

BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

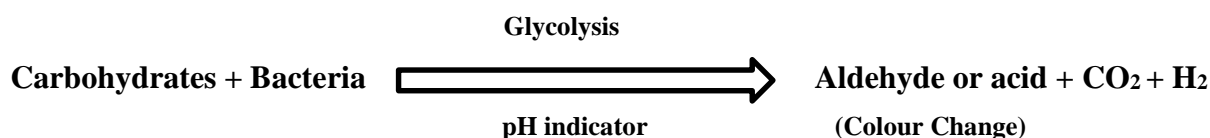
1) Carbohydrate Fermentation

Aim

To determine the ability of microorganisms to degrade and ferment carbohydrate with the production of acid and gas

Principle

Most microorganisms use carbohydrate differently depending on their enzymes components. In fermentation, substrate and alcohols undergo anaerobic dissimilation and produce an organic acid (For example lactic acid, formic acid or acetic acid). The pH indicator Phenol Red is used to detect the production of acid, which is red at a neutral pH 7 and changes to yellow at a slightly acidic pH of 6.8. This indicates a positive reaction.



In some cases, acid production is accompanied by the evaluation of gas such as Hydrogen or Carbon dioxide. To detect the presence of gas produced or Durham's tube (an inverted inner vial) is placed in the fermentation broth, in which the evaluation of gas will be visible as a bubble.

Cultures that are not capable of fermenting any carbohydrate and not producing concomitant evolution of gas are noted. This is a negative reaction.

Materials Required

8 ml Test Tube, Durham's Tube, Phenol Red Indicator, Sugar (Glucose, Lactose, Sucrose)

Procedure

- Using sterile technique, culture was inoculated into its appropriately labeled medium by means of loop inoculation.
- Care was taken during this step not to shake the fermentation tube.
- 1 tube of each fermentation broth was kept uninoculated as a comparative control.
- All the tubes were incubated at 37°C for 24 hours and the reaction was observed.

Observation

All carbohydrate broth cultures were observed for colour and presence or absence of gas bubble by comparing with the uninoculated tube (control).



Fig. 13. Carbohydrate Fermentation Test

Note

A – Only acid is formed; the broth has turned yellow

AG – Acid & Gas formed, the broth turned Yellow and gas bubble is trapped

-ve – No change

Sugar fermentation test

Table 5. Carbohydrate – Fermentation Test		
Glucose	Lactose	Sucrose
Fermented with acid production only eg. <i>S. aureus</i>	Fermented with acid production only Eg: <i>S. aureus</i>	Fermented with acid production only Eg: <i>S. aureus</i>
Fermented with acid and gas production eg. <i>E. coli</i> , <i>Klebsiella</i>	Fermented with acid and gas production Eg: <i>E. coli</i> , <i>Klebsiella</i>	Fermented with acid and gas production Eg: <i>E. coli</i> , <i>Klebsiella</i>
Non- Fermenting eg. <i>Acinoetobacter</i>	Non- Fermenting Eg: <i>S. typhi</i> <i>S. paratyphi</i> <i>Pseudomonas sp.</i>	Non- Fermenting Eg: <i>S. typhi</i> <i>S. paratyphi</i> <i>Pseudomonas sp.</i>

2) Oxidation – Fermentation Test

Aim

To determine the oxidation fermentation characteristics of microorganisms

Principle

This method depends upon the use of semisolid tube medium containing the carbohydrate (Glucose) together with a pH indicator. The acid is produced only at the surface of medium where conditions are aerobic the attack on the medium where conditions are aerobic the attack on the sugar is oxidative. If acid is produced throughout the medium including lower layers and where the conditions are aerobic breakdown is fermentative.

Fermenting organism (*Enterobacteriaceae*, *Vibrio*) produce an acidic reaction throughout the medium in the covered (anaerobic) as well as open (aerobic) tube. Oxidizing organisms (*Pseudomonas*) produce an acidic reaction only in the open tube. Organisms that cannot breakdown carbohydrate aerobically/anaerobically (alkali genes faecalis) produce an alkaline reaction in the open tube and no change in the covered tube. This medium may be used for detecting gas production and motility.

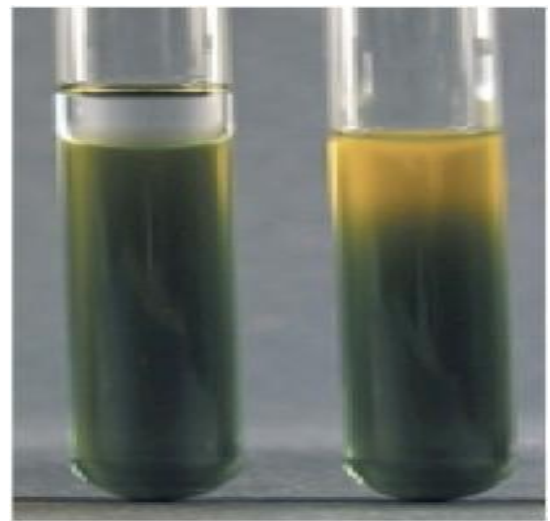


Fig. 14. Oxidation Fermentation Test

Materials Required

Bacterial broth culture, D-F medium, liquid paraffinaol

Procedure

- Using sterile technique, two tubes of medium were inoculated by stabbing with sterile urine.
- Two inoculated tubes were used as control.
- Liquid paraffin was poured over the medium to form a layer about 1cm in depth into one of the tube of each pair.
- The tubes were incubated at 37°C for 24-48hrs was observed.

Observation

The tubes were observed for the colour of the medium and the type of metabolism was recorded.

Table 6. Oxidation – Fermentation Test	
OXIDATIVE	FERMENTATIVE
Eg. <i>Pseudomonas</i>	Eg. <i>Klebsiella</i> <i>S. typhi</i> <i>S. paratyphi A</i> <i>E. coli</i>

3) Indole Production Test

Aim

To determine the ability of microorganisms to decompose the amino acid tryptophan to indole

Principle

Tryptophan an essential amino acid oxidized by some bacteria by the enzyme *tryptophanase* resulting in the formation of indole, pyruvic acid and ammonia. In this experiment, the medium contains the substrate tryptophan which is utilized by the microorganisms.

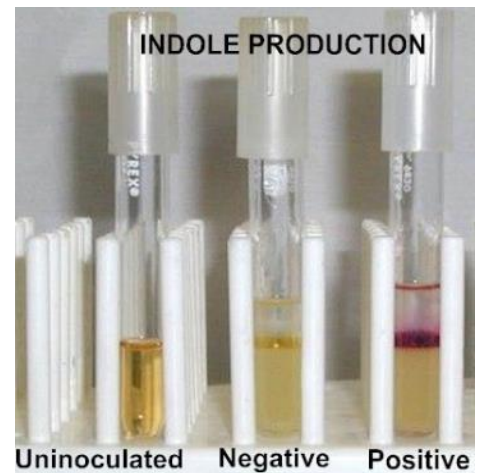
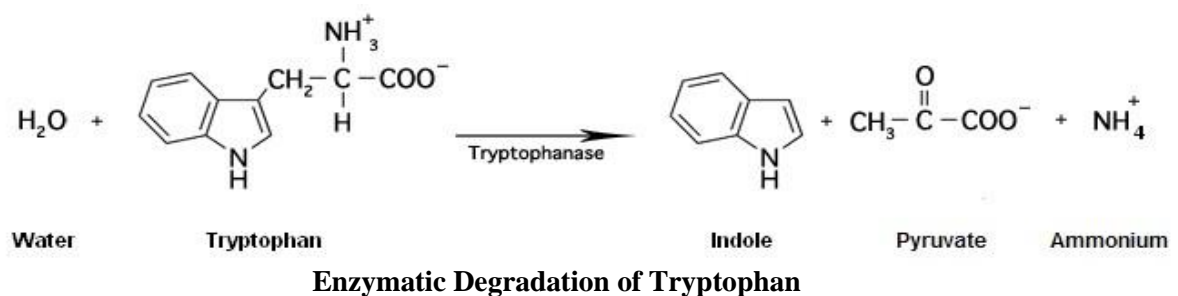


Fig. 15. Indole Production Test



This ability to hydrolyse tryptophan with the production of indole is not a characteristic of all microorganisms and therefore serves as a biochemical mask. The presence of indole is detected by adding Kovac's reagent, which produces a cherry red reagent layers. This colour is produced by the reagent which is composed of Paradimethyl aminobenzaldehyde yielding the cherry red colour

Indole Reaction with Kovac's Reagent

Culture producing a red reagent layers following addition of the Kovac's reagent are indole positive. The absence of red colouration demonstrates that the substrate tryptophan was not hydrolyzed and indicating indole negative reaction.

Another reagent used is Ehrlich's reagent. It's believed to be more sensitive than Kovac's reagent and is recommend for the detection of indole production by anaerobic and non-fermentative Gram negative organism Kovac's reagent was used usually initially to classify the members of *Enterobacteriaceae* family.

Materials Required

15 ml test tubes, bacterial culture, peptone water, Kovac's reagent

Procedure

- The peptone water tubes were inoculated with bacterial broth culture using sterile needle technique.
- An uninoculated tube was kept as control.
- Both tubes were incubated at 37°C for 24-48 hours.
- After proper incubation, 1 ml of Kovac's reagent was added to both tubes including the control.
- The tubes were shaken gently after an interval for 10 – 15 minutes.

Observation

The tubes were observed for the colour in the top reagent layer.

Note

Development of cherry red colour in the top layer of the tube is a positive test. Absence of red colouration is indole negative.

Examples

Positive: *E. coli*, *Proteus vulgaris*

Negative: *Klebsiella sp.*, *Proteus mirabilis*

4) Methyl Red Test

Aim

To determine the ability of microorganism to oxidize glucose with the production and stabilization of high concentrations of acid end products

Principle

All enteric organisms oxidize glucose for energy production and the end products of this process will vary depending on the specific enzymatic pathway present in the bacteria. In this test, the pH indicator methyl red detects the presence of large concentrations of acidic products. The test can be used in differentiating *Escherichia coli* and *Enterobacter aerogenes* (both coliform bacteria) that are used as indicator of the sanitary quality of water, foods etc.

Both of these organisms initially produce organic acid end products

during the early incubation period. The low acid end products produce acidic pH (4) which is stabilized and maintained by *E. coli* at the end of incubation. During the later incubation period *Enterobacter aerogenes* enzymatically converts these acids into nonacid end products such as 2,3 butanedial and acetyl methyl carbinol (pH 6).

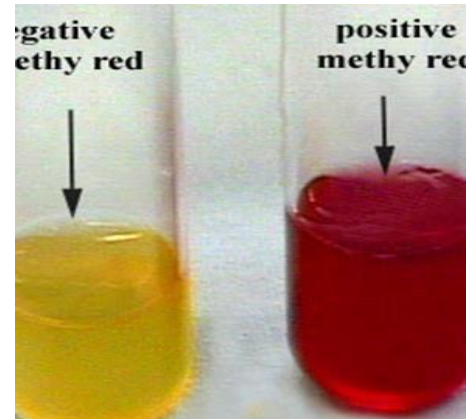
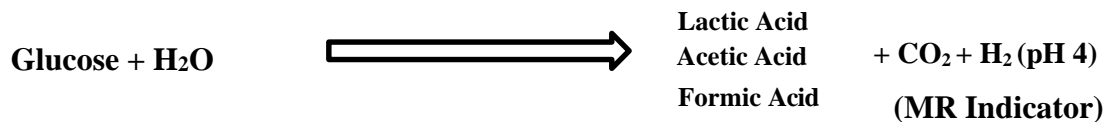


Fig. 16. Methyl Red Test



At a pH of 4, Methyl red indicator will turn red throughout the tube, which is indicating of a positive test. At pH 6, still indicating the presence of acid but with a lower hydrogen ion concentration, the indicators turn Yellow, which is indicating the negative test.

Materials Required

MR broth, 24 hours broth cultures, Methyl red indicator, inoculating loop

Procedure

- Using sterile technique experimental organisms were inoculated into appropriately labeled tubes containing MR broth by means of loop inoculation.
- Uninoculated tube was kept as control
- Both tubes were incubated at 37°C for 24-48 hours.
- After proper incubation 5 drops of MR indicator was added to both tubes including control.
- It was mixed well and colour was observed.

Observation

The tubes were observed for changes in the colour of Methyl Red.

Interpretation

The colour of MR reagents remaining red is a positive test and the colour turning to yellow is negative.

Examples

MR positive – *E. coli*, *Proteus* sp; *Salmonella* sp.

MR negative – *Klebsiella*, *Enterobacter* sp.

5) Voges – Proskauer Test

Aim

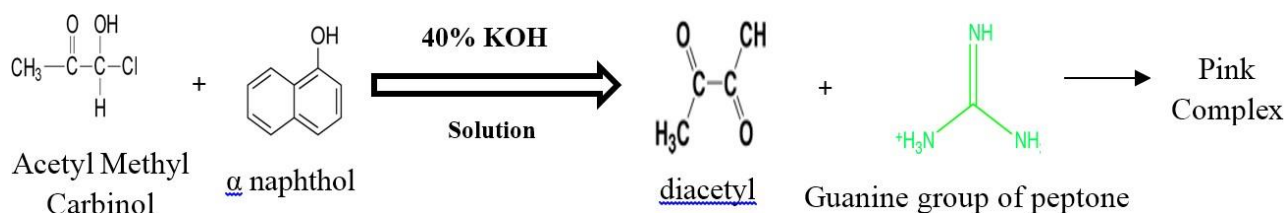
To determine the ability of many microorganisms to produce acetone (acetyl methyl carbinol) during fermentation of glucose

Principle

This determines the ability of many bacteria to ferment carbohydrates with the production of non- acidic / neutral end products, acetyl methyl carbinol or its reduction product, acetyl methyl carbinol or its reduction products, acetyl- methyl carbinol or its reduction product 2,3 Butylene glycol from the organic acids.

The reagent used in this test, Barrett's reagent, consists of a mixture of alcoholic α - naphthol and 40% potassium hydroxide solution. Detection of the acetyl methyl carbinol requires this end product to be oxidized to a diacetyl compound. This reaction will occur in the presence of α - naphthol catalyst and a guanidine group that is present in the peptone. As a result, a pink complex a guanidine group that is present in the peptone. As a result, a pink complex is complex is formed imparting a rose colour to the medium.

Acetyl Methyl Carbinol reaction with Barrett's reagent



Development of deep rose colour in culture with in a minute following the addition of Barrett's reagent is indicative of presence of the acetyl methyl carbinol and represents a positive result. The absence of rose colouration is a negative result.

Procedure

- Using sterile technique, the experimental organism was inoculated into VP broth by means of loop inoculation.
- One tube is kept uninoculated as control.
- The tube will be incubated at 37°C for 24-48 hours.
- After proper incubation, about 3 ml of Barrett's reagent A & 1 ml of Barrett's reagent B was added into both tubes including control.
- The tubes were shaken gently for 30 seconds with the caps off to expose the media to oxygen.
- The reaction was allowed to complete in 15 – 30 minutes and tubes were observed.

Observation

The tubes were observed for the development of crimson red colour.

Note: the colour may be more intense at the surface.



Fig. 17. Voges Proskauer Test

Interpretation

Red colour formation indicates a positive test and colour change is negative.

eg. Positive – *Klebsiella* sp., *Enterobacter*

Negative – *E. coli*, *Proteus* sp.

6) Citrate Utilization Test

Aim

To determine the ability of a microorganism to utilize citrate as the sole source of carbon and as energy source for the growth and ammonium salt as a sole source of nitrogen

Principle

Citrate test is used to differentiate among enteric bacteria on the basis of their ability to utilize / ferment citrate as the sole carbon source. In the absence of glucose or lactose some microorganisms utilize citrate as a carbon source. This ability depends on the presence of citrase enzyme that facilitates the transport of citrate in the cell. Citrate, the first major intermediate in Krebs's cycle is produced by the condensation of active acetyl CoA with oxalo acetic acid and acetate. These products are then enzymatically converted to pyruvic acid and carbon dioxide. During this reaction the medium becomes alkaline; CO₂ combines with sodium and water to form carbonate, an alkaline product. This changes the bromothymol blue indicator in the medium from green to Prussian blue.

Citrate test is preferred / performed by inoculating the microorganisms in to an organic synthetic medium. Simmons citrate agar (solid) or Koser's citrate medium (liquid) in which sodium citrate is the only source of carbon and energy.

Bromothymol blue is green when acidic (pH 6.8 and below). When alkaline (pH 7.6 and above). Formation of blue colour constitutes a positive test. Citrate negative culture will show no growth and the medium will remain green.

Materials Required

Bacterial broth, Simmons Citrate Agar Slants, Inoculation Loop

Procedure

- Using sterile technique Simmons citrate agar slant was inoculated with the test organism by means of a stab and streak inoculation.
- An uninoculated tube was kept as control.
- Both tubes were incubated at 37°C for 24 – 48 hours & was observed

Observation

The tubes were observed for growth and colouration of the medium.

Interpretation

Colour of the medium if turned blue, a positive result is indicated. Colour of the medium remains as green, indicates a negative result.

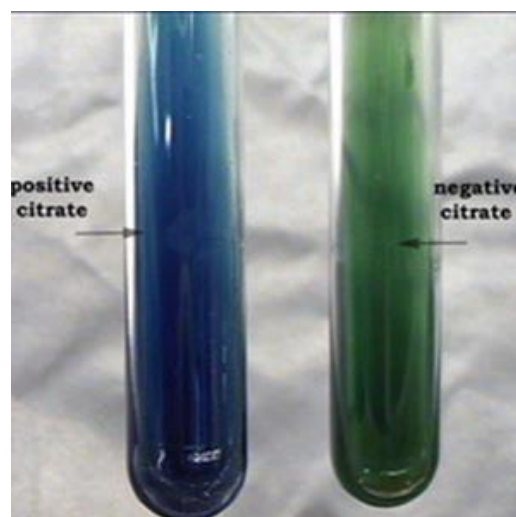


Fig. 18. Citrate Utilization

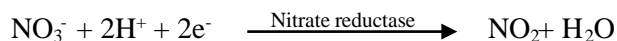
7) Nitrate Reduction Test

Aim

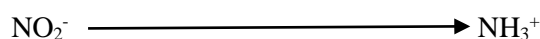
To determine the ability of bacteria to produce an enzyme nitrate reductase

Principle

The reduction of nitrate by some aerobic and facultative anaerobic microorganisms occur in the absence of molecular oxygen an anaerobic process whereby the cell uses in organic substances such as nitrates or sulphates to supply oxygen that is subsequently utilized as a final hydrogen acceptor during energy formation. The biochemical transformation may be utilized as follows:



Some organisms possess the enzymatic capacity to act further on nitrates to reduce them to ammonia or molecular nitrogen. These reactions may be described as follows:



Nitrate reduction can be determined by cultivating organisms a nitrate broth medium. The medium is basically a nutrient broth supplemented with 0.1% potassium nitrate (KNO₃) as the nitrate substrate. In addition, the medium is made into a semisolid by the additional of 0.1% agar. The semisolid impedes the diffusion of oxygen in to the medium, there by favoring the anaerobic requirement necessary for nitrate reduction. An organisms ability to reduce nitrate to nitrite is determined by the addition of two reagent solution A, which is sulphanic acid followed by solution B, which is α -naphthylamine followed reduction, the addition of solution A and B will produce an immediate cherry red colour.



Cultures not producing a colour change suggest one of two possibilities

- Nitrates were not reduced by the organism
- The organism possessed such potent nitrate reductase enzymes that nitrate were rapidly reduced beyond nitrates to ammonia or even molecular nitrogen.

This test determines the production of an enzyme called nitrate reductase, resulting in the reduction of nitrate (NO₃). With this enzyme, nitrate is reduced to nitrite (NO₂). It then forms nitrous acid that reacts with the first reagent sulphanic acid, and that reacts with the other reagent α -naphthylamine to form a red colour. The development of red colour, therefore, verifies that nitrates were not reduced to nitrites by the organism. If nitrites were reduced a negative nitrate reduction had occurred. If the addition of zinc does not produce colour change, the nitrates in the medium were reduced beyond nitrites to ammonia or nitrogen gas. This is a positive reaction or result. Reduction of nitrate is generally an anaerobic respiration in which an organism derives its oxygen from nitrate.

Materials Required

Bacterial broth, Nitrate broth, Nitrate reagent and inoculation loop.

Procedure

- Using sterile technique the test organism was inoculated in to nitrate broth by means of loop inoculation.
- An uninoculated broth was kept as control.
- Both tubes were incubated at 37°C for 24-48 hours.

- After proper incubation equal amounts of nitrate reagent (solution A & B) were added to nitrate broth Cultures and to the control tube and the reaction was observed

Observation

The tubes were observed to see a red colour has been developed or not.

A minute quantity of zinc was added to cultures in which no red colour was developed and it was observed to see if red colour has been developed or not.

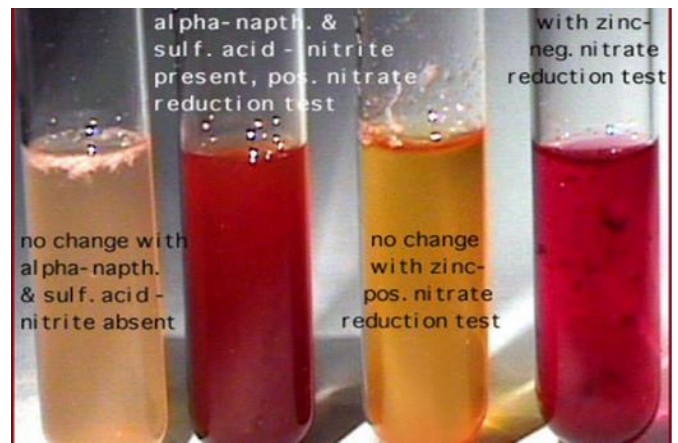


Fig. 19. Nitrate Reduction Test

Interpretation

Development of red colour indicates nitrate positive and no colour change indicates a negative test.

Eg: Positive: all members of *Enterobacteriaceae*

Negative: *Haemophilus duceryi*.

8) Urease Test

Aim

To determine the ability of microorganism to degrade urea by means of the enzyme urease

Principle

Urease is a hydrolytic enzyme that attacks nitrogen and carbon bond in amide compounds such as urea and forms the alkaline end products ammonia. The presence of urease is detectable when the organisms are grown in a urea broth medium containing the pH indicator phenol red. As the substrate urea is split into its products, the phenol red to turn to a deep pink. This is a positive reaction for the presence of urease. Failure of deep pink colour to develop is evidence of negative reaction.

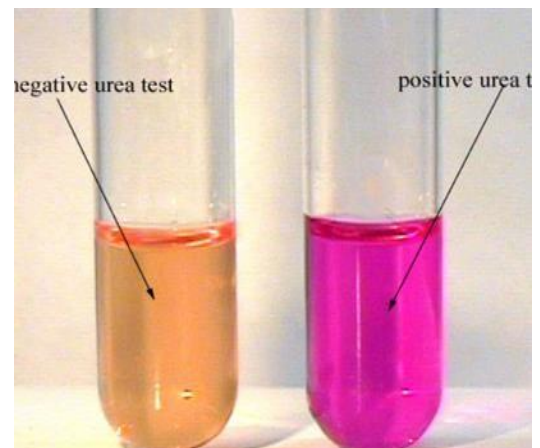
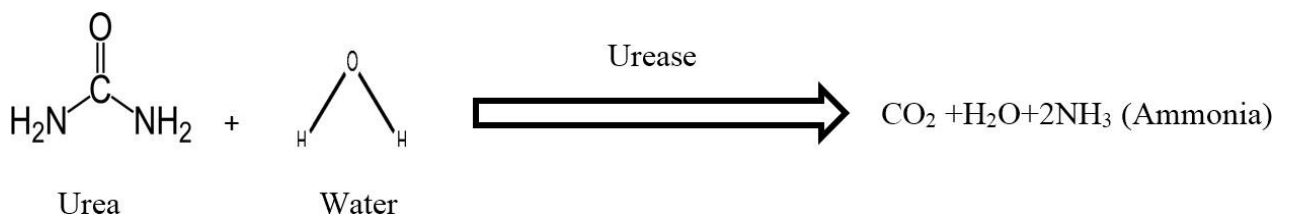


Fig. 20. Urease Test



Materials Required

Bacterial broth cultures, Christener's urea agar slant and the inoculation loop

Procedure

- Using sterile technique, the test organism was inoculated the media by means of loop of inoculation.

- An uninoculated tube was kept as control.
- The tubes were incubated at 37°C for 24-24 hours and the reaction was observed.

Observation

The tubes were observed to see if pink colour has developed or not

Interpretation

Development of pink colour indicator a positive test and no colour change shows a negative test,
Eg Urease Positive – *Klebsiella* sp., *Proteus* sp.

Urease Negative – *E. coli*, *Salmonella* sp.

9) Mannitol Motility Test

Aim

To detect whether the given organism is motile and also mannitol is fermenting or not

Principle

Mannitol motility test medium is an example of semisolid agar media; motile bacteria swarm and give a diffused spreading growth that is easily recognized by the naked eye. The final sterile medium should be quite clear and transparent. After incubating the stabbed culture, non-motile bacteria generally give growth that are confined to stab line and have sharply defined margins leaving the surrounding medium clearly transparent. Motile bacteria typically give diffused, hazy growth that spreads throughout the medium rendering it slightly opaque. This test also helps to identify whether the microorganisms ferment Mannitol or not. It produces acidic end products which in turn change the red colour of phenol red indicator to yellow.

Materials Required

Bacterial culture broth, mannitol fermentation media (semisolid) and inoculation loop

Procedure

- Using sterile technique the test organism was inoculated in to the medium using stab inoculation method.
- An uninoculated tube was kept as control.
- Both tubes were incubated at 37°C for 24-48 hours and the reaction was observed.

Observation

The tubes were observed for motility and also for colour changes from red to yellow.

Interpretation

Diffused growth – Motile bacteria eg:
Pseudomonas sp.

Growth at stab line only – Non-motile bacteria
eg: *Staphylococcus aureus* only

Red colour – Mannitol non-fermenting eg: *Bacillus cereus*

Yellow colour - Mannitol fermenting
eg: *E. coli*

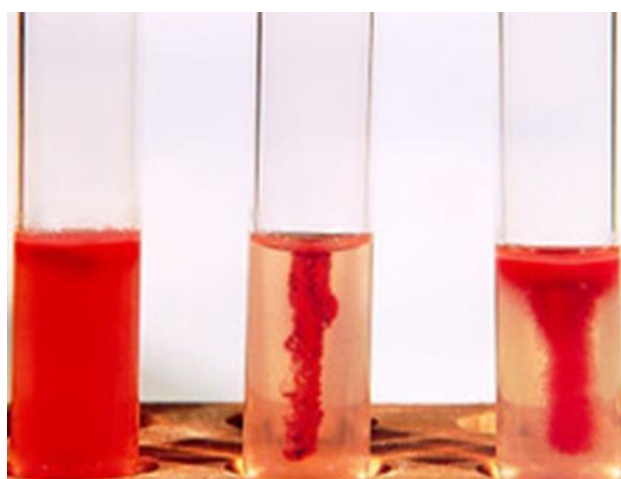


Fig. 21. Mannitol Motility Test

10) Triple Sugar Iron Agar Test

Aim

To identify the microorganisms based on the ability to ferment the carbohydrates (Glucose, Sucrose and Lactose)

Principle

The triple sugar- iron agar test is designed to differentiate among the different groups or genera of the *Enterobacteriaceae*, which are all Gram negative bacilli capable of fermenting glucose with the production of acid and to distinguish them from other gram negative intestinal bacilli. This differentiation is based on the differences in carbohydrate fermentation patterns and hydrogen sulfide production by the various groups of intestinal organisms. Carbohydrate fermentation is indicated by the presence of gas and a visible colour change of the pH indicator, phenol red. The production of hydrogen sulphide in the medium is indicated by the formation of a black precipitate that will blacken the medium in the butt of the tube.

To facilitate the observation of carbohydrate utilization patterns, TSI Agar contains three fermentative sugars, lactose and sucrose in 1% concentrations and glucose in 0.1% concentration. Due to the production of acid during fermentation, the pH falls. The acid base indicator Phenol red is incorporated for detecting carbohydrate fermentation that is indicated by the change in colour of the carbohydrate medium from orange red to yellow in the presence of

acids. In case of oxidative decarboxylation of peptone, alkaline products are produced and the pH rises. This is indicated by the change in colour of the medium from orange red to deep red. Sodium thiosulfate and ferrous ammonium sulfate present in the medium detects the production of hydrogen sulfide and is indicated by the black colour in the butt of the tube.

Carbohydrate fermentation is indicated by the production of gas and a change in the colour of the pH indicator from red to yellow. To facilitate the detection of organisms that only ferment glucose, the glucose concentration is one-tenth the concentration of lactose or sucrose. The amount of acid production in the slant of the tube during glucose fermentation

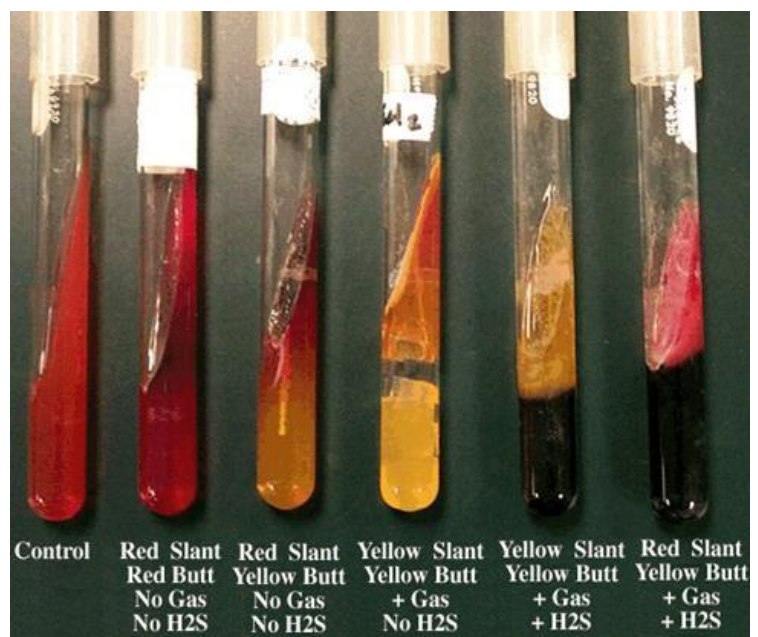


Fig. 22. Triple Sugar Iron Agar Test

oxidizes rapidly, causing the medium to remain orange red or revert to an alkaline pH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube since it is under lower oxygen tension.

After depletion of the limited glucose, organisms able to do so will begin to utilize the lactose or sucrose. To enhance the alkaline condition of the slant, free exchange of air must be permitted by closing the tube cap loosely. If the tube is tightly closed, an acid reaction (caused solely by glucose fermentation) will also involve the slant.

Materials Required

Bacterial broth cultures, TSI agar slants, Inoculation Loop.

Procedure

- Using sterile technique, the test organism was inoculated into the media by means of stab and streak inoculation.
- An uninoculated tube was kept as control
- Both tubes were incubated at 37°C for 24 hours and the reaction was observed

Observation

The tubes were observed for the colour of both the butt and slant and also gas production by means of cracks or bubble or blackness of butt.

Observation	Interference	Examples
A/A without gas and H ₂ S production	Acid Slant / Acid butt without gas & H ₂ S production	<i>Staphylococcus aureus</i>
A/A with gas and without H ₂ S production	Acid Slant / Acid butt with gas & without H ₂ S production	<i>E. coli, Klebsiella</i>
K/A with gas and without H ₂ S production	Alkaline slant / Acid butt with gas & without H ₂ S production	<i>Salmonella paratyphi A</i>
K/K without gas and H ₂ S production	Alkaline slant / Acid butt without gas & H ₂ S production	<i>Pseudomonas sp.</i>
K/A with H ₂ S production	Alkaline slant / Acid butt with H ₂ S production	<i>Salmonella typhi</i>

Interpretation

- A/A: ferments glucose and either sucrose, lactose, or both.
- K/A: does not ferment lactose or sucrose; does ferment glucose.
- K/K: a non-fermenter.
- Black precipitate in stab: produces H₂S (and ferments glucose).

11) Catalase Test

Aim

To demonstrate the presence of catalase in an organism.

Principle

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to



Fig. 23. Catalase Test

neutralize toxic forms of oxygen metabolites and H₂O₂. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. Anaerobes generally lack the catalase enzyme.

Catalase mediates the breakdown of hydrogen peroxide H₂O₂ into oxygen and water. To find out if a particular bacterial isolate is able to produce catalase enzyme, small inoculums of bacterial isolate is mixed into hydrogen peroxide solution (3%) and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production.

Catalase-positive bacteria include strict aerobes as well as facultative anaerobes. They all have the ability to respire using oxygen as a terminal electron acceptor.

Catalase-negative bacteria may be anaerobes, or they may be facultative anaerobes that only ferment and do not respire using oxygen as a terminal electron acceptor (ie. *Streptococci*).

Uses

- The catalase test is primarily used to distinguish among Gram-positive cocci: Member of the genus *Staphylococcus* is catalase-positive, and members of the genera *Streptococcus* and *Enterococcus* are catalase-negative.
- Catalase test is used to differentiate aero tolerant strains of *Clostridium*, which are catalase negative, from *Bacillus* species, which are positive.
- Semi quantitative catalase test is used for the identification of *Mycobacterium tuberculosis*
- Catalase test can be used as an aid to the identification of *Enterobacteriaceae*. Members of Enterobacteriaceae family are Catalase positive.

Materials Required

24 hours old bacterial culture, glass slide, petridish, 3% H₂O₂, applicator sticks

Procedure

- Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
- Place a drop of 3% H₂O₂ on to the slide and mix.
- A positive result is the rapid evolution of oxygen (within 5-10 s) as evidenced by bubbling.
- A negative result is no bubbles or only a few scattered bubbles.
- Dispose of your slide in the biohazard glass disposal container.

Precautions

- Do not use a metal loop or needle with H₂O₂; it will give a false positive and degrade the metal.
- If using colonies from a blood agar plate, be very careful not to scrape up any of the blood agar as blood cells are catalase positive and any contaminating agar could give a false positive.

Observation

The release of bubbles was observed and compared with control.

Interpretation

Bubble Formation : Catalase Positive No

Bubble Formation: Catalase Negative

Examples

Catalase Positive : *Staphylococcus aureus*

Catalase Negative: *Streptococcus pyogenes*

Note

Care must be taken while performing catalase test of growth from blood agar plate because blood (RBC) contains RBC catalase.

12) Oxidase Test

Aim

To test the production of oxidase bacteria

Principle

The oxidase test is a key test to differentiate between the families of *Pseudomonadaceae* (ox +) and *Enterobacteriaceae* (ox-), and is useful for speciation and identification of many other bacteria those that have to use oxygen as the final electron acceptor in aerobic respiration. The enzyme cytochrome oxidase is involved with the reduction of oxygen at the end of the electron transport chain.

There may be different types of oxidase enzymes produced by bacteria. The colorless redox reagent, tetra methyl-p-



Fig. 24. Oxidase Test

phenylenediamine dihydrochloride (or dimethyl) used in the test will detect the presence of the enzyme oxidase and reacting with oxygen, turn a colour. The oxidase reagent contains a chromogenic reducing agent, a compound that changes color when it becomes oxidized, so it acts as an artificial electron acceptor for the enzyme oxidase. The oxidized reagent forms the coloured compound indophenol blue.

Materials Required

Oxidase disc, 24 hours old test organism, applicator stick or glass rod.

Procedure

- The test organisms was rubbed over the reagent impregnated, filter paper disc using sterile applicator sticks or glass rod.

- Controls were also kept along with the test and the reaction was observed within 10 seconds.

Observation

The colour changes to purple were observed with the prescribed time.

Important

Acidity inhibits oxidase enzymes activity therefore the oxidase test must not be performed on colonies that produce fermentation on carbohydrates containing media like Mac Conkey Agar.

Interpretation

Formation of purple colour indicates a positive test. No colour changes show a negative test.

eg. Oxidase Positive: *Pseudomonas* sp., *Vibrio* sp.

Oxidase Negative: *E. coli*, *Klebsiella*

Precautions

- The test reagent is to be freshly prepared
- Nichrome wire is not used to take bacterial growth
- Cultures should not be very cold
- Culture from selective media should not be used
- The colour changes should be observed within the prescribed time

13) Coagulase Test

Aim

To distinguish coagulase producing *Staphylococcus aureus* from other species of *Staphylococcus*

Principle

Staphylococcus aureus is known to produce coagulase, which can clot plasma into gel in tube or agglutinate cocci in slide. This test is useful in differentiating *S. aureus* from other coagulase-negative staphylococci. Most strains of *S. aureus* produce two types of coagulase, free coagulase and bound coagulase. While free coagulase is an enzyme that is secreted extracellular, bound coagulase is a cell wall associated protein. Free coagulase is detected in tube coagulase test and bound coagulase is detected in slide coagulase test. Slide coagulase test may be used to screen isolates of *S. aureus* and tube coagulase may be used for confirmation. While there are seven antigenic types of free coagulase, only one antigenic type of bound coagulase exists. Free coagulase is heat labile while bound coagulase is heat stable.

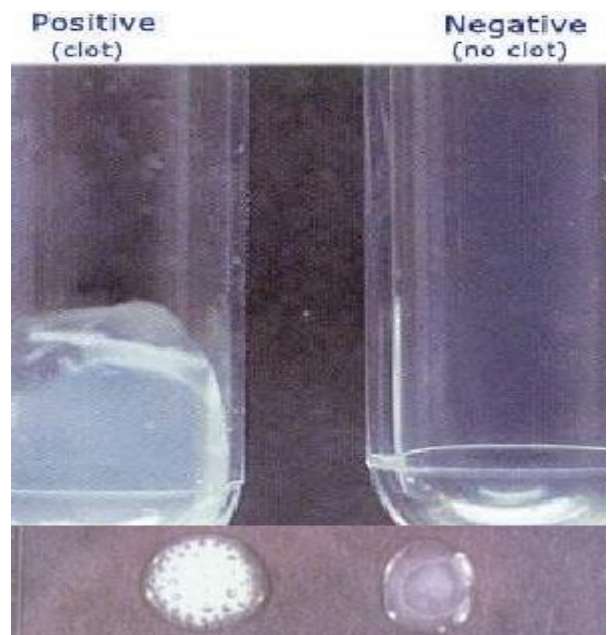


Fig. 25. Coagulase Test

Slide Coagulase Test: The bound coagulase is also known as clumping factor. It cross-links α and β chain of fibrinogen in plasma to form fibrin clot that deposits on the cell wall. As a result, individual coccus sticks to each other and clumping is observed.

Tube Coagulase Test: The free coagulases secreted by *S. aureus* act with coagulase reacting factor (CRF) in plasma to form a complex, which is thrombin. This converts fibrinogen to fibrin resulting in clotting of plasma.

Materials Required

EDTA anticoagulant human plasma, clean glass slide, test tubes, pipettes, distilled water and inoculation loop.

Procedure

Slide Coagulase Test: Dense suspensions of Staphylococci from culture are made on two ends of clean glass slide. One should be labeled as “test” and the other as “control”. The control suspension serves to rule out false positivity due to auto agglutination. The test suspension is treated with a drop of citrated plasma and mixed well. Agglutination or clumping of cocci within 5-10 seconds is taken as positive. Some strains of *S. aureus* may not produce bound coagulase, and such strains must be identified by tube coagulase test.

Observation

The slides were observed for clumping or not within prescribed time.

Interpretation

Clumping formation - Positive reaction

No clumping formation – Negative reaction

Tube Coagulase Test

Three test tubes are taken and labeled “test”, “negative control” and “positive control”. Each tube is filled with 0.5 ml of 1 in 10 diluted rabbit plasma. To the tube labeled test, 0.1 ml of overnight broth culture of test bacteria is added. To the tube labeled positive control, 0.1 ml of overnight broth culture of known *S. aureus* is added and to the tube labeled negative control, 0.1 ml of sterile broth is added. All the tubes are incubated at 37°C and observed up to four hours. Positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube. If the test remains negative until four hours at 37°C, the tube is kept at room temperature for overnight incubation.

Observation

The tubes were observed for clotting in the prescribed time.

Interpretation

Clot formation - Positive reaction

No clot formation – Negative reaction

Examples

Coagulase Positive: *Staphylococcus aureus*

Coagulase Negative: *E. coli*

