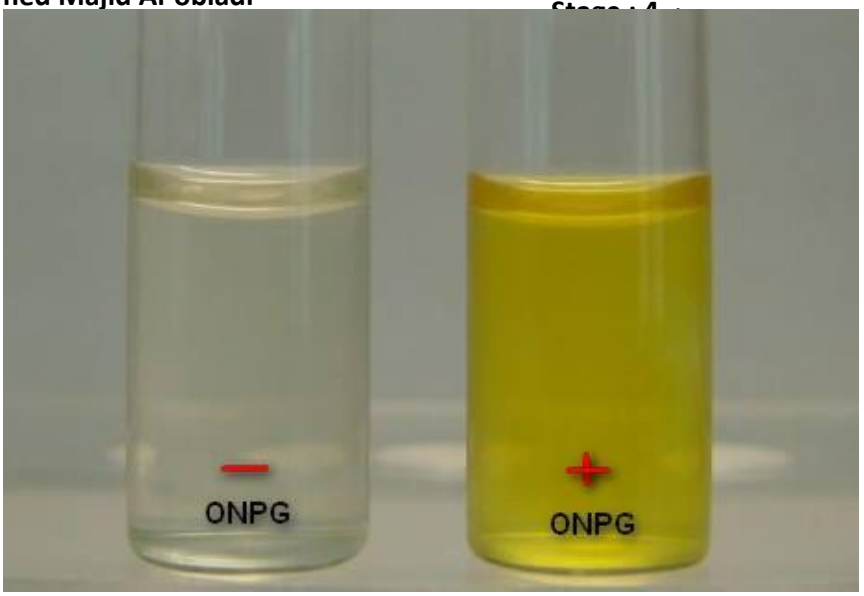
1. Place an ONPG disk into a sterile tube and add 0.2 mL saline.
2. Heavily inoculate the tube with a loopful of the test isolate.
3. Incubate at 35 C for up to 4 hours.

Results:

The substrate diffuse ONPG into the cell, if organism posses B-Galactosidase, the enzyme will splits the B-Galactosidase bond, releasing of o-nitrophenol yellow color compound. If organism lacks enzyme. The Galactoside bond remains intact, the medium remains colorless.

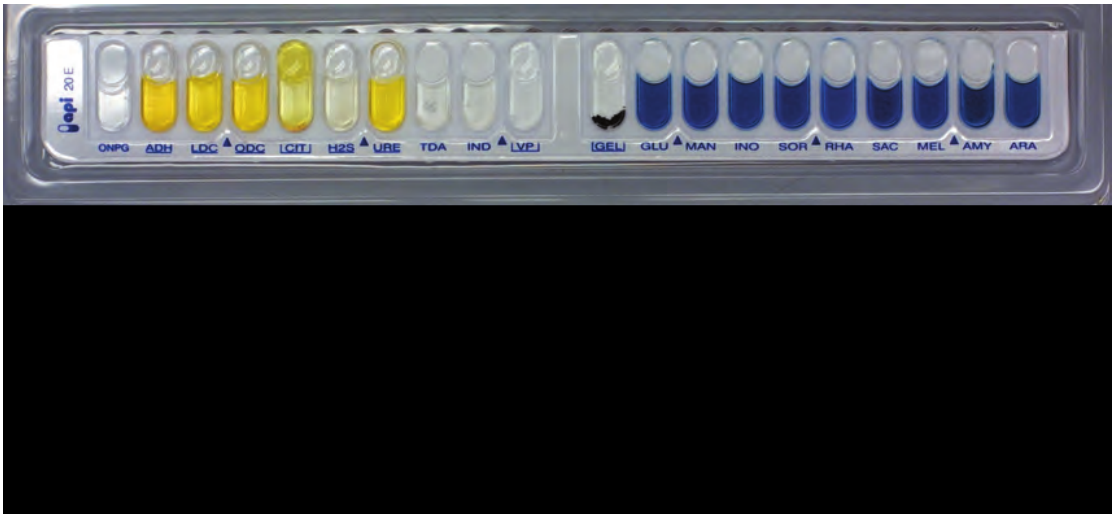
Positive: yellow color within 4 hours

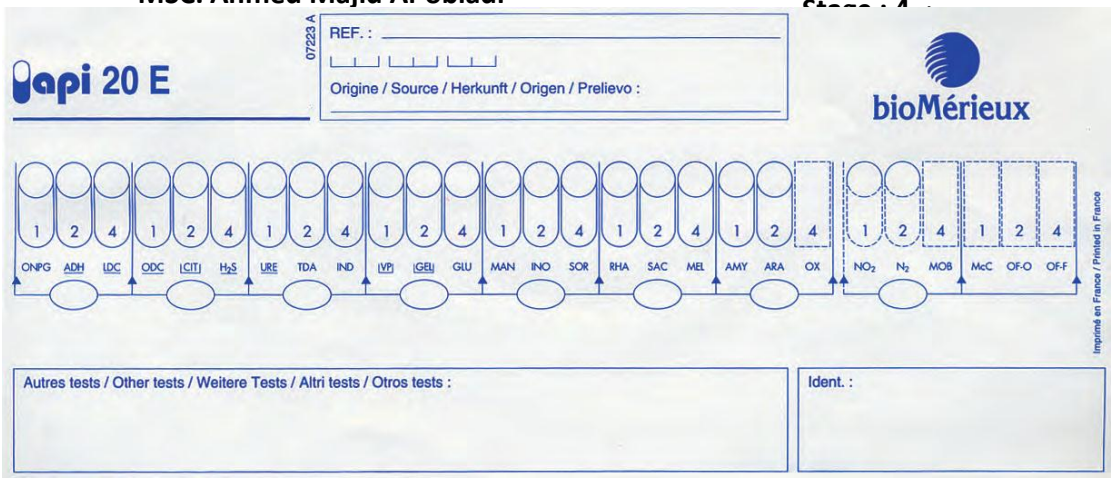
Negative: colorless at 4 hours



Analytical profile index (API) test

The API system is a plastic strip of microtubes, partially filled with different dehydrated substrates. Bacterial suspension is added to the microtubes, rehydrating the media and inoculating them at the same time. As with the other biochemical tests in this section, color changes take place in the tubes either during incubation or after addition of reagents. These color changes reveal the presence or absence of chemical action and, thus, a positive or negative result.



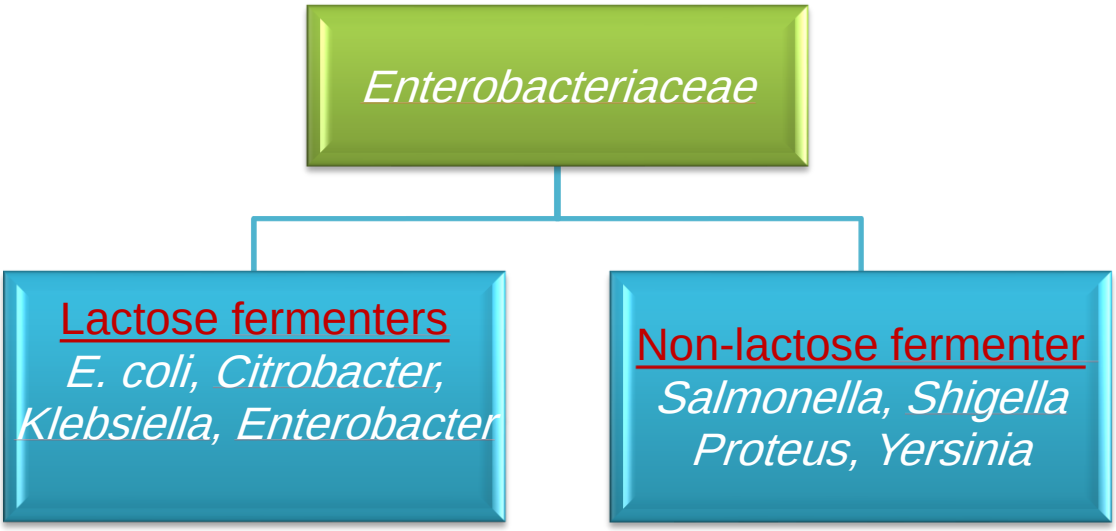


Enterobacteriaceae

Some members of the family enterobacteriaceae are found frequently on external and internal surface of man and his environment. These species may act as opportunistic pathogens to cause wide variety of infections in parts of the human body where there is some abnormality or impairment of host defenses.

All members Oxidase (-), Catalase (+) and ferment sugar(glucose).

Classification of Enterobacteriaceae



The genus *Escherichia coli*

G-ve coccobacilli cells see under the microscope and it give **I**ndole, **M**ethyl Red (+), **V**oges-Prosakaur, **C**itrate (-) (**IMVC**) Tests

Culture characters:-

Most strains of *E. coli* can grow on sample media containing glucose. Optimal growth temperature 36 -37 C, it is lactose fermenter give smooth, glossy, pink colonies on macConkey agar. They grow as yellow colonies on cysteine lactose electrolyte- deficient (CLED) agar or xylose lysine deoxycholat (XLD) agar a minority of strains are capsulated forming mucous colonies on solid media, motile. *E. coli* have many serotype such as (O cell wall lipopolysaccharide) H (flagellar protein) K (capsular polysaccharide or envelope) antigen

E. coli cause frequent opportunistic infection such as

1. Appendix abscsses
2. Peritonitis
3. Cholecystitis
4. Septic wounds
5. Bacteremia
6. Meningitis in neonates
7. UTI lower and upper

Klebsilla

Klebsiella are non motile members of enterobacteriaceae, capsulated and can be recognized by their large, greyish –white, mucous colonies on laboratory medium, they are phenylalamine deaminase negative and oxidase negative, don't produce H_2S in TSI, liquefy gelatin, hydrolysis urea but do so much less rapidly than species of *proteus*. VP+.

Species of *klebsiella*

K. pneumonia sub spp pneumonia

K. pneumonia sub spp ozoenae

K. pneumonia sub spp rhinoscleromatis

That caused severe bronchopneumonia or more chronic lesions with multiple abscesses in lung.

Proteus

G-ve , rods, motile, lactose non fermenter, MR+, urease +(hydrolysis urease rapidly). They are gelatinase and lipase (+).

They are four species:

Proteus mirabilis

Proteus myxofaciens isolated from (gypsy- mouth larvae)

Proteus pennerri

Proteus vulgaris

Swarming phenomena

In clinical lab. Proteus species are often regarded as nuisance because their characteristic swarming growth on many solid media. The cells of Proteus in young culture seen as long, curved and filamentous, sometimes reaching up to 80 m in length. It has (fishy) smell.

Swarming can be prevented by

1. The concentration of agar
2. Interdicting growth inhibitors
3. macConkey agar inhibitor it.

***Proteus* in human infection**

1. caused Urinary tract infection
2. wound(infection and abscess)
3. septicemia and osteomyelitis

Biochemical of three genera

st	dole	IR	P	trate	2S	rease	o2	ctose
coli								

lebsiella								
roteus								

Haemophilus

General characteristics

The genus *Haemophilus* consists of gram-negative pleomorphic coccobacilli or rods that can vary microscopically from small coccobacilli in direct smears of clinical material to long filaments occasionally seen in stained smears of colony growth. They are non-motile and facultative anaerobic, ferment carbohydrates, are generally oxidase and catalase positive, reduce nitrates to nitrites and are obligates parasites on the mucous membranes of humans and animal.

Culture

On chocolate agar, flat, grayish, translucent colonies with diameters of 1-2mm are present after 24 h of incubation. *H. influenzae* does not grow on sheep blood agar except around colonies of *Staphyloococci* "(satellite phenomenon)".

Growth characteristics

Identification of organisms of the *Haemophilus* group depends partly on demonstrating the need for certain growth factors called X and V acts physiologically as hemin; factor V can be replaced by nicotinamide adinine dinucleotide (NAD) or other coenzymes, colonies of *Staphylococci* on sheep blood agar cause the release of NAD, yielding the satellite growth phenomenon.

Haemophilus spp	**X**	**V**
H. inffluenzae	+	+
H. haemolyticus	+	+
H. parainfluenzae	+	v
H. parahaemolyticus	+	v
H. ducreyi	+	-
H.aphrophilus	-/+	-

Virulence factors

H. influenzae, the major pathogen within the genus, has a wide range of pathogenic potential. The following virulence factors play a role in the initiation of infection and the invasiveness of this organism:

*Capsule *immunoglobulin A (IGA) proteases *Adherence by fimbriae and other structures *Outer membranes proteins and lipopolysaccharide (LPS)

Antigenic structure

1. Capsule: composed of polyribose ribitol phosphate (PRP) so *Haemophillus influenza* can be typed into 6 types from A-F, the most important one and pathogenic is type B, while the others are rarely pathogenic.
2. Somatic Ag which are proteins and one of types M,P
3. Endotoxine.

#infections caused by *Hamophilus influenzae*

ncapsulated strains	**on capsulated strains**
epticemia	titis media with effusion
eptic arthritis	onjunctivitis
leningitis	inusitis
steomyelitis	Bacteremia
ellulitis pneumonia	neumonia
ericarditis	
piglottitis	

H. ducreyi causes chancroid (soft chancre) (a sexually transmitted disease)

H. aegypticus cause contagious conjunctivitis

Specimen

Common sources include blood, CSF, middle ear exudate, joint fluids, upper and lower respiratory tract specimens, swabs from conjunctivae, vaginal swabs, and abscess drainage. Nasopharyngeal swabs, sputum.

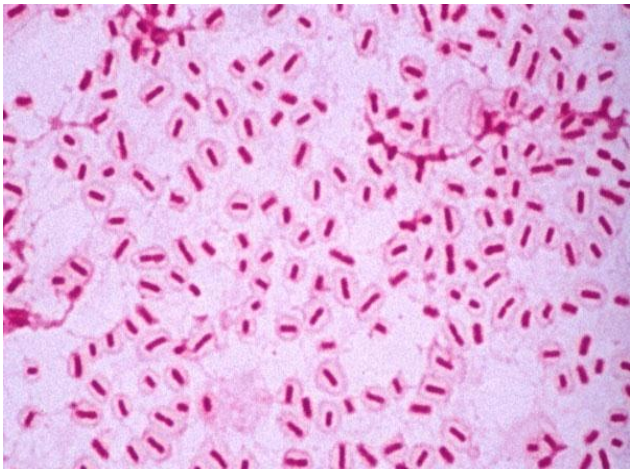
Al-safawa College

MSC. Ahmed Majid Al-obiadi

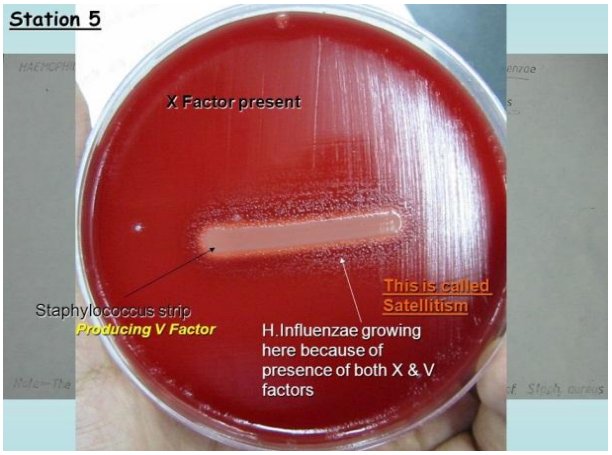
Diagnostic bacteriology

Stage : 4 st

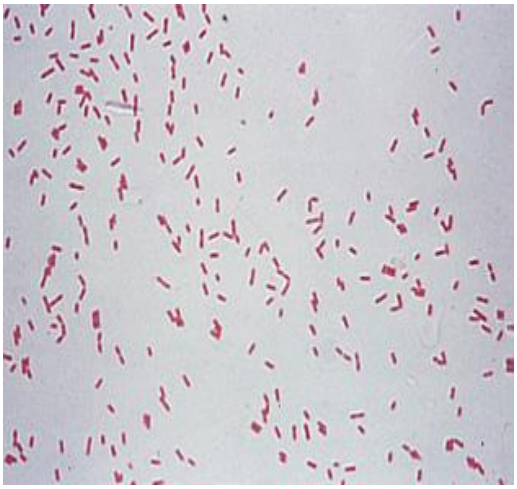
Haemophilus influenza

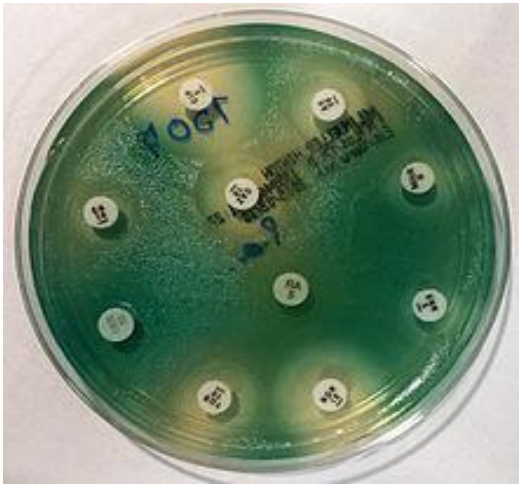


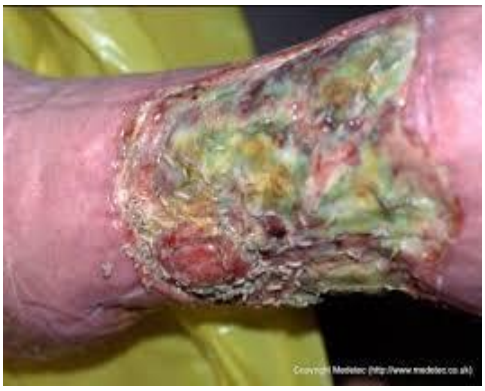
satellite phenomenon



Pseudomonas aeroginosa







Neisseria gonorrheae , Neisseria meningitidis and Moraxella catarrhalis

- **General characteristics**

✓ Gram-negative diplococcic

✓ Oxidase positive, thought of as aerobic,catalase positive, non-motile, optimal growth 35-37C, inhabit mucous membranes

Neisseria gonorrheae

Often referred to as gonococcus or GC, always considered pathogen, complex growth requirements, growth requires increased CO2.

Neisseria species differentiated by: patterns of acid detected from oxidation of carbohydrates, and other tests

Clinical significance

Causative agent of gonorrhea cause acute urethritis in males; infection of endocervix in females 50% of infections in females are asymptomatic untreated can cause infertility.

Pharyngeal infections, anorectal infections, ocular infections, PID (pelvic inflammatory disease), disseminated gonococcal disease,

Specimen Handling and Collection

Proper collection and transport are critical

Transport swab of (swab in non-nutrient buffer, can't allow specimen to dry out) endocervix, urethra, rectum, pharynx, conjunctiva, blood, joint, fluid, aspirates from skin lesions

Culture

Best method for maximal recovery of gonococci is to plate directly onto growth medium and incubates at 35C in a CO2 enriched environment. Specimens from sites with normal flora should be inoculated onto Thayer-Martin medium, also called VCN medium, Exhibit several colony types

Gram stain and oxidase test:

Identification – acid detected from carbohydrate oxidation; cysteine tryptic digest agar base (CTA) medium containing 1% carbohydrates and phenol red PH indicator

Glu	Lac	Mal	Suc
+	-	-	-

In cases of sexual assault and child abuse it is extremely important to be able to correctly identify *N. gonorrhea*

Gonococci are difficult to identify- sometimes they are so fastidious they don't grow. Can use fluorescent antibody test

Nucleic acid probe testes and nucleic acid amplification testes are available for *N. gonorrhea*

Neisseria meningitidis

Often referred to us meningococcus or MC

May colonize nasopharyx as non-pathogens in a carrier state; some strains cause epidemic and acute meningitis

Can grow on blood agar not on nutrient agar

Growth enhanced by humidity and CO2, encapsulated

Clinical significance

Carries in the population (5-15%), spread person or by aerosols

Meningococcemia-characterized by small hemorrhagic skin lesions (petechial) meningitis

Virulence is related to capsular polysaccharide serogroup (13serogroups) from bloodstream may get meningococcal arthritis, osteomyelitis, and pericarditis

Specimen collection

Collect cerebrospinal fluid (CSF) and blood, swab skin lesions and nasopharynx

Culture

Culture and incubation conditions are those described for gonococcus

Gram stain and oxidase test

Identification- acid detected from carbohydrate oxidation

Glu	Lac	Mal	Suc
+	-	+	-

Also direct antigen test for capsular polysaccharides

Moraxella catarrhalis

1-5% of adults carry this organism; children and older adults may have higher frequency

Can grow on nutrient agar

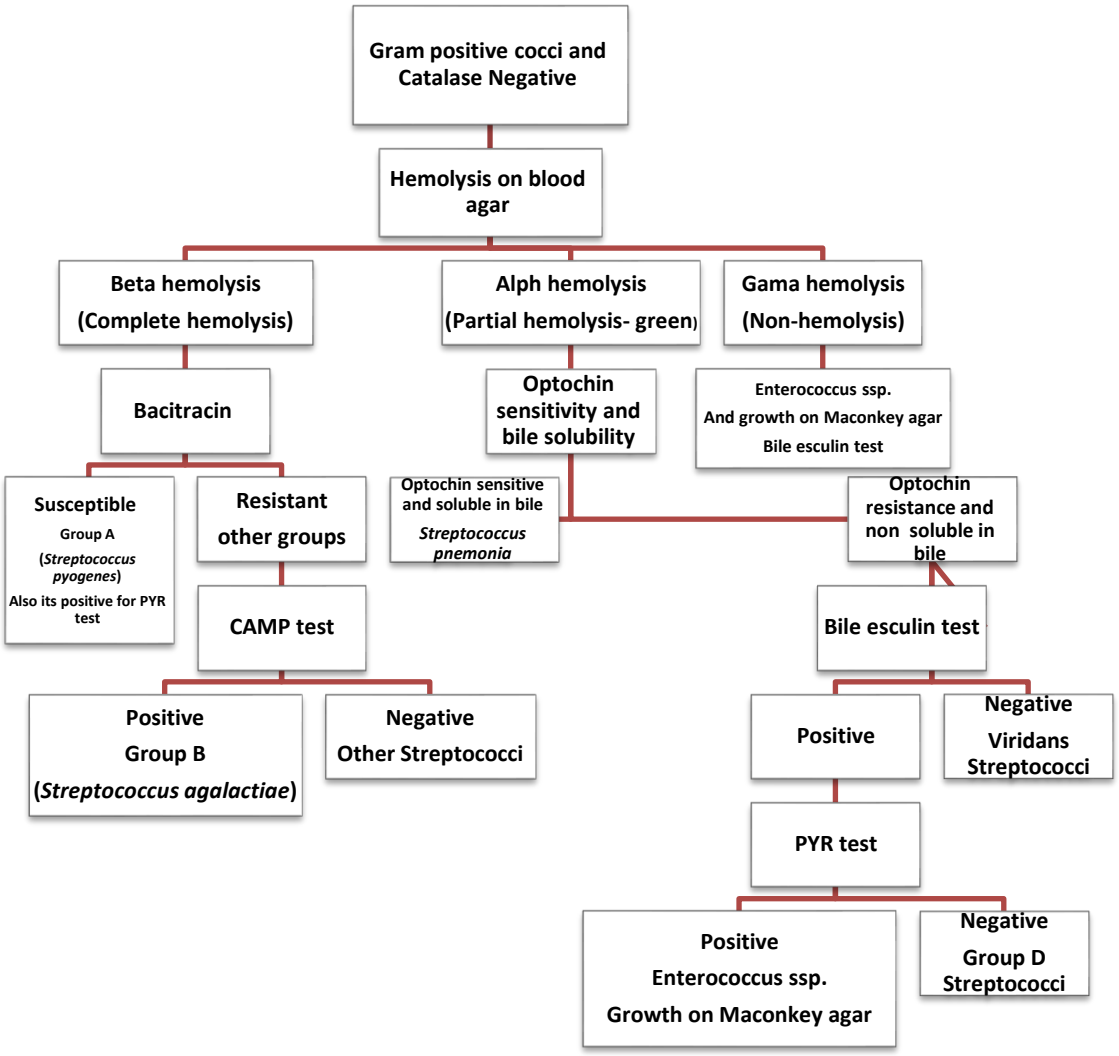
Must be distinguished from Neisseria species in the lab

Clinical significance

Otitis media, sinusitis, bronchitis, pneumonia

Differentiation of the various *Neisseria* species may be done by: observing growth on nutrient agar (pathogenic species are fastidious and do not grow on this medium, whereas, non-pathogenic species are not fastidious and will grow on this medium),

Identification of Streptococcus and Enterococcus



Hemolysis on blood agar

The three major types of hemolysis are α-hemolysis, β-hemolysis, and gama-hemolysis.

β-hemolysis, the complete destruction of RBCs and hemoglobin, results in a clearing of the medium around the colonies. **α-hemolysis** is the partial destruction of RBCs and produces a greenish discoloration of the agar around the colonies.

gama-hemolysis is nonhemolysis and appears as simple growth with no change to the medium

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The bacitracin test is used to differentiate and presumptively identify Beta-hemolytic group A streptococci (*Streptococcus pyogenes*) from other Beta-hemolytic streptococci.

Inoculate Blood Agar plate, then place bacitracin antibiotic discs in the center of the inoculum. Following incubation (at 37 C for 24 h), measure the clear zones surrounding the discs to determine susceptibility or resistance of the test organisms to the antibiotic.

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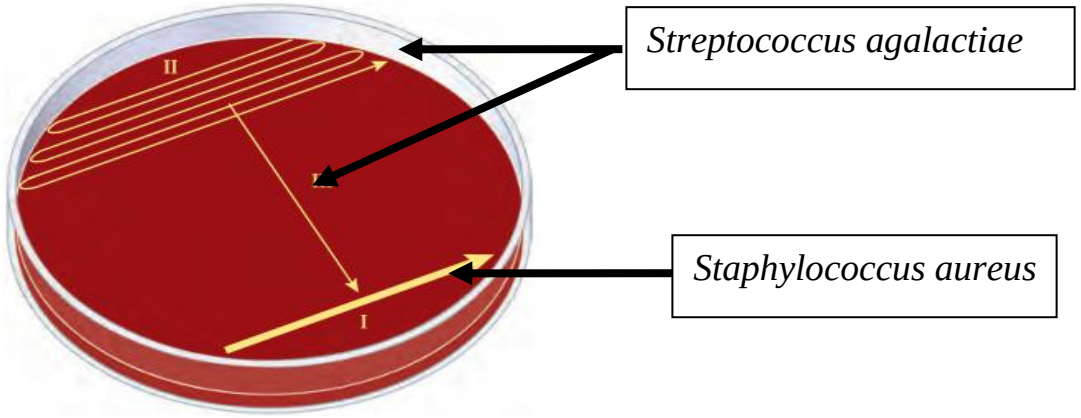
Group B *Streptococcus agalactiae* produces the CAMP factor—a hemolytic protein that acts synergistically with the beta-hemolysin of *Staphylococcus aureus*.

When streaked perpendicularly to an *S. aureus* streak on blood agar, an arrowhead-shaped zone of hemolysis forms and is a positive result.



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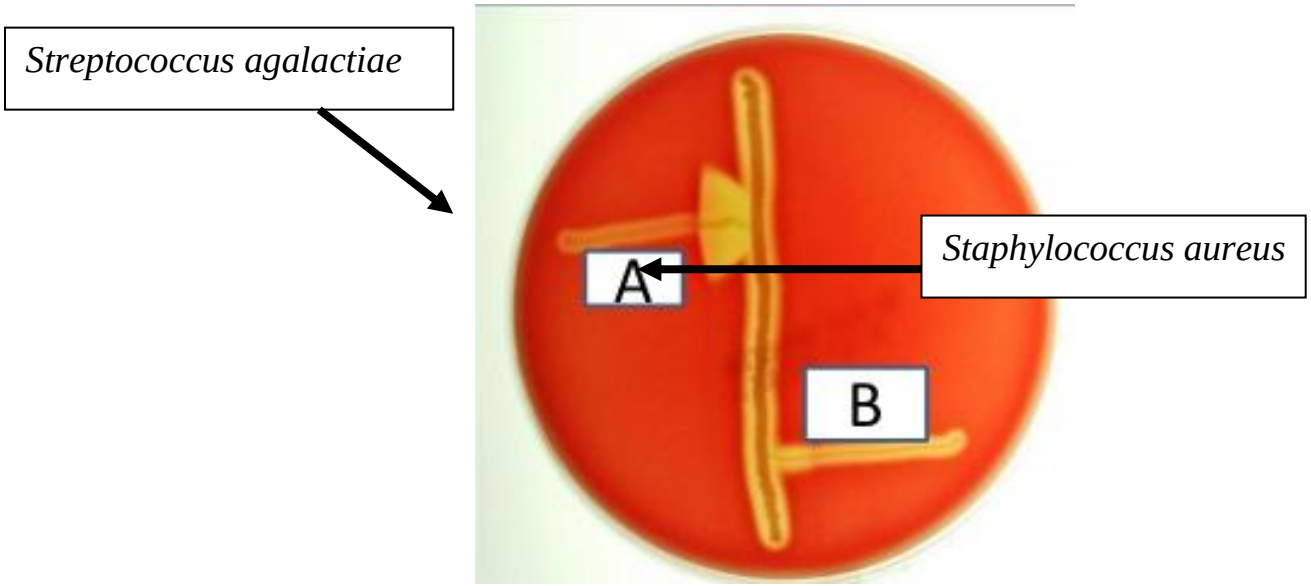

Bile Esculin Test

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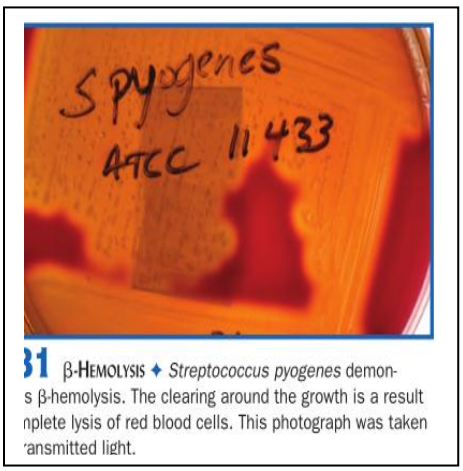
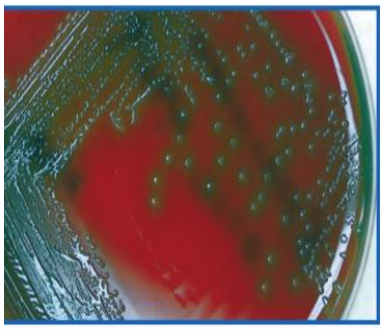


β-HEMOLYSIS ✦ *Streptococcus pyogenes* demonstrates β-hemolysis. The clearing around the growth is a result of complete lysis of red blood cells. This photograph was taken with transmitted light.

Beta-hemolysis



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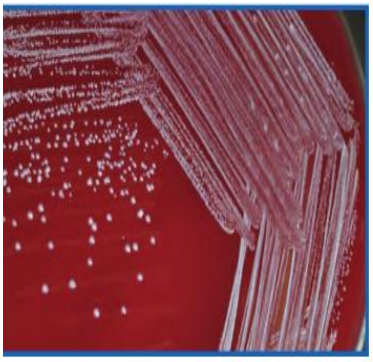
Alpha-hemolysis



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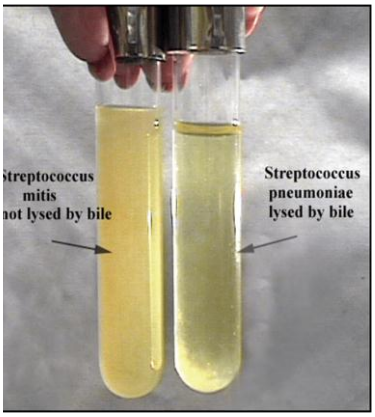
Gama- hemolysis

BACITRACIN SUSCEPTIBILITY ON A SHEEP BLOOD AGAR
The organism above has no clear zone and is resistant. The organism below has a clear zone larger than 10 mm

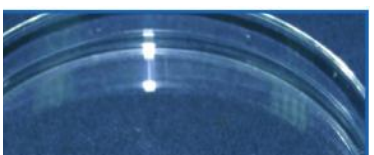
Bacitracin test

OPTOCHIN SUSCEPTIBILITY TEST ✦ The zone of inhibition around the disk indicates susceptibility to optochin and

Optochin test



Bile solubility



Al-safawa College

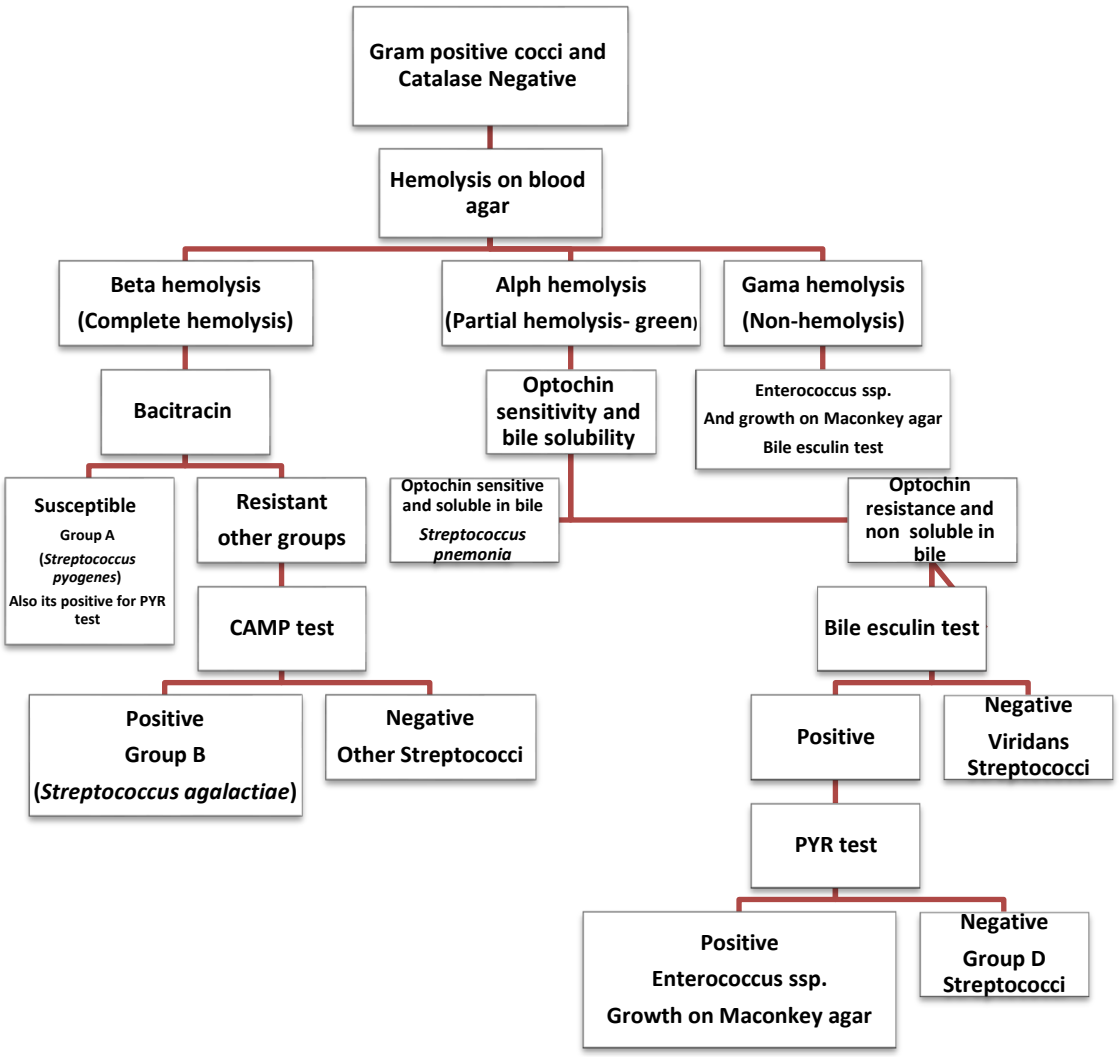
MSC. Ahmed Majid Al-obiadi

Diagnostic bacteriology

Stage : 4 st

CAMP Positive test Bile esculin test PYR test

Identification of Streptococcus and Enterococcus



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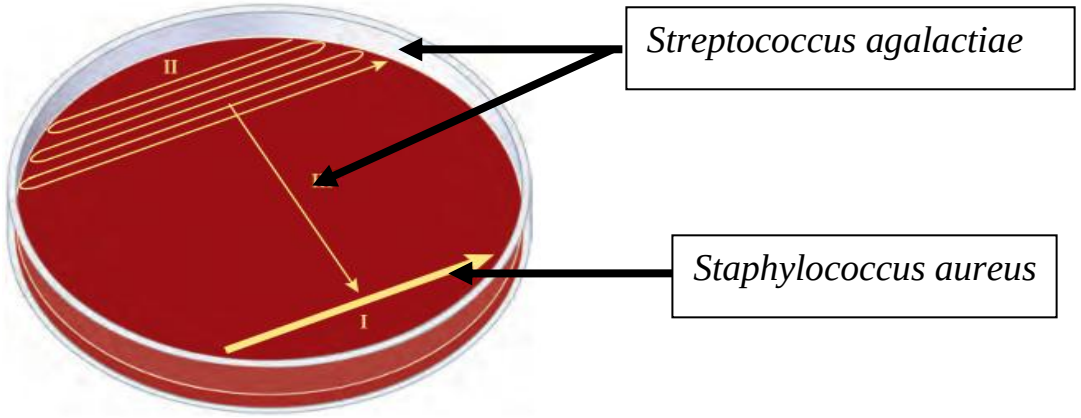

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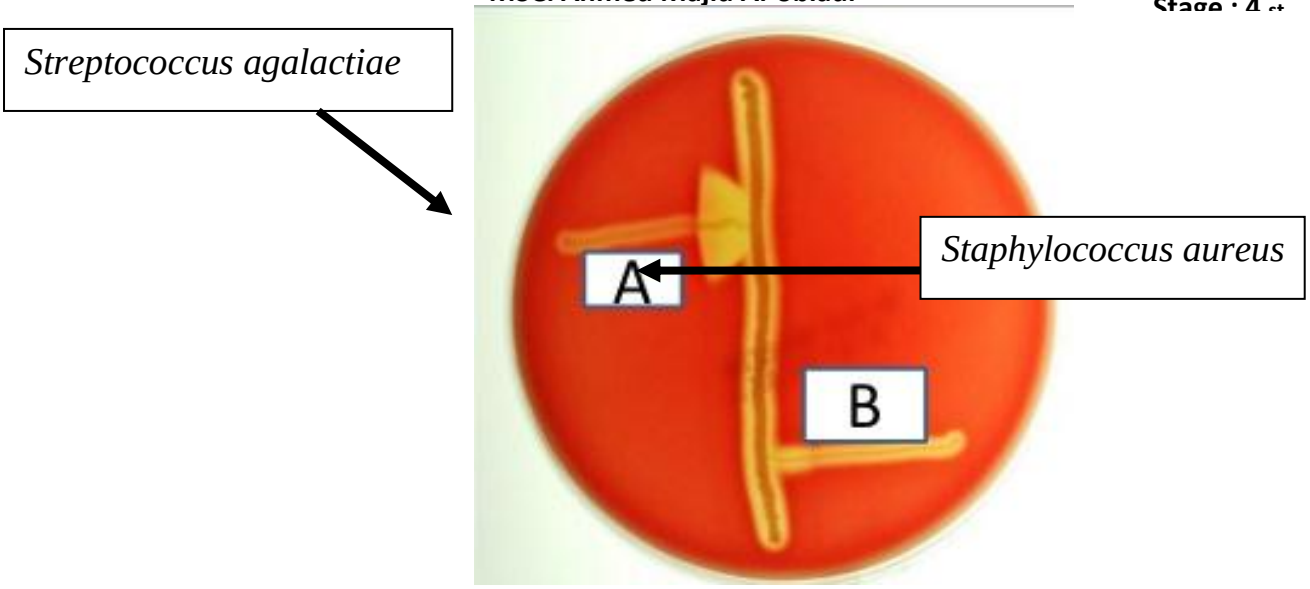

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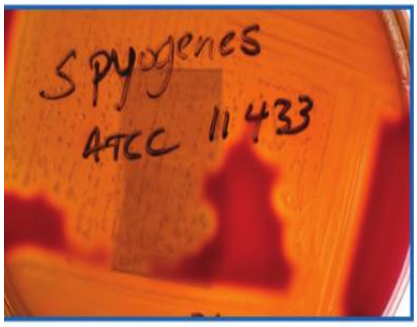

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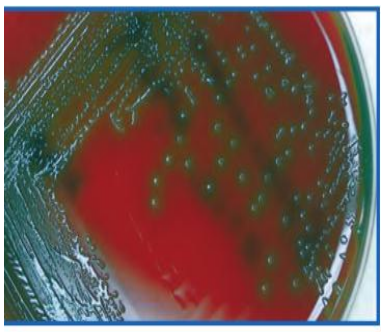

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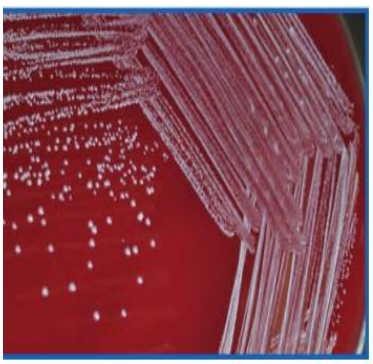

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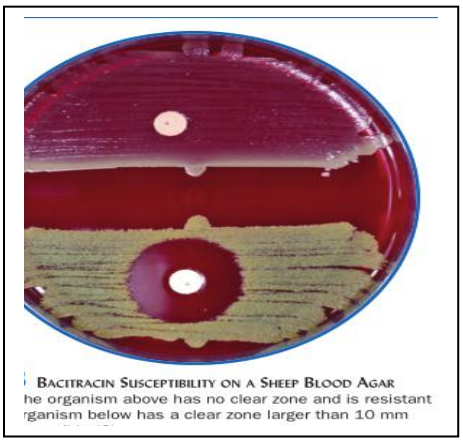
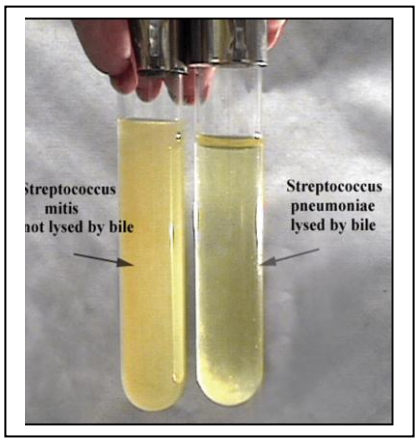
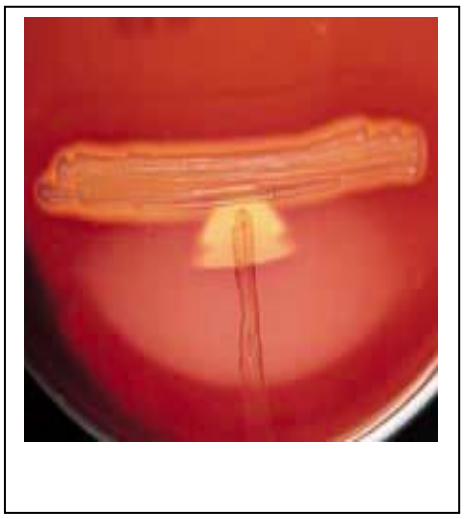
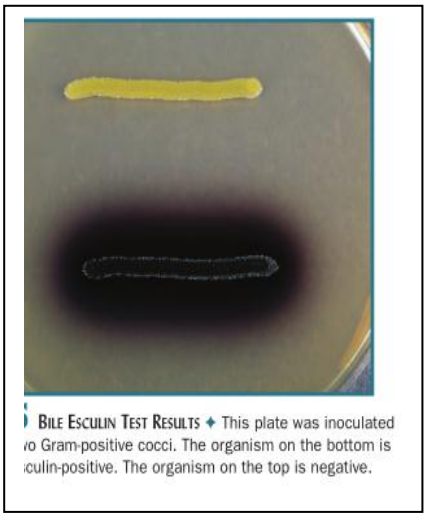
Beta-hemolysis Alpha-hemolysis Gama- hemolysis

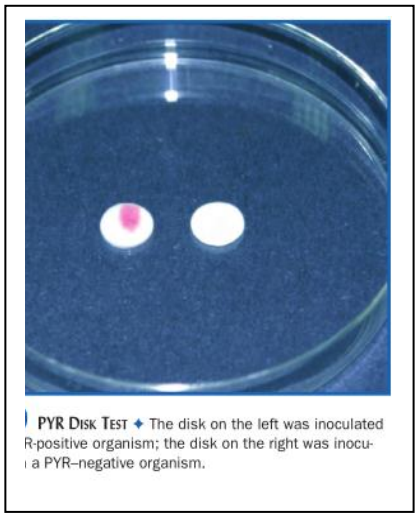




Bacitracin test Optochin test Bile solubility







CAMP Positive test Bile esculin test PYR test

Vibrio

Vibrio are gram negative bacteria, facultative anaerobic, motile, curved or straight. Vibrios either require NaCl or have their growth stimulated by its addition. Oxidase positive and reduce nitrates t nitrites. Within the Vibrionaceae are many different species, most of which are normal inhabitants the aquatic (water) environment

Vibrio spp.

1. *V. cholera*
2. *V. parahemolytica*

3. *V. vulnificus*
4. *V. alginolyticus*

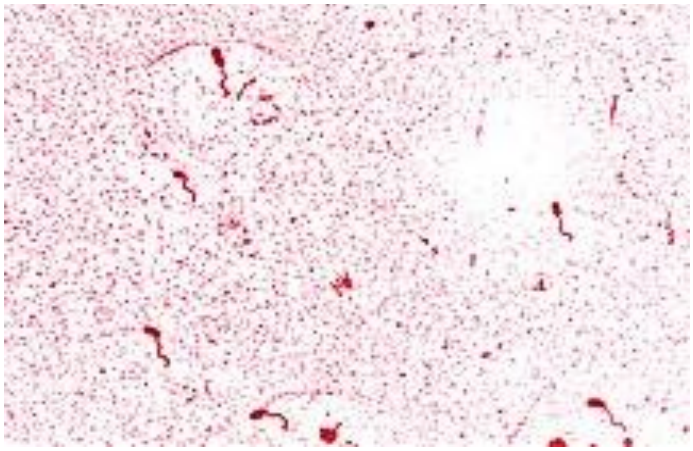
V. cholera infected GIT Cause watery diarrhea (rice watery diarrhea), wound infection.

V. cholera serotype O1, O139 and non -O1

Vibrio grow in nutrient broth with add NaCl. Selective media Thiosulfate citrate Bile salt sucrose) (TCBS) give green colonies

Basic Characteristics	**Properties (*Vibrio cholerae*)**
Capsule	Non-Capsulated
Citrate	+ve
Gas	-ve
Gelatin Hydrolysis	+ve
H2S	-ve
Hemolysis	Beta Hemolysis
Indole	+ve
MR (Methyl Red)	-ve
Nitrate Reduction	+ve
Oxidase	+ve
Spore	Non-Sporing
String Test	+ve
Urease	-ve
VP (Voges Proskauer)	Variable
Fermentation of	
Glucose	+ve
Lactose	Variable
Sucrose	+ve
Enzymatic Reactions	
Arginine Dehydrolase	-ve

Lysine	+ve
Ornithine Decarboxylase	+ve



Aeromonas

The genus has been placed in the new family Aeromnaceae, 4 species were recognized:

A. hydrophila

A. Salmonicida

A. veronii biovar sobria

A. caviae

The following 3 species are of primary clinical importance in human infections:

A. hydrophila, A. caviae, A.veronii biovar sobria cause gastroenteritis and wound infection.

Properties of Aeromonas:

The organisms are straight G-ve rods, about 4.5 x1 mm, with occasional filaments , motile by polar flagella or few have lateral flagella, temperature range for growth (4-42) with optimum about 32 C, PH range 4.5-9 they grown most laboratory media, on blood agar *Aeromonas* give wide zones of ß hemolytic. Non halophilic, they will not grow in 6% NaCl and produce oxidase (+), catalase (+).

Biochemical reaction

character	*A. hydrophila*	*A. caviae*	*A.Veronii biovar sobria*
Aesculin hydrolysis	+	+	-
Voges-proskauer	+	-	+
Lysine decarboxylase	+	-	+
Ornithine decarboxyla	-	-	-
Indole production	+	+	+
glucose	+	-	+
arabinose	+	+	-
mannitol	+	+	+
sucrose	+	+	+