Lab 11 (Biochemical Tests)

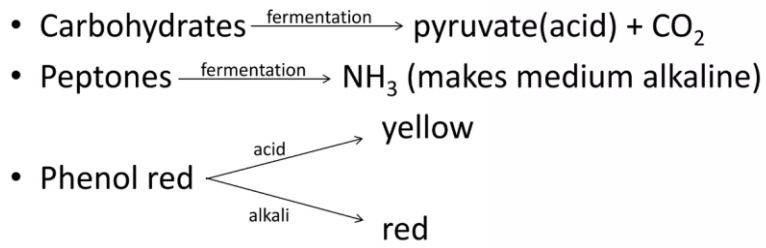
TSIA test (Triple Sugar Iron Agar)

- The **triple sugar iron agar** (TSIA) test is a **biochemical test** used to differentiate bacteria based on their ability to ferment three sugars (**glucose, lactose, and sucrose)** and release acid and **hydrogen sulfide (H_2S)** gas which reacts with ferric ions in the medium to produce iron sulfide (black insoluble precipitate).
- Objectives of TSI include:

1. Identification of enteric pathogens among Enterobacteriaceae (Gram-negative bacilli)
2. To test the bacteria's ability to utilize glucose, lactose, and/or sucrose and produce H_2S gas.
3. To differentiate lactose fermenters from non-lactose fermenters.

Principle of TSI

- TSIA test is based on the distinct metabolic pattern of the different bacterial genera to metabolize glucose, lactose, sucrose, and sodium thiosulfate (a sulfur compound).



- Fermentation of carbohydrates result in the production of acid which decrease the pH of the medium and change color from reddish-orange to yellow.
- Utilization of peptones result in alkalization of the medium due to the production of NH_3.
- The production of hydrogen sulfide is indicated by the presence of black ppt. formed by the reaction of H_2S with ferric ions.
- Slant is aerobic while butt is anaerobic.
- Gas production is indicated by the splitting of the agar and lifting of it to the top.
- The formation of H_2S requires an acidic environment; this means that even if the color of the pellet cannot be seen because of the darkening of the medium, the bacterium is glucose (+) because if there was no consumption of glucose and acidification of the medium the formation of H_2S could not take place.

Results Interpretation

1. Glucose fermenter:
   tube reaction: alkaline over acid (K/A); red slant, yellow butt;

➔ with H_2S production → (K/A, H_2S +ve); red slant, black butt

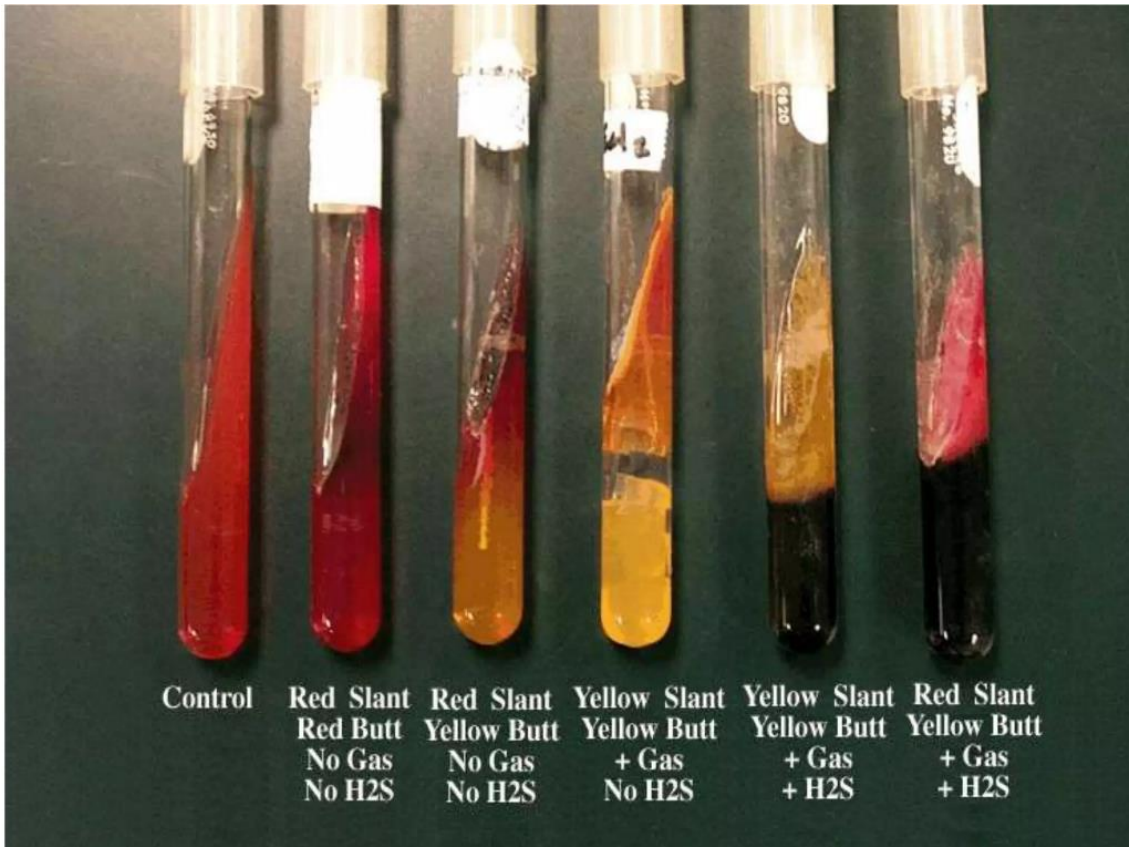
2. Glucose, lactose and/or sucrose fermenter:
   tube reaction: acid over acid (A/A)
   → with H_2S production →(A/A, H_2S +ve); yellow slant, black ppt. in the butt.
3. Glucose, lactose, and sucrose non-fermenters:

   Tube reaction:

a. Alkaline over alkaline (K/K) if the bacteria can metabolize peptones both aerobically and anaerobically; both slant and butt are red

b. Alkaline over no change (K/NC) if peptones can only be metabolized aerobically; slant red, butt no change.

➔ With H_2S production → alkaline over no change (K/NC, H_2S +ve); black ppt. in the butt.



Expected Culture results:

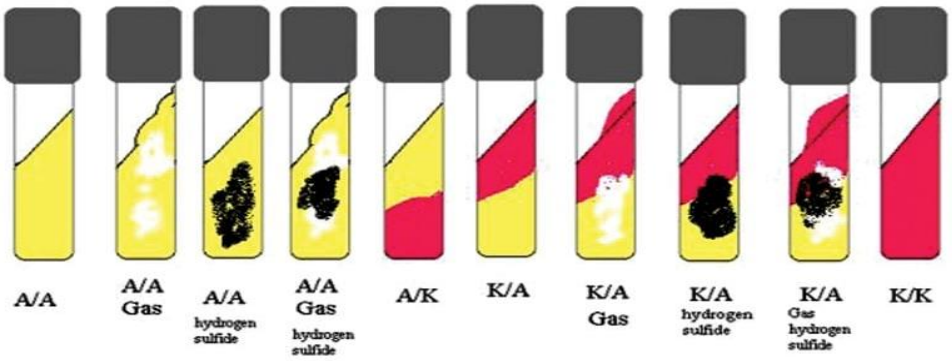
	Slant	Butt	Gas	H_2S
Proteus mirabilis	K	A	-	+
Pseudomonas aeruginosa	K	K	-	-
E. coli	A	A	+	-
Shigella flexneri	K	A	-	-

Procedure

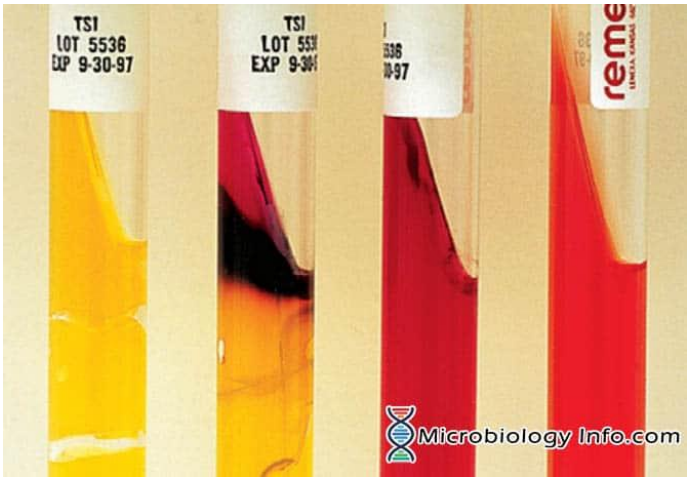
1. Touch a well-isolated colony from a fresh culture of the test bacterium that is 18 to 24 hours old using a sterile inoculating wire.
2. Stab the bottom up to 3 to 5 mm above the base of the test tube using the inoculating wire and while withdrawing, streak the slant.
3. Incubate the tube aerobically (with a loose cap) at 35±22°C for about 24 hours.
4. Examine for color change of the slant and bottom and report the color within 24 hours of incubation. (If you want to read the H_2S production, incubate it for another 24 to 48 hours, but read sugar fermentation and color change within the first 24 hours of inoculation and incubation.)

Results:

1. The red slant and yellow bottom (Red/Yellow or Alkaline (K)/Acidic (A)) indicate only glucose is fermented.
2. Yellow slant and yellow bottom (Yellow/Yellow or Acidic (A)/Acidic (A)) indicate lactose and/or sucrose fermentation or fermentation of all three sugars.
3. Red slant and red bottom (Red/Red or Alkaline (K)/Alkaline (K)) indicate none of the three sugars are fermented.



4. Blackening of the media or formation of the black-colored spots indicates H_2S production.
5. Cracking of the media, gas bubbles of the media, or forming a gap in the media indicates gas production.



Triple sugar iron agar tubes: from the left:
1, Acid slant/acid bottom with gas, no H_2S (A/A).

2, Alkaline slant/acid butt, no gas, H_2S-positive (K/A H_2S+).

3, Alkaline slant/alkaline butt, no gas, no H_2S (K/K).

4, Uninoculated tube (negative control).

IMViC test

IMViC test is a series of four different biochemical tests used in identifying and differentiating bacteria, especially the members of *Enterobacteriaceae*. Though it can be (and is) used for the identification of any type of bacteria, it is mainly used for identifying Gram-negative bacteria. It is the key to identifying and differentiating members of the *Enterobacteriaceae* family.

IMViC is an acronym for four different biochemical tests; each letter except "i" represents an individual test making this series of biochemical tests. IMViC series contains the following biochemical tests:

1. **"I" = Indole Test**
2. **"M" = Methyl Red (MR) Test**
3. **"V" = Voges – Proskauer (VP) Test**
4. **"C" = Citrate Utilization Test (simply Citrate Test)**

Objectives of IMViC Test

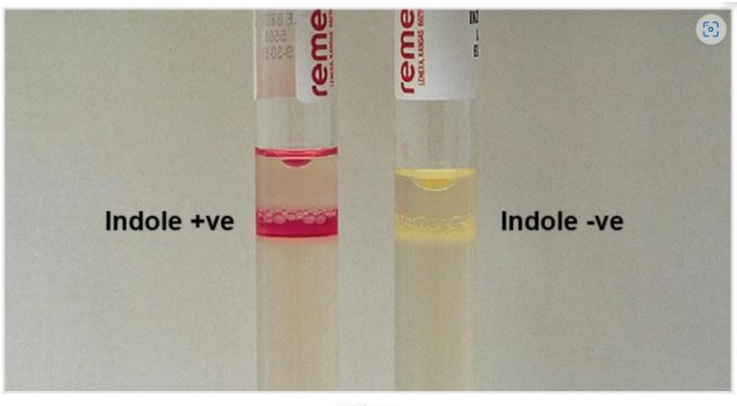
1. To study some biochemical properties – indole production, acid production, acetylmethylcarbinol (acetoin) production, and citrate utilization – of isolated unknown bacteria in order to characterize and identify them.
2. To selectively differentiate and identify members of the *Enterobacteriaceae* family

1. The indole test

The indole test is a biochemical test in the IMViC test series which detects the ability of organisms (bacteria) to produce indole as a metabolic product utilizing tryptophan. It is indicated by the letter "I" of the IMViC.

Principle of Indole Test

Some bacteria can produce an enzyme called 'tryptophanase' which helps them to metabolize the amino acid 'tryptophan' into 'indole, pyruvic acid, and ammonia'.



Indole Test

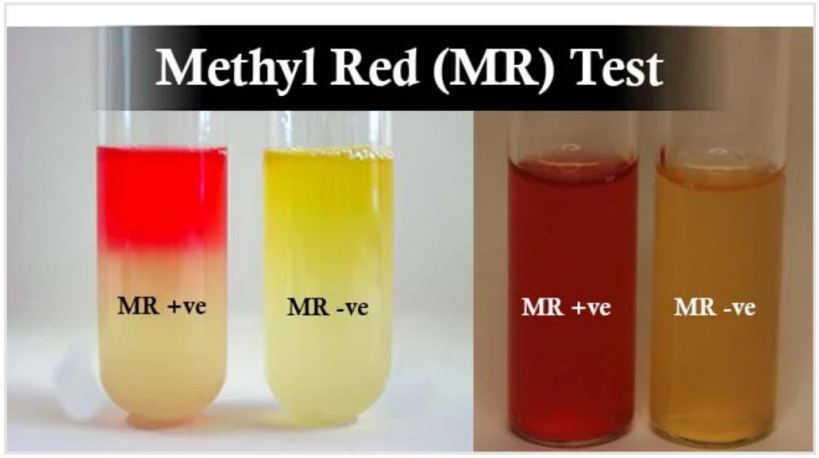
Indole Test results in different Bacterial strains.

2. **Methyl Red (MR) Test**

Methyl Red (MR) Test is a biochemical test that detects the ability of organisms (bacteria) to produce stable mixed acids as metabolic end products of glucose metabolism. It is indicated by the letter "M" of the IMViC.

Principle of MR Test

Some species of bacteria use the mixed acid fermentation pathway as their glucose metabolism process. Following this metabolic pathway, they convert pyruvate into stable mixed acids.



MR Test.

Methyl Red Test (MR)

Left, result on agar media.

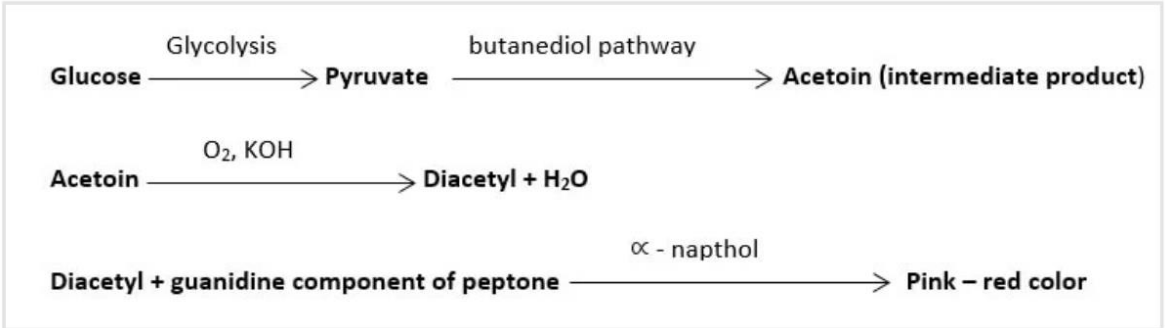
Right: results in liquid media (broth)

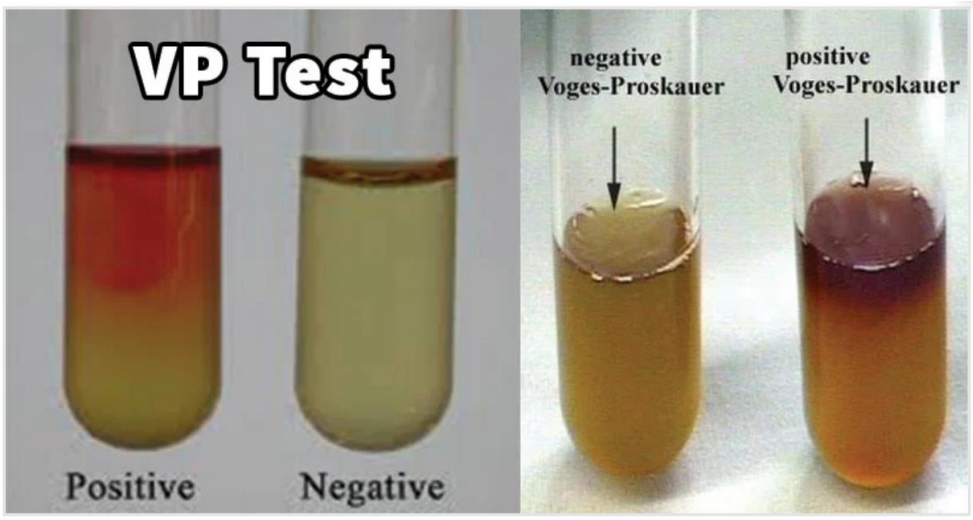
3. **Voges-Proskauer (VP) Test**

Voges-Proskauer (VP) Test is a biochemical test in the IMViC test series which detects the ability of organisms (bacteria) to metabolize the pyruvate into a neutral intermediate product called 'acetylmethylcarbinol' or 'acetoin'. It is indicated by the letter "V" of the IMViC.

Principle of VP Test

Pyruvate can be metabolized into a neutral intermediate product called 'acetyl methyl carbinol', commonly called the 'acetoin' during the butanediol pathway of 2,3-butanediol production.





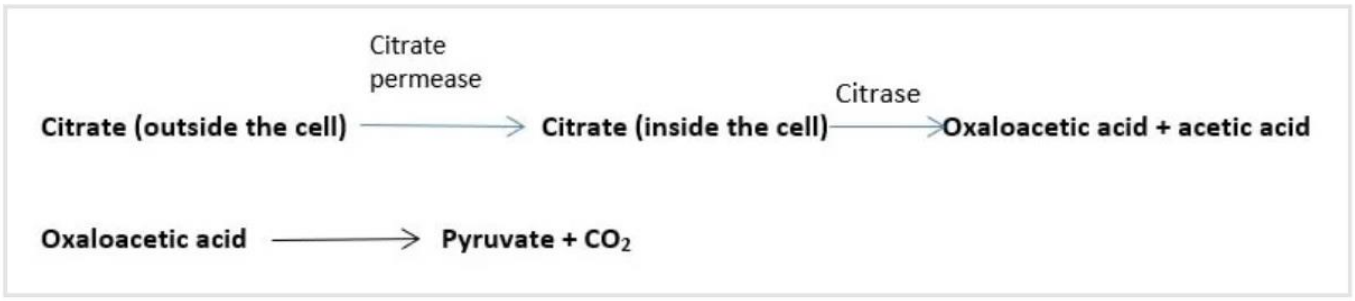
VP Test.

4. Citrate Utilization Test

Citrate Utilization Test is a biochemical test in the IMViC test series which detects the ability of organisms (bacteria) to utilize citrate as a sole source of energy. It is indicated by the letter "C" of the IMViC.

Principle of Citrate Utilization Test

Some bacteria can utilize 'citrate' as their sole source of carbon. Such bacteria produce citrase enzymes which will break the citrate into oxaloacetic acid and acetic acid. The oxaloacetic acid will then be decarboxylated to produce pyruvate and CO_2



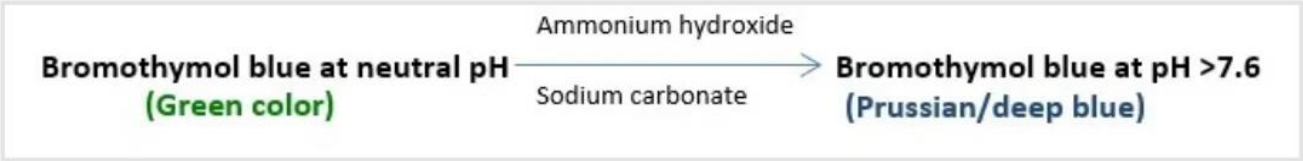
Released CO_2 will combine with H_2O and excess sodium from sodium citrate to produce alkaline 'sodium carbonate'. The sodium carbonate will increase the pH of the medium

$$CO_2 + H_2O + \text{excess sodium from sodium citrate} \rightarrow Na_2CO_3 \text{ (alkaline)}$$

Additionally, the released CO_2 will trigger the metabolism of ammonium salts. Utilization of the ammonium salts as a source of nitrogen will cause the production of ammonia (or ammonium hydroxide).

$$\text{Ammonium salt} \rightarrow \text{Ammonium hydroxide (alkaline)}$$

The combined effect of ammonium hydroxide and sodium carbonate will increase the pH of the media above 7.6. This increase in pH will turn the pH indicator bromothymol blue in the medium from deep forest green (at neutral pH) to Prussian blue.



Following the incubation of 24 – 48 hours (up to 4 days for some), bacterial growth and color change in the slant portion is observed. A positive result is indicated by growth and change in color of slant from green to intense blue. A negative result is indicated by no change in the color of the slant.