Biochemical tests

To identify bacteria, we must rely heavily on biochemical testing. The types of biochemical reactions each organism undergoes act as a " thumbprint "بصمة الإبهام for its identification.

Purposes of biochemical tests

1. **Test for metabolism of carbohydrates and related products**.
    - Sugar fermentation test
2. **Test for specific break down products**
    - Indole test
    - Methyl Red and Voges –Proskauer tests
3. **Test to show ability to utilize a specific substance**
    - Citrate (Simon citrate medium)
4. **Test for enzymes**
   Catalase , oxidase , urease ….
5. **Test for metabolism of protein and amino acids**

1- Fermentation of carbohydrates

A wide variety of carbohydrates may be fermented by various bacteria in order to obtain energy and the types of carbohydrates which are fermented by a specific organism can serve as a diagnostic tool for the identification of that organism.

Procedure: incubate tubes of media containing:

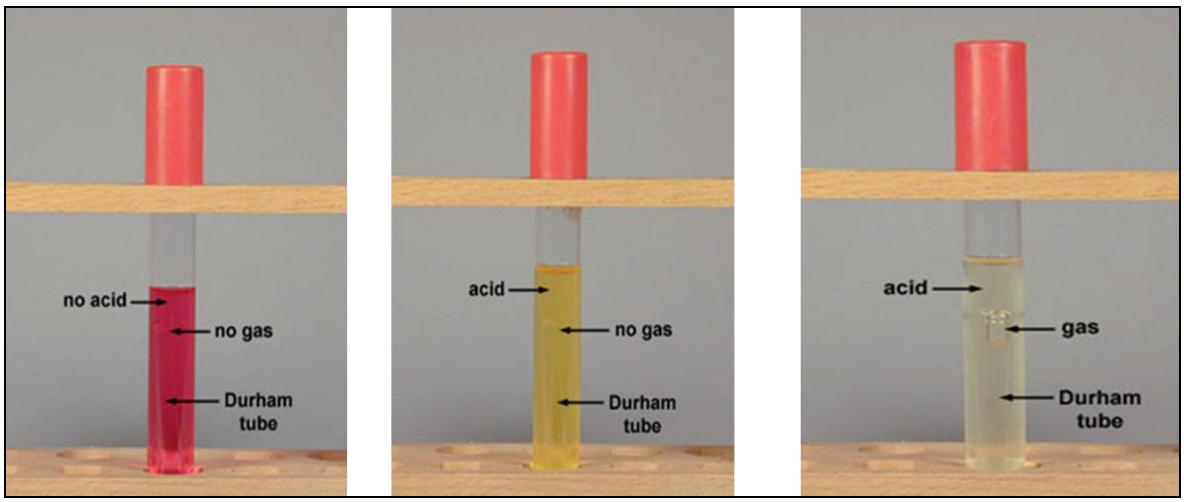
1. A single carbohydrate (such as lactose or glucose)
2. pH indicator (such as phenol red)
3. Durham tube (a small inverted tube to detect gas production).

If the particular carbohydrate is fermented by the bacterium ,acid end products will be produced which lowers the pH , causing the pH indicator to

change color **(phenol red turns yellow)** .If gas is produced along with the acid , it collects in the Durham tube as a gas bubble.

Serratia marcescens (produce acid) , *E coli* (produce acid & gas)

Alcaligenes faecalis (no acid or gas)



2- CATALASE TEST

Bacterial cells produce hydrogen peroxide (H_2O_2) during aerobic respiration. If hydrogen peroxide accumulates in the cell , it becomes toxic. For this reason , Most aerobic and facultatively anaerobic bacteria possess an enzyme called catalase , which breaks down hydrogen peroxide. However ,some bacteria,

such as species of *Streptococcus and Enterococcus* , lack this enzyme. These bacteria are easily distinguished from catalase-positive bacteria, such as species of *Staphylococcus and Micrococcus*. The catalase test is performed by adding **3% hydrogen peroxide** (H_2O_2) to an 18-24 hour culture on an agar slant or glass slide. The culture is observed for the immediate appearance of bubbles.

Tips for Success

1- Do not use media containing blood , because red Blood cells contain catalase.

2- Use afresh bottle of hydrogen peroxide, because hydrogen peroxide is unstable.

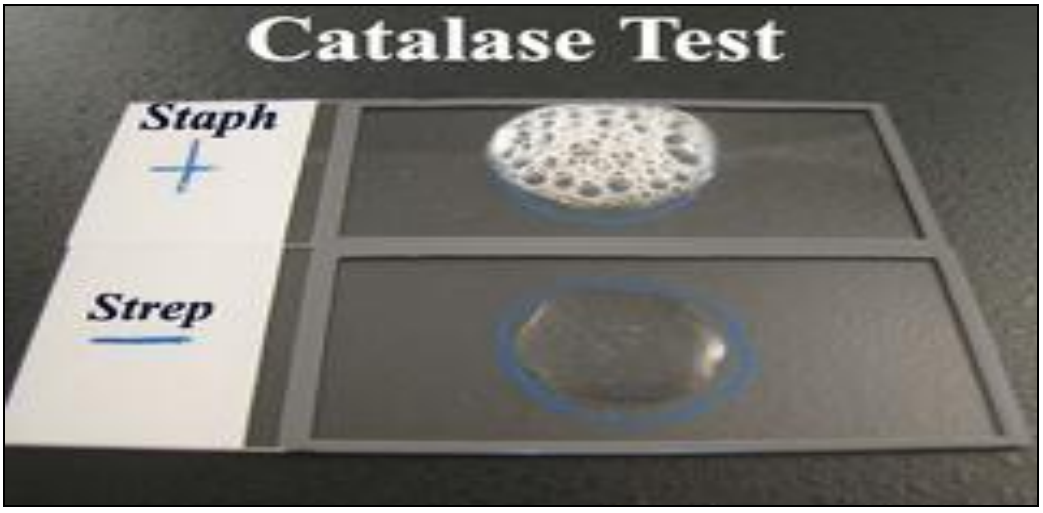
3-To ensure its chemical reactivity, test the bottle's contents on a known catalase positive organism.

Expected Results

The catalase enzyme breaks down hydrogen peroxide in to water and oxygen The oxygen causes bubbles to form within seconds , indicating a positive test. The absence of bubbles is considered a negative test

Staphylococcus epidermidis (+)

Enterococcus faecalis , Streptococcus (-)



3- GELATIN Utilization

Purpose and Procedure Summary

Gelatin is a protein that is digested by bacterial extracellular enzymes called gelatinase . The end products of this reaction are amino acids that are transported in to the cell for utilization. Some bacteria , such as ***Pseudomonas aeruginosa*** (+) produce gelatinases, while others , such as ***Alcaligenes faecalis*** do not

Nutrient gelatin tubes are used to determine whether an organism produces gelatinases , Nutrient gelatin contains beef extract and **peptone** to support growth , and enough gelatin (**120g / liter**) to cause the medium to **gel**

An isolate is inoculated in to a nutrient gelatin tube with a sterile transfer needle .The tube is incubated at **35C° for 24-48** hours. The medium is chilled thoroughly in a refrigerator before examination. Chilling is essential because gelatin is liquid at temperatures above **20**c°. After chilling, the medium is observed for gelling by carefully tilting the tube to the side .

Tips for Success

1- Compare results to an uninoculated control tube.

2- Do not shake the tube when transferring it to the refrigerator ; gelatin digestion may have occurred only at the surface.

3- Incubate tubes for up to **7 days** for slow gelatin utilize

4-Coagulase test

The **coagulase test** is used to differentiate ***Staphylococcus aureus*** from coagulase-negative staphylococci. *S.aureus* produces two forms of coagulase (i.e., bound coagulase and free coagulase)**. 1- Bound coagulase**, otherwise known

as "clumping factor", can be detected by carrying out a slide coagulase test**,** **2- free coagulase** can be detected using a tube coagulase test.

Slide test

A slide coagulase test is run with a negative control to rule out auto agglutination. Two drops of saline are put on to the slide labeled with sample number, Test (T) and control (C). The two saline drops are emulsified with the test organism using a wire loop, straight wire, or wooden stick. **A drop of plasma (rabbit plasma anticoagulated with EDTA is recommended)** is placed on the inoculated saline drop corresponding to test, and mixed well , then the slide is rocked gently for about 10 seconds.

- If 'positive', macroscopic clumping would be observed in the plasma within 10 seconds, with no clumping in the saline drop.
- If 'negative', no clumping will be observed..

Tube test

A fibrin clot formed in a test tube by the coagulase reaction The tube test uses rabbit plasma that has been inoculated with a staphylococcal colony (i.e., Gram-positive cocci which are catalase positive).

The tube is then incubated at 37C° for 1.5 hours. If negative, then incubation is continued up to 18 hours.

- If 'positive' (e.g., the suspect colony is ***S. aureus***), the plasma will coagulate, resulting in a clot (sometimes the clot is so pronounced, the liquid will completely solidify).

- If 'negative', the plasma remains a liquid..

- *List of coagulase-positive staphylococci: Staphylococcus aureus subsp. anaerobius, S. a. aureus, S. a. delphini, S. hyicus, S. intermedius, S. lutrae, and Staphylococcus schleiferi subsp. coagulans.*

- *List of coagulase-negative staphylococci of clinical significance: S. saprophyticus, S.cohnii subsp. cohnii*

5-HYDROGEN SULFIDE (H_2S) PRODUCTION

Purpose and Procedure Summary

Bacteria use the enzyme cysteine desulfurase to hydrolyze the amino acid cysteine , forming hydrogen sulfide **(H_2S)** is also an end-product of the bacterial reduction of thiosulfate. Hydrogen sulfide is produced by ***Proteus vulgaris*** *and* ***Salmonella typhimurium*** , but not by ***Escherichia coli*** *and* ***Shigell aflexneri***

cysteine desulfurase

cysteine ---------- NH_3+ pyruvic acid + H_2S

H_2S+$FeS0_4$ ---------- FeS+H_2S0_4 (blackening of medium)

No cysteine desulfurase

cysteine ---------- cysteine (no blackening of medium)

SIM medium ,also used to detect motility and indole production, is used to demonstrate hydrogen sulfide production. SIM medium contains **peptone** and beef extract to support growth. Peptone contains the **amino acid cysteine**. Sodium thiosulfate is also included in the medium. Ferrous sulfate is the source of iron used to detect hydrogen sulfide production from either cysteine or **sodium thiosulfate Agar** is added to make the medium semisolid Triple sugar iron agar and **Kligler iron agar**, are also used to demonstrate hydrogen sulfide production

An isolate is inoculated into a tube with a sterile transfer needle, The tube is incubated at 35C° for 24-48 hours before examination The medium is observed for blackening

Tips for Success

1- Read hydrogen sulfide results first if determining indole production in the same tube.

2- Any blackening of the medium is considered a positive test.

6-INDOLE PRODUCTION

Purpose and Procedure Summary

Indole production is the "I" portion of the four IMViC tests used to characterize enteric bacteria The amino acid **tryptophan** can be broken down by the enzyme tryptophanase to form indole , pyruvic acid , and ammonia as end-products**. Tryptophanase** differentiates the indole -positive enterics , such as ***Escherichia coli*** *and* ***Proteus vulgaris*** , from the indol-negative enterics , such as ***Serratia marcescens , Proteus mirabilis*** and ***Enterobacter aerogenes*** .

SIM medium ,also used to detect motility and hydrogen sulfide production, is used to demonstrate indole production. SIM medium contains peptone and beef extract to support growth . Peptone contains the amino acid tryptophan. Agar is added to make the medium semisolid An isolate is inoculated into a tube with a sterile transfer needle .The tube is incubated at 35C° for 24-48 hours. After incubation , five drops of **Kovac's reagent** are added to the agar surface Kovac's

amyl alcohol , hydrochloric acid , and para-dimethyl aminobenzaldehyde , which reacts with **indole** to form a **red color (Ring)**

Tips for Success

-Test the reactivity of the Kovac's reagent by using it on a known indole - positive organism

- Watch for color development in the alcohol layer on the surface .

7- OXIDASE TEST

Purpose and Procedure Summary

The electron transport chain is a sequence of reactions that represent the final stage of bacterial cell respiration. The final reaction in this sequence is catalyzed by the enzyme **cytochrome oxidase**. In this final step, cytochrome oxidase oxidizes the electron transport molecule, **cytochrome c** , while reducing **oxygen** to form water. Bacteria that contain cytochrome oxidase, such as species of ***Pseudomonas***, are

oxidase positive, while those that lack this enzyme, such as ***Escherichia coli*** and other enterics, are oxidase negative.

The oxidase test requires the use of a reduced chemical reagent. This reagent does not interact directly with cytochrome oxidase, but instead interacts with the enzyme's product, **oxidized cytochrome c** .Cytochrome c changes the reduced reagent to an oxidized form. The oxidase test can be performed either by adding oxidase reagent to bacterial growth on an agar plate or by transferring growth to a Dry Slide TM that already contains the oxidase reagent. After the growth is combined with oxidase reagent, color change is observed for up to 60seconds.

cytochrome oxidase

cytochrome $C_{(reduced)}$ $+O_2$ **…….... c**ytochrome $C_{(oxidized)}$ $+H_20$

cytochrome $C_{\textbf{(oxidized)}}$ +oxidase reagent **(reduced)** …cytochrome $C_{\textbf{(reduced)}}$ + oxidase reagent (oxidized)

(colorless) (dark purple)

8-PHENYLALANINE DEAMINASE TEST

Purpose and Procedure Summary

The amino acid **phenylalanine** can be **broken down** by the enzyme **phenylalanine deaminase to** form **phenylpyruvic acid** and **ammonia** . Phenylalanine deaminase is produced by some **enterics**, such as ***Proteus vulgaris***,

but not by others, such as ***Escherichia coli and Enterobacter aerogenes***. Phenylalanine agar is used to detect the deamination of phenylalanine. This agar contains yeast extract to support growth , DL-phenylalanine, and agar.

An isolate is inoculated onto a slant with a sterile transfer loop. The slant is incubated at 35C° for 18-24 hours. After incubation , four or five drops of 10% ferric chloride ($FeCl_3$) are added and rolled Over the surface of the slant. The slant is then observed for color change.

phenylalanine deaminase

- phenylalanine …….. phenylpyruvic acid+NH3

phenylpyruvic acid+ added $FeCl_3$=green color (***Proteus vulgaris***)

no phenylalanine deaminase

- phenylalanine ……..…….. phenylalanine

phenylalanine +added $FeCl_3$=no green color (***Escherichia coli***)

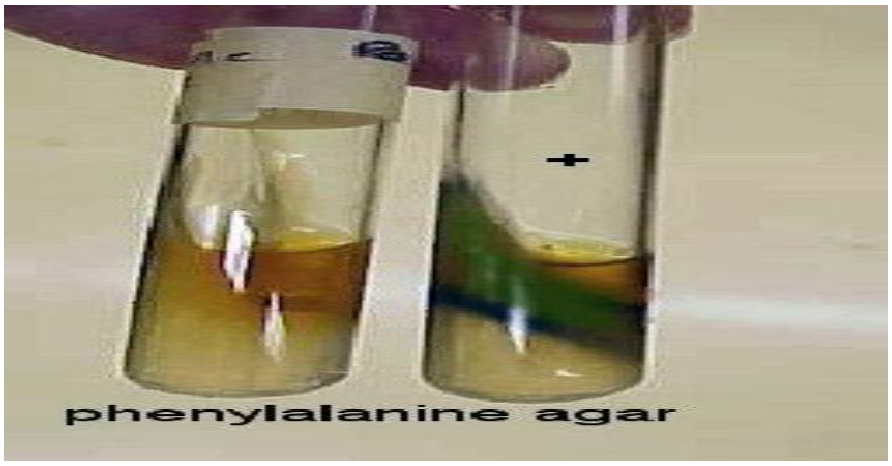
Tips for Success

- Test the reactivity of the ferric chloride on a known positive.

- Read this test within1-5 minutes ,because the green color disappears.

Expected Results

Ferric chloride reacts with **phenyl pyruvic acid** to form a green color , A green color represents a positive test. No green color represents a negative test.



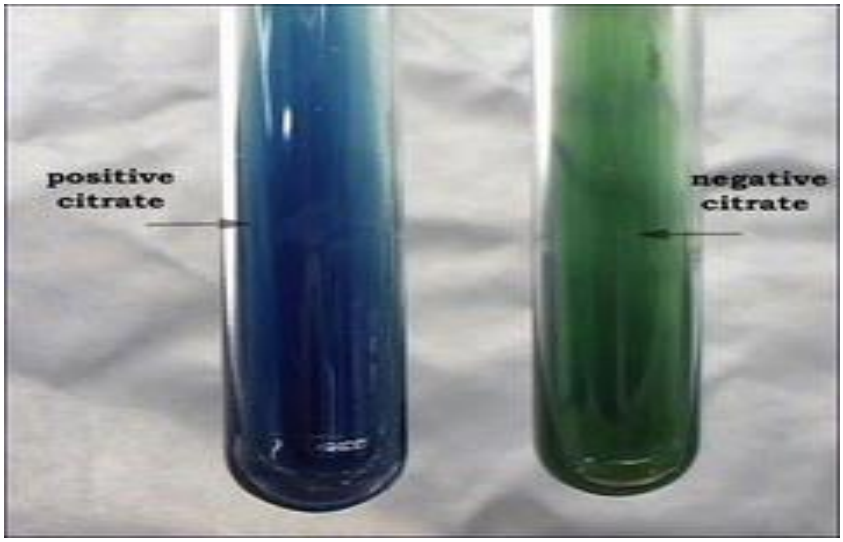
9-Citrate Utilization Test

Citrate utilization is the "C" portion of the four IMViC tests , which are used to characterize enteric bacteria. Citrate is an organic molecule that can be utilized by bacteria that produce the enzyme citrase

Simmons Citrate agar is a defined medium containing **sodium citrate** as the sole carbon source. The pH indicator bromothymol blue , will turn from green at neutral pH **6.9** to blue when a pH higher than **7.6** is reached (alkaline). If the citrate is utilized, the resulting growth will produce alkaline products changing the color of the medium from green to blue. (Blue color= positive reaction *Klebsiella)* ; (green color = negative reaction *E.coli*)

Tips for Success

Compare results to an uninoculated control to aid in the detection of color changes , Look for growth as well as color change, because only citrase - positive bacteria will grow on a medium that contains citrate as the only carbon source.



10- MOTILITY TEST

Purpose and Procedure Summary

Although the motility test is not a biochemical test, it is included here because it is often used to distinguish certain bacteria. The motility test determines the presence of flagella , external appendages used by bacteria for movement.

Bacteria with flagella , such as ***Citrobacter freundi*** , are called motile ,while bacteria without flagella, such *as* ***Staphylococcus epidermidis***, are called non motile, Motility test medium, used to detect bacteria With flagella , contains beef extract and peptone to support growth, and **0.5% agar** .The medium is semisolid because of the low concentration of agar, allowing movement of motile organisms through the medium. SIM medium is another semisolid medium used to determine bacterial motility.

An isolate is inoculated in to a tube with a sterile transfer needle .The needle is inserted and withdrawn in a straight line in the center of the medium. The tube is incubated at 35C° for 24-48 hours before examining the growth along the line.

Tips for Success

- Hold the tubes up to the light for better contrast When examining growth.

- Compare results to an uninoculated tube held up to the light.

Expected Results

Bacteria with flagella spread away from the line of inoculation. When the tube is held up to the light, growth is seen macroscopically as turbidity extending through the semisolid medium. Growth away from the line of inoculation indicates that the

organism is motile Bacteria without flagella do not spread away from the line of inoculation , so their growth does not extend into the medium .Growth along the line of inoculation only indicates that the organism is non motile .

11- UREA UTILIZATION

Purpose and Procedure Summary

Some bacteria produce Urease , an enzyme capable Of breaking down urea. The breakdown of urea Within 24hours is a trait used to distinguish species of Proteus from other enteric bacteria. Urea broth contains yeast extract, urea, and the pH

indicator phenol red. Phenol red is yellow orange at the initial pH of 6.8 but changes to pinkish-red at a pH of 8.4. An isolate is inoculated into a tube with a sterile transfer loop. The tube is incubated at 35C°for 24 hours before examination for color change.

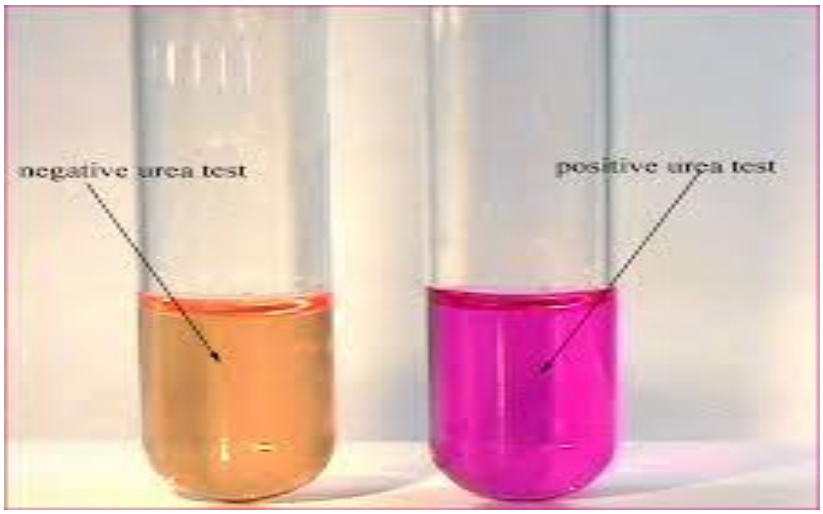
Tips for Success

- Examine the color of broth with in 24hours; a pinkish-red color within 24hours distinguishes

Species of Proteus; a pinkish-red color after24 hours indicates the slow urease activity of other enterics.

Expected Results

The enzyme urease breaks down urea in to alkaline end-products that raise the pH of the medium, causing the phenol red to turn a pinkish-red color There fore , a pinkish-red color represents a positive test for urea utilization. No color change represents a negative test.



12- Methyl red test & Voges-Proskauer test MR-VP test

MR test:

Principle to test the ability of the organism to produce **acid end product** from glucose fermentation , this is a qualitative test for acid production.

VP test:

To determine the ability of the organisms to produce **neutral end product (acetoin) from glucose fermentation.**

procedure

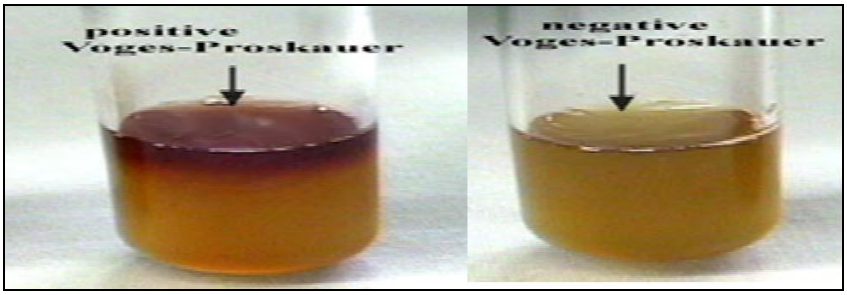
1. Inoculate the tested organism in to 2 tubes of MR-VP broth
2. Incubate the tubes at 37°C for 24 hours
3. AFTER INCUBATION: Run the MR test in the tube 1, and the VP test in tube 2.
   - For methyl red: Add 6-8 drops of methyl red reagent.
   - For Voges-Proskauer: Add 12 drops of **Barritt's A** (α-naphthol), mix , 4 drops of **Barritt's B** (40% KOH), mix
   - Let sit , for at least 1hour

Results 1- MR results:

Red: Positive MR (*E. coli*); Yellow: Negative MR (*Klebsiella*)

2-Voges-Proskauer results

Pink: Positive VP (*Klebsiella*), yellow: Negative VP (*E. coli*)



13- DECARBOXYLASE TESTS

Purpose and Procedure Summary

Decarboxylase are bacterial enzymes that remove the carboxyl (-COOH) group from amino acids.

This process, called **decarboxylation** , is a step in the metabolism of amino acids. A specific **decarboxylase enzyme** exists for each amino acid , but only three are used to distinguish enteric bacteria:

lysine decarboxylase , ornithine decarboxylase , and arginine decarboxylase , Lysine decarboxylase , for example, is produced by ***Enterobacter aerogenes*** , but not by ***Proteus vulgaris***. Decarboxylase medium is supplemented with one of the three amino acids lysine , ornithine , or arginine to detect decarboxylation .

This medium also contains **peptone** and **beef extract** to support bacterial growth. The presence of a **coenzyme called pyridoxal** enhances decarboxylase activity. Glucose provides a fermentable carbohydrate. The **pH indicator bromcresol purple**, turns purple at a pH **above 6.8** and yellow at a pH below 5.2.

An isolate is inoculated into a tube with a sterile transfer loop. The tube is covered with **2-3 ml of sterile mineral oil** to exclude oxygen. The lack of oxygen promotes the production of acids from glucose fermentation, thus creating the acidic environment necessary for the formation of decarboxylase.

The tube is incubated at 35C° for 24-48 hours before examination. The medium is observed for color change. from yellow to purple

A purple color represents a **positive test**. The medium remains acidic, and yellow, in tubes inoculated with bacteria that lack decarboxylase. A yellow color represents a negative test. are the same regardless of the amino acid tested.

Tips for Success

Compare results to an uninoculated control tube to determine whether changes have occurred in the medium. Any trace of purple is considered a positive test;

color may vary from light purple to dark purple.

Expected Results

Acids from glucose fermentation lower the pH of the medium, causing the bromcresol purple to turn yellow. The acidic environment promotes the formation of decarboxylase in those bacteria that produce these enzymes. The resultant

decarboxylation results in alkaline end-products that raise the pH of the medium ,causing the bromcresol purple to turn

14- STARCH HYDROLYSIS TEST

Purpose and Procedure Summary

Starch is a polysaccharide composed of repeating alpha-D-glucose subunits. Bacteria that produce the extracellular enzyme **amylase**, such as ***Bacillus cereus***, break down starch into single subunits of alpha-D-glucose. These are transported in to the cell, where they are broken down in cell respiration.

Starch agar is used to test for the breakdown of starch by amylase. This medium contains beef extract and peptone to support growth, soluble starch and agar **.**

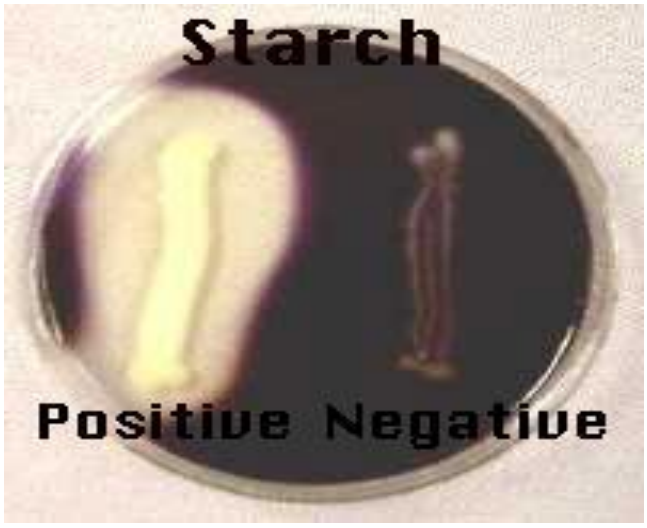
An isolate is inoculated on to a plate with a sterile transfer loop. The plate is incubated at 35C° for 48hours. After incubation, the plate is flooded with Gram's iodine , which reacts with starch to produce a purple -blue color.

Tips for Success

- Test the reactivity of the Gram's iodine by testing it on a known amylase-positive organism. Examine for color immediately after addition of Gram'siodine, because the color may fade.

Expected Results

Gram'siodine reacts with starch to produce a purple-blue color throughout the agar medium. A clear zone around bacterial growth indicates starch hydrolysis A purple-blue color to the edge of bacterial growth indicates no starch hydrolysis.



15-LITMUS MILK REACTIONS

Purpose and Procedure Summary

Bacteria can act on several different substrates in **litmus milk** , including lactose, casein, and litmus, causing a variety of reactions that are specific for each species of bacteria . Litmus milk contains **skim milk** ,the source of lactose and casein, and litmus, the pH/oxidation reduction indicator. Litmus is purplish-blue at the initial pH of **6.8** in the uninoculated medium. Several other colors for litmus are possible after bacterial growth occurs, depending on the action of the isolate. An isolate is inoculated into a tube with a sterile transfer loop. The tube is incubated at 35°C for24-48 hours before it is examined for changes.

Tips for Success

- Compare results to an uninoculated control tube to determine whether changes have occurred in the medium.

An additional incubation of 24-48 hours may Be necessary for some reactions to develop.

The blue of the alkaline reaction is most evident at the top of the medium ,while the white of litmus reduction is most evident at the bottom

Expected Results

The variety of reactions possible in litmus milk, are the following:

lactose fermentation (A): Lactose fermentation releases lactic acid, which lowers the pH of the medium to 4.5, causing the litmus to turn pink. A pink color indicates lactose fermentation

alkaline reaction (Alk): The action of bacteria on the nitrogen-containing components of skim milk causes ammonia to be released into the medium. As

a result, the pH increases to 8.3-and the litmus turns blue .A blue color indicates an alkaline reaction clot formation (C): Either the precipitation of casein by lactic acid or the action of the enzymerenn in on casein may cause the formation of a clot , which appears as a white mass in the bottom of the tube

reduction of litmus (R): If litmus is used as an electron acceptor during lactose fermentation, it is


Litmus Milk Reaction
Uninoculated
(-)
E. coli
Ps. aeruginosa
S. aureus
K. pneumoniae

16- Triple sugar-iron test

Triple sugar iron (TSI) agar and Kligler iron agar

(KIA) are used to determine both carbohydrate fermentation and hydrogen sulfide production in enteric bacteria. These media contain a variety of substrates that enteric bacteria can utilize, including several carbohydrates, proteins, and thiosulfate. Different enteric bacteria utilize these substrates differently, so they can be distinguished based on their pattern of utilization .

Both media contain beef extract, yeast extract, and peptone to support bacterial growth. The ferment able carbohydrates in TSI are glucose, lactose, and sucrose. KIA contains only glucose and lactose.

Sodium thiosulfate is the source of sulfur for H_2S production in both media .Both TSI and KIA contain ferrous sulfate, which reacts with$H_2S$to form a black precipitate called ferrous sulfide. The pH indicator in both media is phenol red. Phenol red is red at the initial pH of 7.4 but turns yellow at an acidic pH and dark red at an alkaline pH. Agar is present in these two media as a solidifying agent.

An isolate is inoculated on to a ***TSI or KIA slant*** using a sterile transfer needle. First ,the butt is stabbed. Then ,the needle is with drawn and the slant is streaked. The slant is incubated at 35°C for18-24 hours before examination for color changes.

Procedure:-

1. Sterilize the inoculating needle in the blue flame of the Bunsen burner till red hot and then allowed to cool.

2. From the rack , take the broth tube containing the 24-48 hour culture remove the, cap and flame the neck of the tube.

3. Using aseptic technique, take the culture of the organism from the tube with the needle.

4. Again flame the neck of the tube and replace the tube in the test tube rack.

5. Take a sterile TSI slant tube from the rack, remove the cap and flame the neck of the tube.

6. Stab the needle containing the pure culture in to the medium , up to the butt of the TSI tube, and then streak the needle back and forth along the surface of the slant.

7. Again flame the neck of the TSI tube, cap it and place it in the test tube rack.

8. Incubate at 37^{o}c for 18 to 24 hours.

Expected Results:

1. **Alkaline slant (red) and acid butt (yellow) with or without gas production (breaks in the agar butt):**

Only glucose fermentation has occurred. The organisms preferentially degrade glucose first. Since this substrate is present in minimal concentration, the small amount of the acid produced on the slant surface is oxidized rapidly. The peptones in the medium are also used in the production of alkali. At the butt, the acid reaction is maintained because of the reduced oxygen tension and slower growth of the organisms.

2. **Acid slant (yellow) and acid butt (yellow) with or without gas production:**

Lactose or sucrose fermentation has occurred. Since these substances are present in higher concentrations, they serve as substrates for continued fermentative activities with maintenance of an acid reaction in both the slant and the butt.

3. **Alkaline slant (red) and alkaline butt (red) or no change (orange-red) butt:**

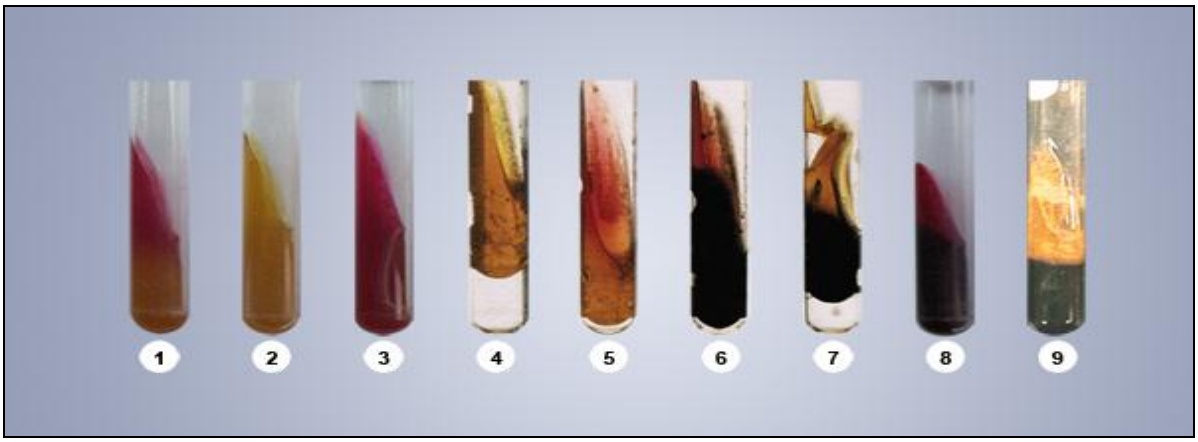
No carbohydrate fermentation has occurred. Instead; peptones are catabolized under anaerobic and /or aerobic conditions resulting in alkaline pH due to production of ammonia. If only aerobic degradation of peptones occurs, the alkaline reaction is evidenced only on the slant surface. If there is aerobic and anaerobic utilization of peptone, the alkaline reaction is present on the slant and the butt.

4. **Hydrogen sulfide (H_2S) production:**

Some bacteria utilize thiosulfate anion as a terminal electron acceptor, reducing it to sulfide. If this occurs, the newly-formed hydrogen sulfide (H2S) reacts with ferrous sulfate in the medium to form ferrous sulfide, which is visible as a black precipitate. The blackening of the medium is almost always observed in the butt (bottom) of the medium.

5. **Carbon dioxide (CO_2) production:**

It is recognized simply as bubbles of gas between the agar and the wall of the tube or within the agar itself. The carbon dioxide production is sufficient to split the agar into two or more sections. To obtain accurate results, it is absolutely essential to observe the cultures within 18-24 hours following incubation. This will ensure that the carbohydrate substrates have not been depleted and that degradation of peptones yielding alkaline end products has not taken place.



	Result (slant/butt)	Symbol	Interpretation
1	Red/Yellow	K/A	Glucose fermentation only, peptone catabolized.
2	Yellow/Yellow	A/A	Glucose and lactose and/or sucrose fermentation.
3	Red/Red	K/K	No fermentation, Peptone catabolized.
4	Yellow/Yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation, Gas produced.
5	Red/Yellow with bubbles	K/A,G	Glucose fermentation only, Gas produced.
6	Red/Yellow with bubbles and black precipitate	K/A,G,H_2S	Glucose fermentation only, Gas produced, H_2S produced.
7	Yellow/Yellow with bubbles and black precipitate	A/A,G,H_2S	Glucose and lactose and/or sucrose fermentation, Gas produced, H_2S produced.
8	Red/Yellow with black precipitate	K/A,H_2S	Glucose fermentation only, H_2S produced.
9	Yellow/Yellow with black precipitate	A/A,H_2S	Glucose and lactose and/or sucrose fermentation, H_2S produced.

13-LITMUS MILK REACTIONS

Purpose and Procedure Summary

Bacteria can act on several different substrates in litmus milk , including lactose, casein, and litmus, causing a variety of reactions that are specific for each species

of bacteria . Litmus milk contains skim milk ,the source of **lactose and casein, and litmus,** the pH/oxidation reduction indicator. Litmus is purplish-blue at the initial pH of **6.8** in the uninoculated medium. Several other colors for litmus are possible after bacterial growth occurs, depending on the action of the isolate. An isolate is inoculated into a tube with a sterile transfer loop. The tube is incubated at 35°C for24-48 hours before it is examined for changes.

Tips for Success

- Compare results to an uninoculated control tube to determine whether changes have occurred in the medium.

An additional incubation of 24-48 hours may Be necessary for some reactions to develop.

The blue of the alkaline reaction is most evident at the top of the medium ,while the white of litmus reduction is most evident at the bottom

. **17- Oxidation - Fermentation Test (O-F test)**

Principle

To determine the oxidative or fermentative metabolism of a carbohydrate or its non-utilization.

Fermentation is an anaerobic process and bacterial fomenters of carbohydrates are usually facultative anaerobes.

Oxidation is an aerobic process and bacterial oxidizers' are usually strict aerobes.

Hugh and Leif son medium is a semi-solid medium in tubes containing glucose and a pH indicator bromothymol blue which turn to yellow in acids production and turn to blue in alkalinity.

Carbohydrate conversion to acidic products can occur either aerobically by oxidation or an aerobically by fermentation. Bacteria that oxidize carbohydrates are called aerobes, while bacteria that ferment carbohydrates are called facultative anaerobes. The oxidation-fermentation (O-F) test is used to determine whether bacteria are aerobes or facultative anaerobes. The O-F test distinguishes Gram negative rods that are aerobes, such as Pseudomonas aeruginosa , from those that are facultative anaerobes, such as Escherichia coli. The O-F test also distinguishes Gram-positive cocci that are aerobes, such as species of Micrococcus ,from those that are facultative anaerobes, such as species of Staphylococcus.

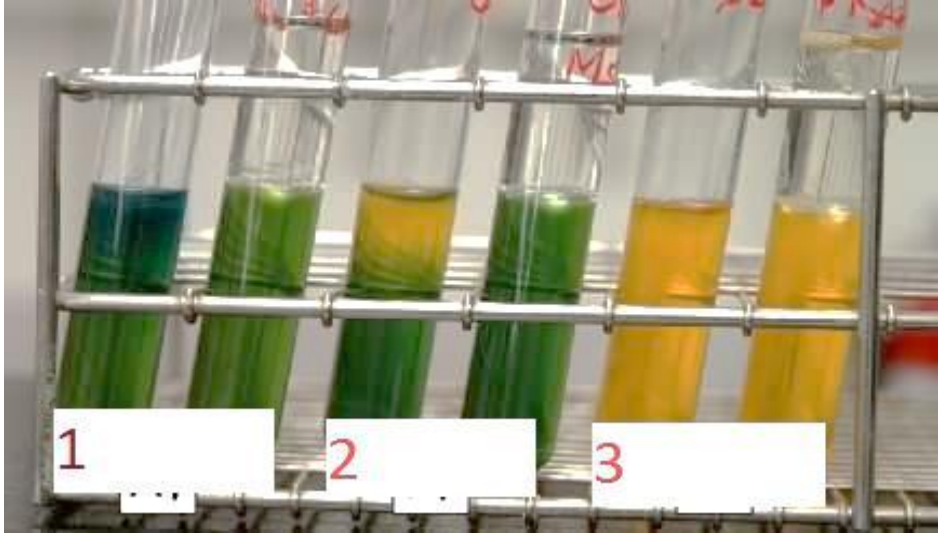
Oxidation-fermentation (O-F) medium is used for this test. This medium contains a low concentration of peptone, but enough to support growth. A low concentration of peptone is essential to limit the formation of alkaline products that would neutralize the effect of acidic products. A carbohydrate, such as glucose, lactose, maltose, sucrose, mannitol ,or xylose , is added at a high concentration. This high concentration promotes carbohydrate utilization, with the resulting formation of acidic products. The pH indicator, bromothymol blue ,is green at the initial pH of 7.1 and yellow at a pH of 6.0. Agar is added at a low concentration to make the medium semisolid. Although phenol red broth can also be Used for the O-F test, it is not recommended because it has a high concentration of peptone, which May result in the formation of sufficient alkaline products to neutralize the effect of acidic products.

An isolate is inoculated into two tubes of O-F Medium with a sterile transfer needle. The medium in one tube is then covered with 2-3 ml of sterile mineral oil to create an anaerobic environment. The medium in the other tube is left open to the air to. provide an aerobic environment. The two tubes are incubated at 35C° for 48 hours before examination for color change.

Procedure

Two tubes are inoculated by stabbing and one is immediately sealed with oil to produce anaerobic conditions

1. Organisms that cannot break down the carbohydrate aerobically or anaerobically eg *Alcaligenes feacalis* produce an alkaline reaction in the open tube and no change in the covered tube
2. Oxidizing organisms ***Pseudomonas* spp** produce an acid reaction in the open tube only
3. Fermenting organisms **Enterobacteriaceae** produce an acid reaction throughout the medium in both tubes


1
2
3