Identification of medical bacteria by Biochemical tests

In the microbiology lab, biochemical test relays on enzymes, which is glycoprotein or protein that act as catalyst by lowering the activation energy of certain biological reaction.

Substrate $\xrightarrow{\text{enzyme}}$ product

We can use our knowledge in bacterial enzymes to identify the causative agent of diseases and distinguish between bacterial species.

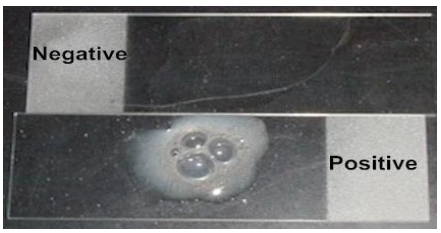
1- **Haemolysis**: Some types of pathogenic bacteria are able of producing haemolysin enzyme that lyses Erythrocytes (RBCS). This can be detected *in vitro* on blood agar plates. There are three types of haemolysis:

A- β-haemolysis: Complete clear circular zone around the bacterial colonies due to complete lysis of red cells. e.g. *Streptococcus pyogenes and Staphylococcus aureus*.

B-: **α-haemolysis:** appear as greenish zone around the colonies· due to partial haemolysis of RBCs. e.g. *Streptococcus viridian* .

C- γ-haemolysis: (no haemolysis) no any obvious changes around the colonies e.g. *Enterococcus faecalis.*

2- **Catalase test:** The Catalase Test is used to identify organisms that produce the enzyme **catalase**. It is most commonly used to differentiate members of staphylococci from streptococci. Catalase acts as a catalyst in the breakdown of hydrogen peroxide (H_2O_2) to oxygen gas and water.

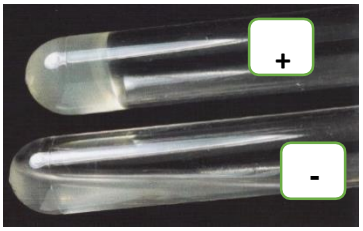


$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

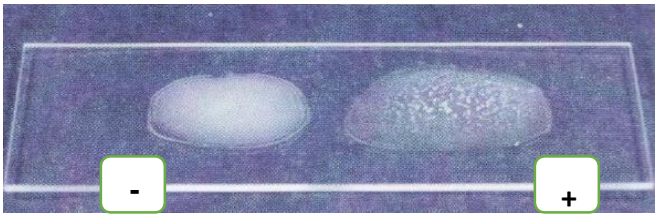
Procedure: A small amount of bacterial culture to be tested is picked from nutrient agar by stick or glass rod and put it on the surface of a clean slide, where a drop of (3 %H2O) was added. Formation of gas bubbles indicates **a positive result**. A false positive reaction may obtain if the culture medium contain catalase (Blood agar) or if iron loop is used.

3- **Coagulase test:** Some bacteria produce coagulase enzyme that converts soluble fibrinogen protein to insoluble fibrin protein (coagulation of plasma). The Coagulase Test is typically used to differentiate *Staphylococcus aureus* from other Gram-positive cocci. The formation of clot around an infection caused by this bacterium will protects it from phagocytosis.

Procedure: Coagulase enzymes occur in two forms—**bound Coagulase (**also called **"clumping factor")** and **free coagulase**. Two forms of the Coagulase Test have been devised to detect the enzymes: the Tube Test and the Slide Test.

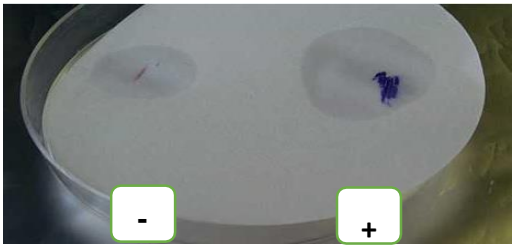


(a) <u>Tube coagulase test</u>: to detect coagulase, suspend several colonies in 0.5 ml of rabbit plasma, incubate the inoculated plasma for one, four, and 24 hours and record the levels of coagulation. Clot formation indicates *S. aureus* (positive +ve).



(b) <u>Slide coagulase test</u>: a more rapid and simple method in which a drop of plasma is added to a suspension of bacteria on a glass slide; visible clumping indicates the presence of coagulase.

Note: The Tube Test detects the presence of either bound or free coagulase, while the Slide Test detects only bound coagulase.

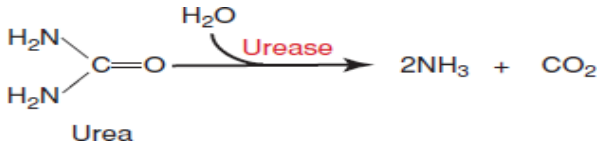


4- **Oxidase test:** The Oxidase Test is used to identify bacteria containing the respiratory enzyme **cytochrome c oxidase**, which related to respiratory electron transport chain and it produced by strictly aerobic (or facultatively anaerobic) bacteria e.g. *Pseudomonas, Vibrio* and *Neisseriae*.

Procedure: A small area of filter paper is soaked with a freshly prepared 1% oxidase reagent (Tetramethyl-*P*-Phenylenediamine Dihydrochloride) bacterial colony to be tested is picked from agar by stick or glass rod and put it on the soaked area. **A positive result** is indicated by formation of deep purple color due to reduction of this dye by oxidase enzyme .

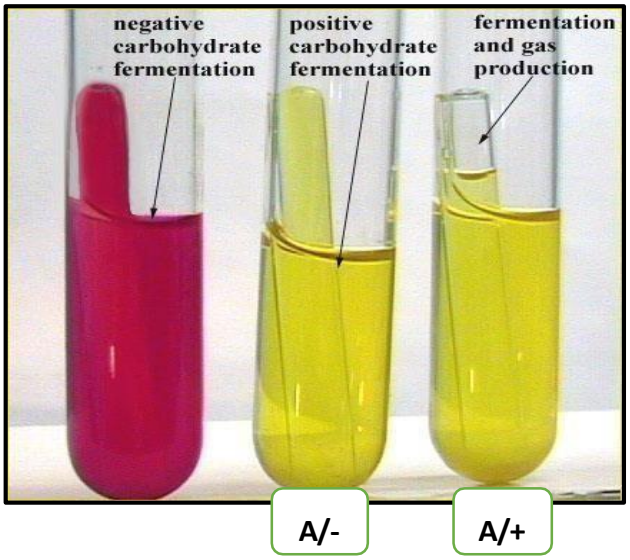
5- **Urease test:** The Urease Test is used to differentiate organisms based on their ability to hydrolyze urea with the enzyme **urease** to make ammonia and carbon dioxide. Urinary tract pathogens from the genus *Proteus* may be distinguished from other enteric bacteria

by their rapid urease activity. Also *Helicobacter pylori & Y. enterocolitica* are urease positive, while *Salmonellae* and *shigellae* do not produce urease.



The hydrolysis of urea raises the pH of the medium (urea agar) to above 7.0 and the pH indicator (phenol red) turns the medium from yellow to red pink (positive). Urease-negative organisms either produce no color change in the medium or turn it yellow from acid products.

6- **Carbohydrate fermentation test**: this test detect the ability of microorganisms to ferment a specific carbohydrate (break down sugars whether mono-, di- or even polysaccharide and produce acid or acid and gas). Fermentation patterns can be used to differentiate among bacterial groups or species of ***Enterobacteriaceae*** and to distinguish them from other Gram- negative rods. pH indicator (phenol red) containing media with suitable sugar are reliable to confirm fermentation, depending on the color changes of pH indicator due to acid production. Gas production can be detected using **Durham tube** (small inverted tube placed into the liquid media to collect gas bubbles).



Positive result for acid production as a color change from red to yellow. **Positive result for gas production** is a bubble in the Durham tube **Completely negative** result has no color change or reddish color and no bubble.

7- **Triple sugar iron (TSI) and Kligler iron agar (KIA):** Both of these tests are primarily used to differentiate members of *Enterobacteriaceae* and to differentiate them from other Gram-negative rods such as *Pseudomonas*. These media are composed from 1) Sugars: glucose, lactose and sucrose (KIA contain only glucose and lactose). 2) pH indicator: phenol red (red in alkaline pH and yellow in acidic pH). 3) Ferrous sulfate as an indicator of H_2S production. 3) Others: includes beef extract, yeast extract, and peptone as carbon and nitrogen sources, and sodium thiosulfate as a reducible sulfur.

Procedure: The medium is prepared as a shallow agar slant with a deep butt, thereby providing both aerobic and anaerobic growth environments. It is inoculated by a stab in

the agar butt followed by a fishtail streak of the slant. The incubation period is 18 to 24 hours for carbohydrate fermentation and up to 48 hours for hydrogen sulfide reactions. Three criteria can be detected:

l- Bacterial ability to produce gas from sugar fermentation. This makes the media to push up or break up.

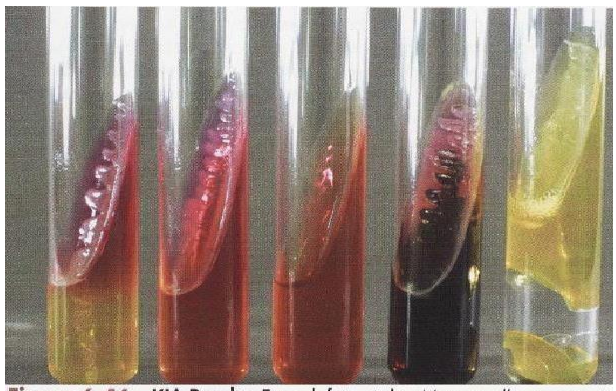


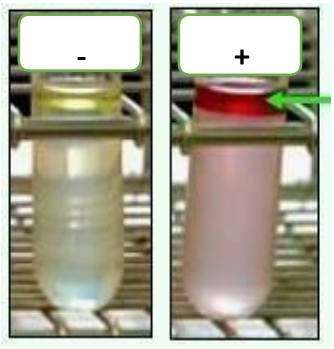
Figure 6-46. KIA Results. From left to right: *Morganella morganii* (K/A, atypically not producing gas), *Pseudomonas aeruginosa* (K/NC), uninoculated control, *Proteus mirabilis* (K/A,H_2S), and *Escherichia coli* (A/A,G).

2- H_2S gas production can be detected by the production of black precipitate in the bottom of the media. As H2S react with iron in the media to form black ferrous sulfide in the butt .

3- Ability to ferment sugars that can be detected by color changes from red to yellow. Position of the color change distinguishes the acid production associated with glucose fermentation from the acidic products of lactose or sucrose fermentation. Bacteria that ferment glucose produce acid that turn the color of the pH indicator to yellow in the butt but not in the slant (result——> K/A). While lactose or sucrose fomenters produce more acid that turn both butt and slant to yellow (result—> A/A). No fermentation will appear as Red slant/red butt (k/k) or No change in slant/ No change in butt (NC/NC).

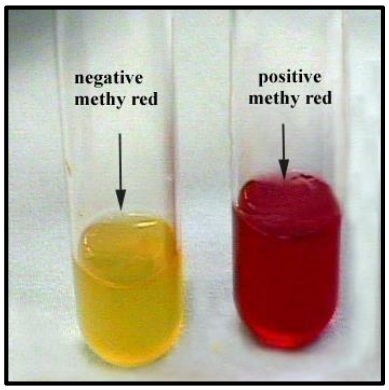
8-**IMViC**: A battery of biochemical tests known as IMViC are used in the clinical lab to distinguish between enteric microorganisms (enterobacteriaceae). The acronym IMViC stands for indole, methyl red, VogesProskauer and citrate. The "i" in the acronym is added for pronunciation purposes.

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



Peptone liquid medium containing tryptophan is inoculated with the tested bacteria and incubated at 37 °C for 24 hrs. Few drops of **Kovac's reagent** are added to the bacterial growth. The presence of red ring in the

superficial layer of the medium indicate +ve result of indole production e.g. *E.coli.* Yellow ring indicate -ve result e.g. *Klebsiella* .

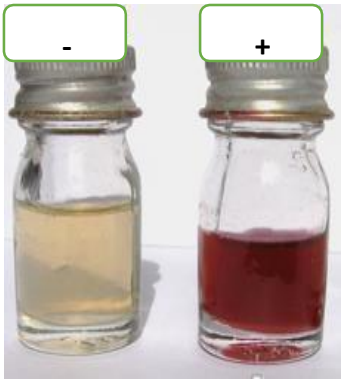


B- Methyl red/ Voges-Proskauer tests: Both MR and VP tests are used to determine what end products result when the tested organism degrades glucose (for energy production) and this depend on the type of enzyme that the bacteria have.

MR- used to detect acid as an end product from complete glucose fermentation. VP- used to detect acetoin (acetyl methyl carbinol) production from partial glucose fermentation.

MR-VP medium is used for both tests; it's inoculated with the test bacteria, alter incubation at 37 °C for 24hrs:

•In MR; 5 drops of methyl red indicator are added. Color changes of the medium to red indicate positive result e.g. *E. coli* and yellow in negative result e.g. *Kebsiella*



•ln VP; Voges proskauer reagent (Barritt's reagent) is added to the medium. This reagent is consists of reagent A (5% α-naphtbol) and reagent B (40% KOH). Positive reaction can be detected by developing a pink-burgundy color within 20-30 min. e.g. of +ve result is *Enterabacrer aerogener and Klebsiella* while -ve result as *E. coli*.

C- Citrate utilization: It used to test the ability of bacteria to consume citrate as a sole source of carbon. Simmon's citrate agar can be used with bromthymol blue as pH indicator.The tubes will be incubated after inoculation by stabbing and streaking, +ve result is blue)meaning the bacteria metabolised citrate) e.g. *Enterobacter and Klebsiella* and –ve result remains green e.g. *E coli.*