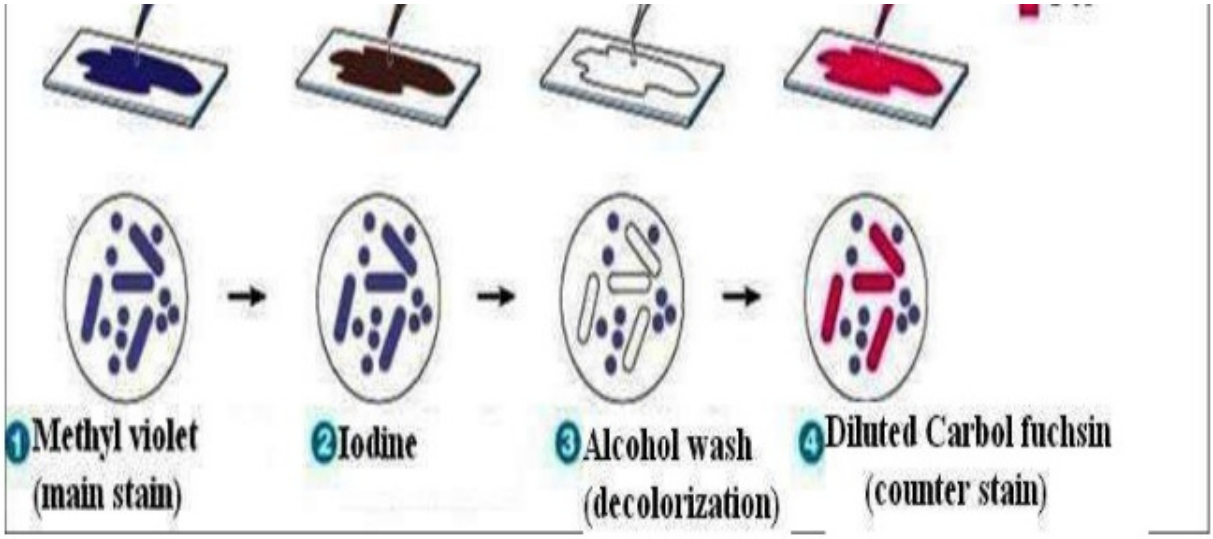
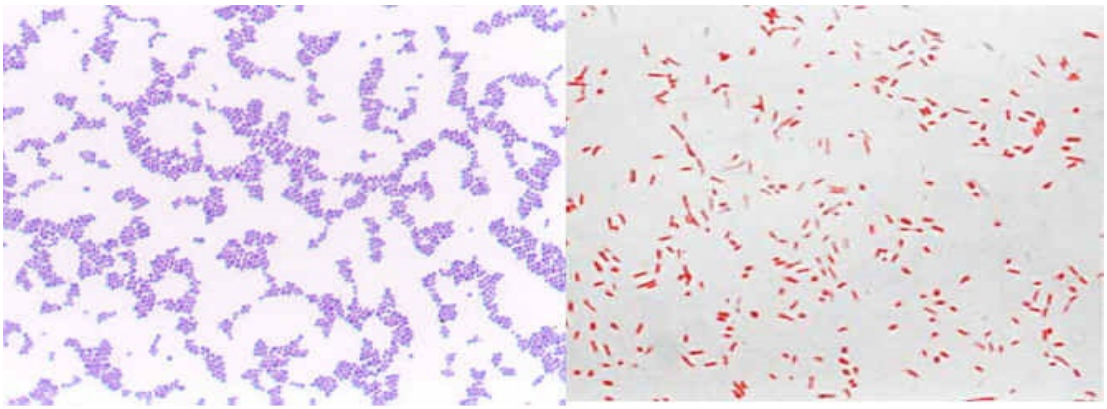
Microbe Identification

- Identification measures include:
  - **Microscopy (staining)**
  - **growth on enrichment, selective, differential or characteristic media**
  - **specimen biochemical test (rapid test methods)**
  - **immunological techniques**
  - **molecular (genotypic) methods**.
- After the microbe is identified for clinical samples it is used in **susceptibility tests** to find which method of control is most effective.

MICROSCOPY



Gram Staining technique



Gram +ve Bacteria

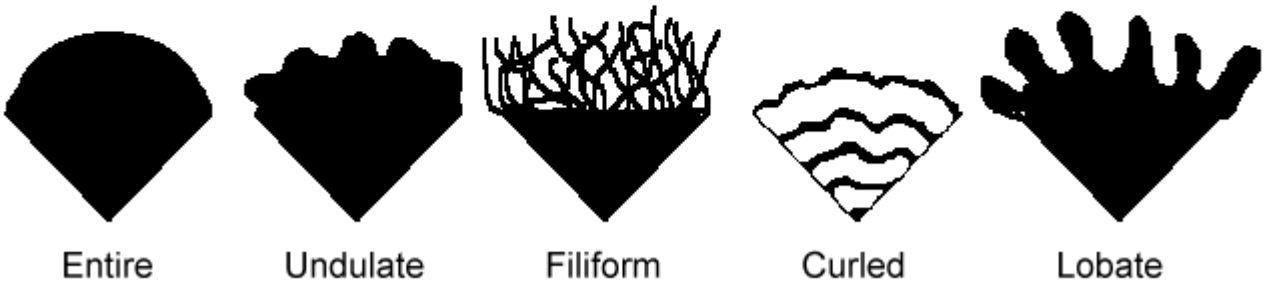
Gram -ve Bacteria

COMMON COLONY CHARACTERISTICS

Form

Elevation

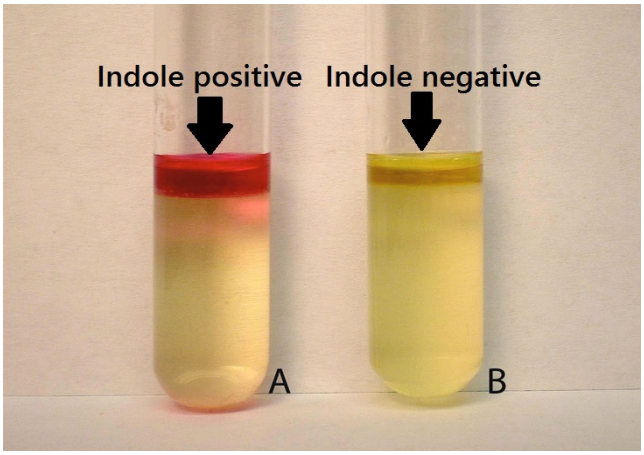
Margin



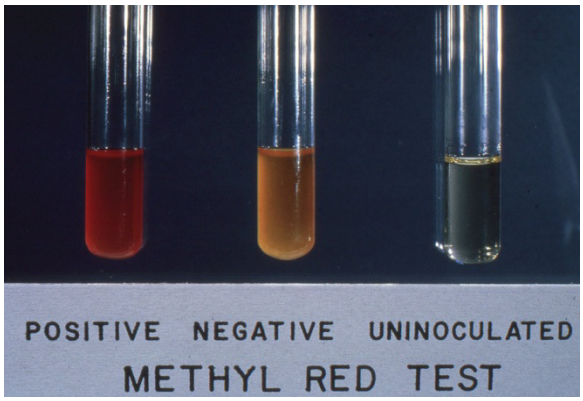
BIOCHEMICAL TESTS FOR THE IDENTIFICATION OF BACTERIA

A series of tests have been developed based on biochemical and metabolic activities of bacteria.

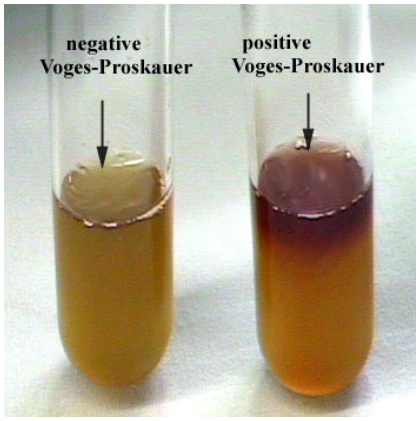
1. INDOLE PRODUCTION TEST- Indole is the break down product of the amino acid tryptophan. When an organism is grown in media containing tryptophan, it breaks down tryptophan producing indole. This indole when combines with compound paradimethyl aminobenzaldehyde (Ehrlichs or Kovac's reagent) gives a pink colored ring on the surface.
   Procedure- Add a few drops of Kovac's or Ehrlich's reagent into the overnight broth culture of the test organism in peptone water.
   Observation- a. Pink coloured ring – positive test (eg. E.coli)
   b. Yellow coloured ring- negative test (eg. Shigella)



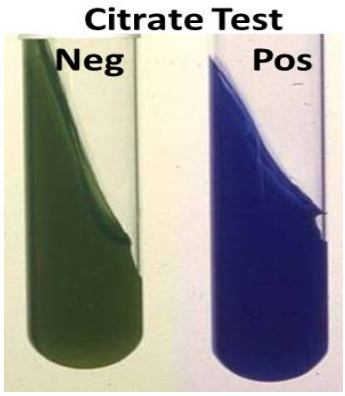
2. METHYL RED TEST- Some Gram negative bacilli produce large amount of acid in Glucose phosphate broth. The acidity can be determined by adding a few drops of methyl red indicator.
   Procedure – add a few drops of methyl red indicator into the overnight broth culture of the test organism in Glucose phosphate broth.
   Observation- a. Red colour – positive (eg. E.coli)
   b. Pale yellow- negative (eg. Klebsiella)



3. VOGES-PROSKAUER TEST- Some Gram negative bacilli produce a compound called acetyl methyl carbinol from glucose. This break down product reacts with alpha naphthol in the presence of alkali to give a cherry red colour.
   Procedure- Add a few drops of alpha naphthol and a few drops of 40%KOH into an overnight culture of test organism in Glucose phosphate broth. Shake well and keep for 10 minutes.
   Observation- a. Cherry red colour – positive (eg. Klebsiella)
   b. Pale yellow colour – negative (eg. E.coli)



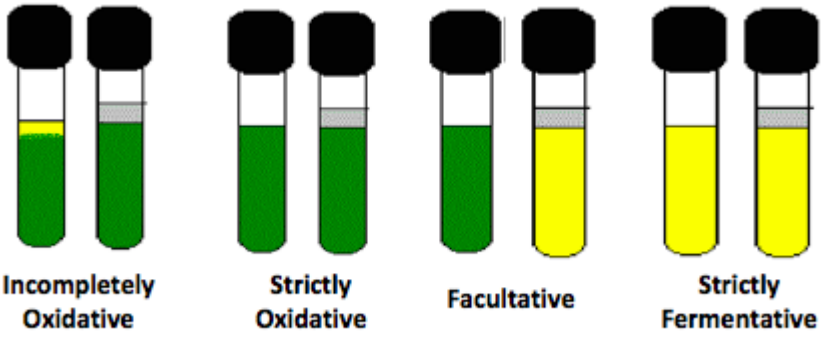
4. CITRATE UTILIZATION TEST- Some bacteria can utilize simple organic salts like sodium citrate or sodium acetate as a sole source of carbon. During metabolism it releases carbon dioxide which makes the medium alkaline. The indicator Bromothymol blue turns blue in alkaline conditions.
   Procedure- Inoculate the test organism over Simmon's Citrate slope and incubate it overnight.
   Observation- a. Growth with deep blue slant- citrate utilized (eg. Klebsiella)
   b. Green slant- citrate not utilized (eg. E.coli)



5. UREASE TEST- Some bacteria decompose urea and liberate ammonia by the action of enzyme urease. The ammonia will make the medium alkaline where the indicator phenol red will turn the medium pink.
   Procedure – Inoculate the test organism over Christensen's urea medium and incubate it.
   Observation – a. Growth with pink coloured slant- positive (eg. Proteus)
   b. Growth with pale yellow coloured slant- negative (eg. E.coli)

6. PRODUCTION OF HYDROGEN SULPHIDE- Some bacteria produce hydrogen sulphide by breaking down aminoacids like cysteine or cystine. The hydrogen sulphide production can be detected by exposing a filter paper dipped in 10% lead acetate solution.
   Procedure- The test is conducted by inserting a paper (dipped in the reagent) at the mouth of test tube containing over night peptone water culture of the test organism.
   Observation- a. Blackening of the lead acetate paper- H_2S positive (eg. Proteus)
   b. No blackening of the lead acetate paper- H_2S negative(eg. E.coli)

7. OXIDATION- FERMENTATION TEST- Saccharolytic bacteria can break down glucose through oxidation pathway or through fermentation. Fermentation of glucose can give strong acids which can easily be detected. Whereas oxidation of glucose yields weak acids which are difficult to detect. So to differentiate oxidation and fermentation Hugh- Leifson's oxidation-fermentation medium is used. The test is done by inoculating two tubes of OF medium by stab inoculation. One tube is sealed with sterile paraffin to provide anaerobic atmosphere.
   Observation- Acid formation will turn the medium to yellow colour.
   a. Yellow colour in both the tubes- organism is fermentative (eg. E.coli)
   b. Yellow colour only in the open tube- organism is oxidative(eg. Pseudomonas)
   c. No colour change in both the tubes- asaccharolytic reaction (eg. Moraxella)

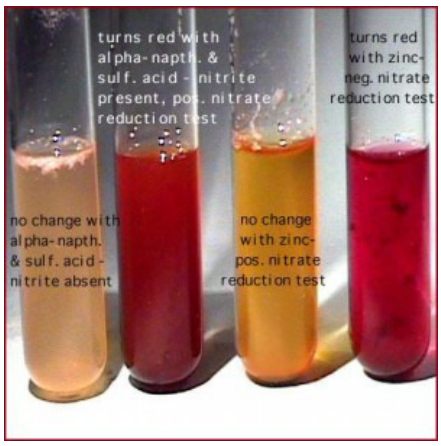


8. NITRATE REDUCTION TEST - The enzyme nitrate reductase produced by bacteria reduces nitrate to nitrite. The nitrate is detected by adding alpha naphthylamine and sulphanilic acid to the nitrate broth.
   Procedure- the test organism is grown in nitrate broth for 24 hours. To this equal amount of alpha naphthylamine and sulphanilic acid is added.
   Observation- a. positive reaction is indicated by formation of an immediate red colour.

If the test is negative it may be due to reduction of nitrite beyond nitrite to ammonia, nitrogen gas or nitric oxide. This is differentiated by addition of zinc dust. If it is a true negative reaction nitrate present in the medium is reduced by zinc to nitrite and develops a red colour.

Examples- Positive- E.coli

Negative- Acinetobacter

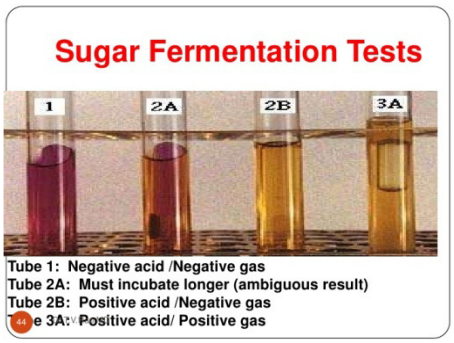


9. SUGAR FERMENTATION TEST - Many bacteria are able to ferment a variety of sugars ultimately breaking them into acids like lactic acid or pyruvic acid, and gases like carbon dioxide or hydrogen. For this media should have a base that supports the growth of bacteria like peptone water and a sugar which can be carbohydrate, glycosides, organic acids or polyhydric alcohols. To detect the change in the pH Andrade's indicator is used which gives a pink colour if the sugar has been fermented. Production of gas is detected in Durham's tube.

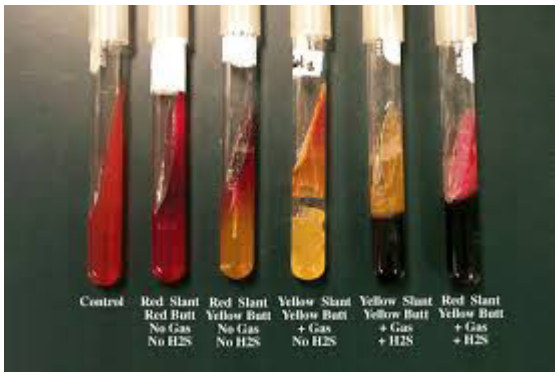
   Procedure- Inoculate the sugar media with the test organism using Pasteur pipette.

   Observation-

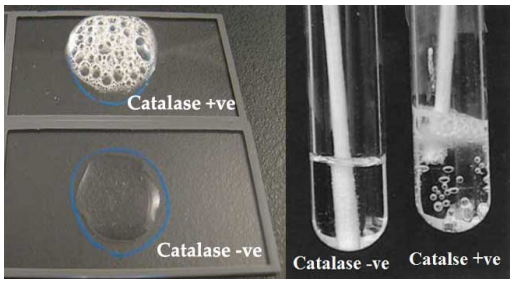
   a. Pink colour with gas in Durham's tube- sugar is fermented with acid and gas (eg. E.coli)
   b. Pink colour with no gas in Durham's tube- sugar is fermented with only acid production (eg. Shigella)
   c. No colour change, no gas- sugar is not fermented (eg. Pseudomonas)



10. TRIPLE SUGAR IRON (TSI)- TSI test facilitates the preliminary identification of Enterobacteriaceae. This medium can detect fermentation of sugar (glucose, lactose and sucrose), gas production and hydrogen sulphide formation. Medium is distributed in tubes with a butt and slant.
    Procedure- inoculate the butt and slant separately with the test organism, incubate overnight.
    Observation-
        a. Yellow colour slant by yellow colour butt- A/A reaction- all sugars are fermented. Example- E.coli
        b. Pink coloured slant by yellow colour butt- K/A reaction – lactose is not fermented. Example- Salmonella
        c. Bubbles in the butt- gas formation. Example- Klebsiella
        d. Blackening of the medium- Hydrogen sulphide formation- Example- Proteus

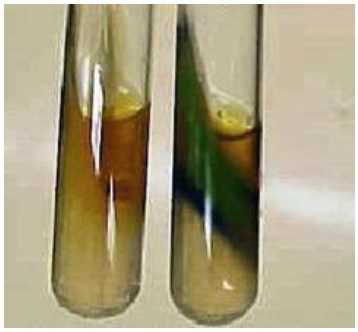


11. CATALASE TEST- Demonstration of the presence of enzyme catalase which catalyses the release of oxygen from hydrogen peroxide.
    Procedure- the test is done by adding a drop of 3%hydrogen peroxide to fresh bacterial growth deposited on a glass slide.
    Observation - The production of immediate bubbles or effervescence indicates a positive test.
    Examples – Catalase positive- Staphylococcus aureus
    Catalase negative- Streptococcus pyogenes.
    Precaution- Nichrome wire should not be used to pick up the colony. A colony grown on blood agar is unsuitable for the test.



12. OXIDASE TEST- Cytochrome oxidase helps in aerobic respiration by transporting electrons from water to oxygen. To detect the presence of this enzyme a dye is used which is colourless in reduced condition but becomes coloured in presence of cytorchrome oxidase.
    Procedure- A filter paper strip impregnated with oxidase reagent (1% acqueous solution of tetramethyl paraphenylene diamine dihydrochloride) is taken. The bacterial colony is smeared with a glass tip.
    Observation – Development of purple colour within 30 seconds indicates a positive reaction.
    Positive Example- Pseudomonas aeroginosa, Vibrio cholera
    Negative example- E.coli, Klebsiella

13. PHENYLALANINE DEAMINASE (PPA) TEST- Certain bacteria deaminate phenylalanine with production of phenyl pyruvic acid.
    Procedure- The test organism is inoculated on PPA agar slant and incubated. Phenyl pyruvic acid is detected by adding a few drops of ferric chloride solution over the growth.
    Observation- production of green colour indicates a positive test.
    Examples – Positive- Proteus
    Negative- E.coli



14. LYSINE IRON AGAR TEST (LIA TEST)- It is used to demonstrate the members of enterobacteriaceae based on their ability to decarboxylate or deaminate lysine and produce hydrogen sulphide gas.

    LIA contains small amount of glucose, lysine, a sulphur salt, hydrogen sulphide indicator and pH indicator. As the organism ferment glucose they produce acid turning the colour of medium to yellow. As this is a fermentation process it occurs in the butt.

    Organism may also decarboxylase or deaminase lysine. Decarboxylation yields alkaline product which neutralize the acid leading the reversion of the butt from yellow to purple. Organism that deaminase lysine causes the medium to turn red in colour.

    Some bacteria are able to produce hydrogen sulphide gas from sodium thiosulphate. This forms black colour precipitate or blackening of the medium.

    Procedure- inoculate the butt and slant separately with the test organism, incubate overnight.

    Observation-

    a. Purple slant by yellow butt- lysine not decarboxylated
    b. Purple slant by purple butt- lysine decarboxylated.
    c. Red slant by red butt- lysine deaminated
    d. Blackening of the medium- hydrogen sulphide produced