
Biochemical tests used for identification of bacteria

Biochemical tests have an important role in the identification of bacteria to classify bacteria and determine the causative agent of diseases.

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

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

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

2- Mannitol fermentation: This can be detected *in vitro* on mannitol salt agar plates. *Staphylococcus aureus* can be ferment the sugar (mannitol) in this media & become yellow, while *S. epidermidis* cannot ferment the sugar & become white.

3 -Pigment production: Some type of bacteria able to produce a characteristic pigments. There are two types of pigments:

Endopigment: Remain bound to the body of the M.O. and doesn't diffuse to the surrounding media e.g. *Serratia* and *Staphylococcus*.

Exopigment: Soluble which readily diffuse into the surrounding media e.g. *Pseudomonas aerogenosa* produce four types of pigments Pyocyanin (blue-gree) Pyoveridin (green), Pyorubin (red) and Pyomelanin (black).

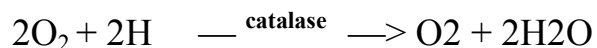
4- Motility test: Motility of bacteria can be detected by several methods; used to determine whether an organism is equipped with flagella or not e.g.:

1-Stabbing of semisolid medium .
technique.

2-Hanging drop

3-Flagellar stain. Motile bacteria such as *Salmonella*, *Proteus* and *E. coli*.

5-Catalase production test: Some aerobic bacteria are able to produce catalase enzyme that catalyses H₂O₂ (Hydrogen peroxide) and releases O₂ and H₂O.



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 %H₂O₂) was added. Formation of gas bubbles indicates a **positive result**. A false positive reaction may obtain if the culture medium contains catalase (Blood agar) or if iron loop is used.

6- Coagulase production: Some bacteria produce coagulase enzyme that converts soluble fibrinogen protein to insoluble fibrin protein (coagulation of plasma). Coagulase is a virulence factor of *Staphylococcus aureus*. The formation of clot around an infection caused by this bacterium will protect it from phagocytosis.

A- Bound coagulase (detected in Slide method): Homogenous suspension of the test organism is made in a drop of saline on a clean slide then mixed with a drop of undiluted human or rabbit plasma. Examine it under the microscope and **look for clumping as positive result, as the enzyme will precipitate the fibrin in the plasma on the cell surface.**

B-Tube method (detected in Free coagulase): It is done by adding 5 drops of an overnight broth culture of the test organism to 1 ml of human or rabbit plasma diluted 1:6 in sterile saline. The tubes are incubated for 4 hours at 37 °C in water bath and inspected hourly for clot formation by tilting the tube. Clot will float in the fluid or the whole plasma converts into gel.

7-Oxidase test: Use to detect the production of cytochrome oxidase which is related to respiratory electron transport chain and it is produced by strictly aerobic bacteria e.g. *Pseudomonas* and *Neisseriae*.

Procedure: A small area of filter paper is soaked with a freshly prepared 1% oxidase reagent (Tetramethyl-p-phenylene Diamine 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.

8-Carbohydrate utilization (Fermentation): Many types of bacteria are able to break down sugars whether mono-, di- or even polysaccharide and produce acid or acid and gas. 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.

8-Triple sugar iron (TSI) and Kligler's gon agar (KIA)

TSI medium contain- :

*Sugars: glucose, lactose and sucrose (KIA contain only glucose and lactose).

*pH indicator: phenol red (red in alkaline pH and yellow in acidic pH).

*Ferrous sulfate as an indicator of H₂S production.

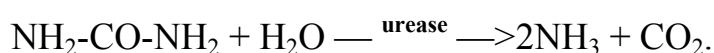
These media are used to detect ability of bacteria to ferment these sugars and this aid in the identification and classification of enteric G-ve bacilli (enterobacteriaceae). Three criteria can be detected:

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

2- H₂S 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 fermenters produce more acid that turn both butt and slant to yellow (result—> A/A).

9- Urease test: This test is used to identify bacteria able of hydrolyzing urea using the enzyme urease to make ammonia and carbon dioxide. The hydrolysis of urea raises the pH to above 7.0 and the pH indicator (phenol red) turns the medium from yellow to red pink.



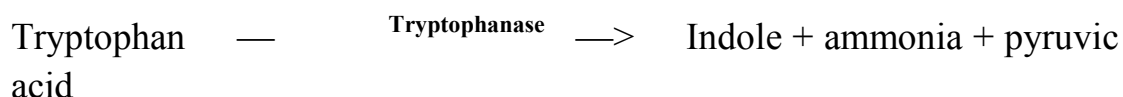
Urea

ammonia

Ex: of urease producer are *Helicobacter pylori* and *V. cholerae*, *Klebsiella* & *Proteus* .

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

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



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

Glucose phosphate peptone water medium is used for both tests; it's inoculated with the test bacteria, after incubation at 37 °C for 24hrs:

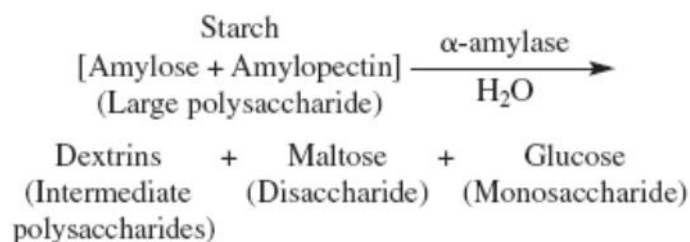
- In MR; 5 drops of methyl red indicator are added. Color changes of the medium to red indicate positive result e.g. *E. coli* and yellow in negative result e.g. *Kebsiella*

- In VP; Voges proskauer reagent (Barritt reagent) is added to the medium. This reagent is consists of reagent A (5% or-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, +ve result is blue (meaning the bacteria metabolised citrate) e.g. *Enterobacter and Klebsiella* and –ve result remains green e.g. *E coli*.

Starch test

Many bacteria produce enzymes called **hydrolases**. Hydrolases catalyze the splitting of organic molecules into smaller molecules in the presence of water. This exercise will present the hydrolysis of the carbohydrate starch. The starch molecule consists of two constituents: **amylose**, an unbranched glucose polymer (200 to 300 units) and **amylopectin**, a large branched polymer. Both amylopectin and amylose are rapidly hydrolyzed by certain bacteria, using their **-amylases**, to yield dextrans, maltose, and glucose, as follows:



Gram's iodine can be used to indicate the presence of starch. When it contacts starch, it forms a blue to brown complex. Hydrolyzed starch does not produce a color change. If a clear area appears after adding Gram's iodine to a medium containing starch and bacterial growth, α -amylase has been produced by the bacteria (figure 22.1). If there is no clearing, starch has not been hydrolyzed.

Figure 20.2 Carbohydrate Fermentation. (a) Possible carbohydrate fermentation patterns of microorganisms, with phenol red as the pH indicator. (b) The tube on the left is the control. The next tube shows alcohol fermentation. Notice the gas bubble at the top. The third tube from the left shows no carbohydrate fermentation (negative). The tube on the right shows acid and gas production.

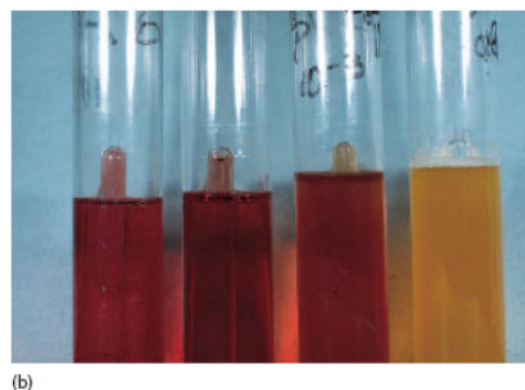
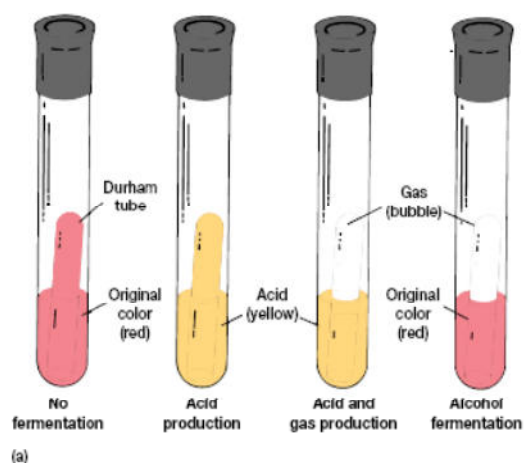
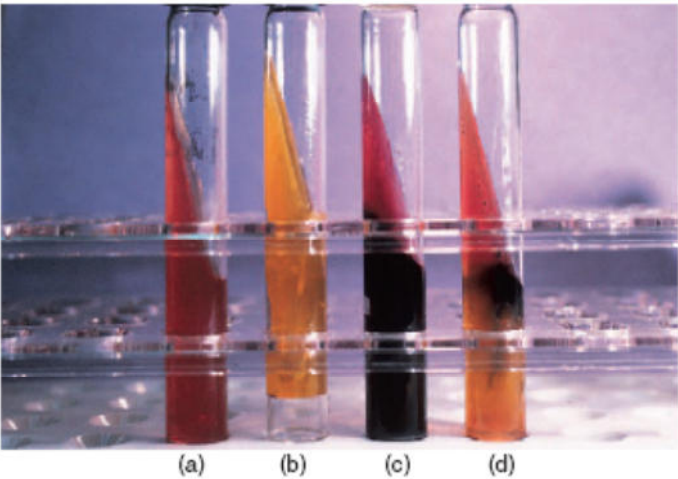


Figure 21.3 Triple Sugar Iron Reactions (TSI-3) and Their Interpretation. (a) The tube on the left is an uninoculated control. Notice the red color. (b) The second tube from the left has a yellow slant (acid), yellow butt (acid), gas production at the bottom of the tube, and no H₂S production. This would indicate a weak lactose fermenter. (c) The third tube from the left has a red slant (alkaline), red butt (alkaline), and the black indicates H₂S production, but no gas. (d) The tube on the right has a red slant (alkaline), yellow butt (acid), H₂S production, but no gas production. This would indicate a nonlactose fermenter.



	Tube a	Tube b	Tube c	Tube d
Slant	-	A	K	K
Butt	-	A	K	A
Gas	-	+	-	-
H ₂ S	-	-	+	+

Figure 22.1 Test for Starch Hydrolysis After Adding Gram's Iodine. (a,c) Positive hydrolysis. The complete breakdown of all starch is shown by the clear (white) halo. (b) Negative hydrolysis. Starch remains intact—no color change as indicated by the purple to brown color around the streak.

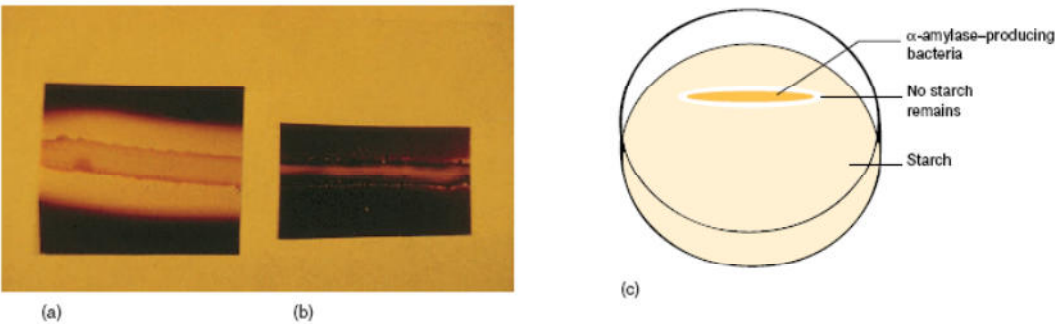


Figure 25.1 Indole Test. The tube on the left is indole negative and the tube on the right is indole positive.

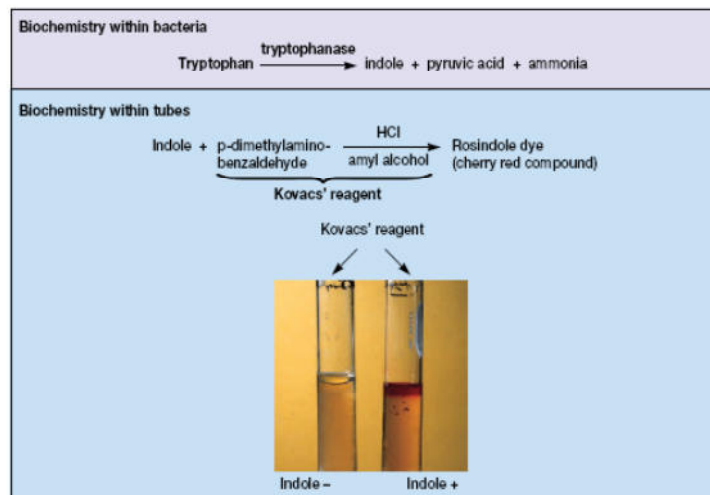


Figure 25.3 Methyl Red Test. (a) *Escherichia coli*, MR+. (b) *Enterobacter aerogenes*, MR-.

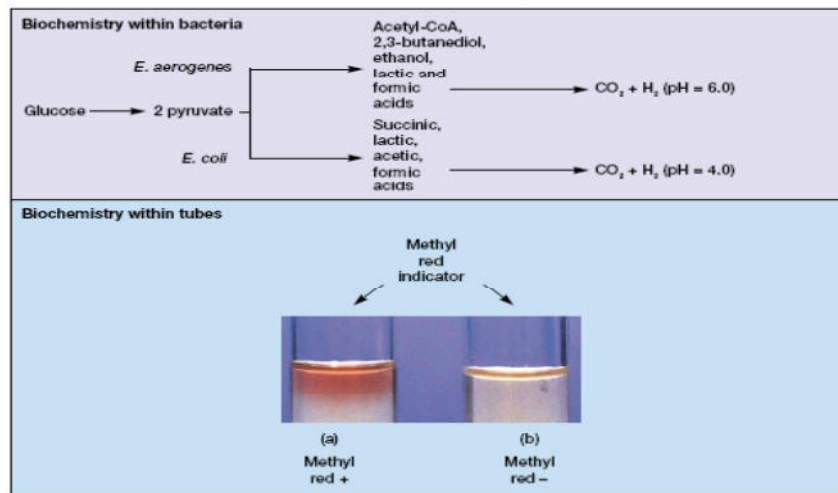


Figure 25.4 Voges-Proskauer Test. (a) *Enterobacter aerogenes*, VP+. (b) *Escherichia coli*, VP-.

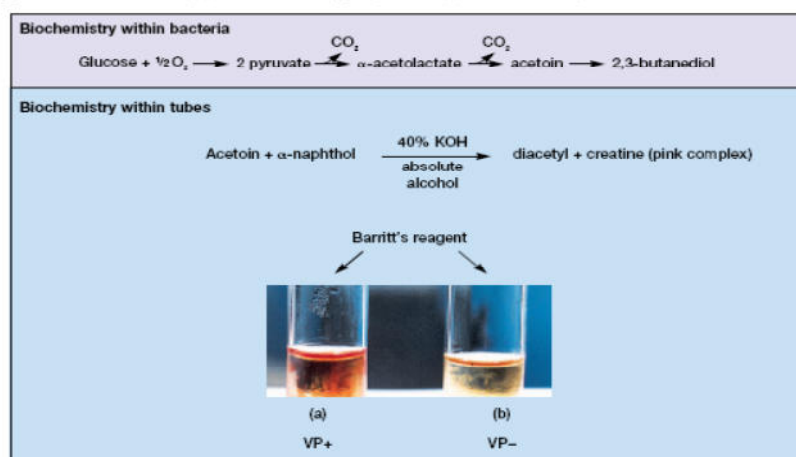


Figure 25.5 Citrate Test. (a) *Enterobacter aerogenes*; blue color is positive. (b) *Escherichia coli*; green color is negative.

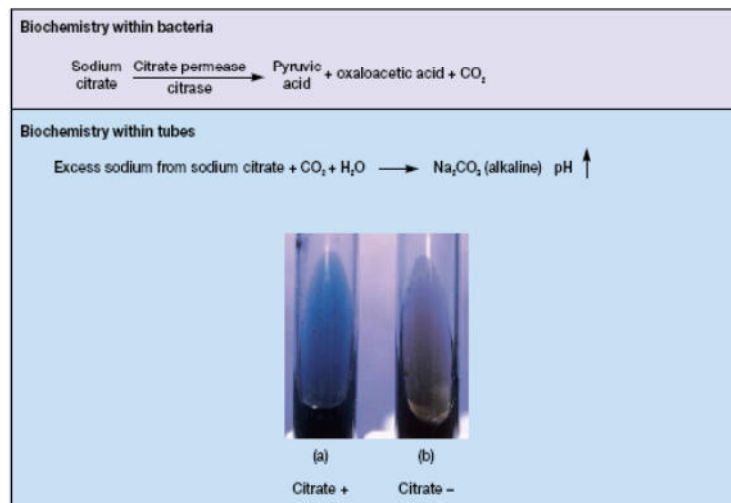


Figure 31.1 Urea Hydrolysis. (a) Uninoculated control. (b) Weakly positive reaction (delayed positive). (c) Very rapid positive reaction. (d) Negative reaction.

