

# Biochemical Test for bacteria

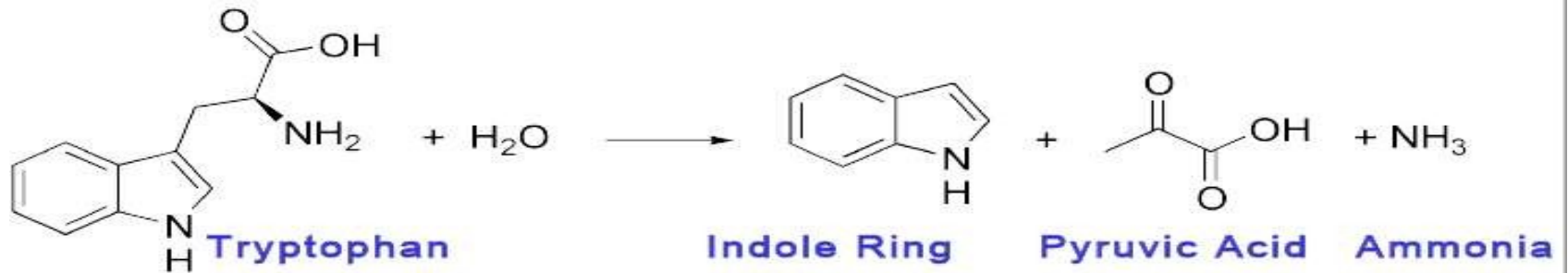
## • IMViC:

- Indole Test
- Methyl Red Test ( MR –VP Test)
- Voges-Proskauer Test
- Citrate Utilization Test
- Some microorganism are differentiated on the basis of **enzyme-catalysed metabolic reactions**
- Presence and absence of certain enzymes, intermediately metabolites and products often give valuable information in identifying and classifying microorganism.
- These tests are useful in distinguishing members of Enterobacteriaceae (Aerobic, non-acid fast, gram negative bacilli found in human and other animal intestine).

# Indole test

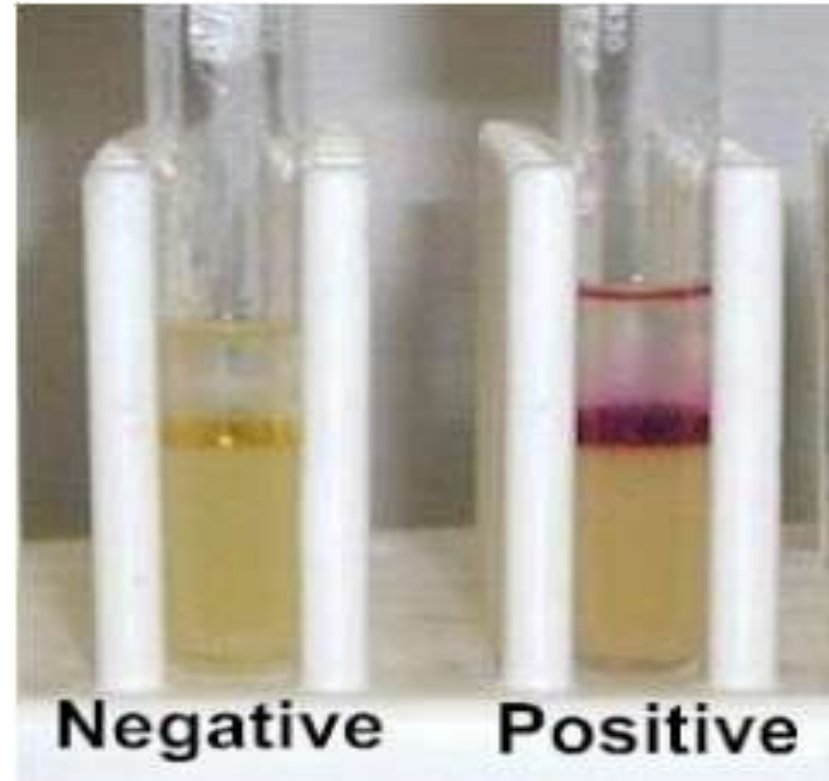
- Identifies bacteria capable of **producing indole**
- Some bacteria are capable of **converting tryptophan (an amino acid) to indole and pyruvic acid by using the enzyme tryptophanase**
- Pyruvic acid can be converted to energy or used to synthesize other compounds required by the cell

## Principle Reaction:



# Procedure

- Indole production is detected by inoculating the test microorganisms into Tryptophan broth and incubating it at 37°C for 48 hrs.
- Then add 0.5ml of **Kovac's reagent(10drops)** and mix gently.
- If indole is produced, A “cherry-red”, band forms on the surface of the media.

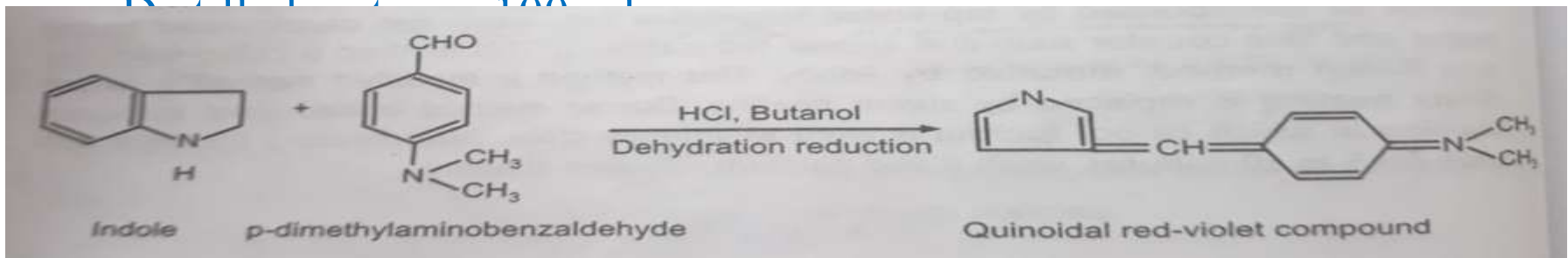


## Tryptophan broth

Tryptophan -1 gm

Sodium chloride -0.5 gm

Distilled water -100 ml



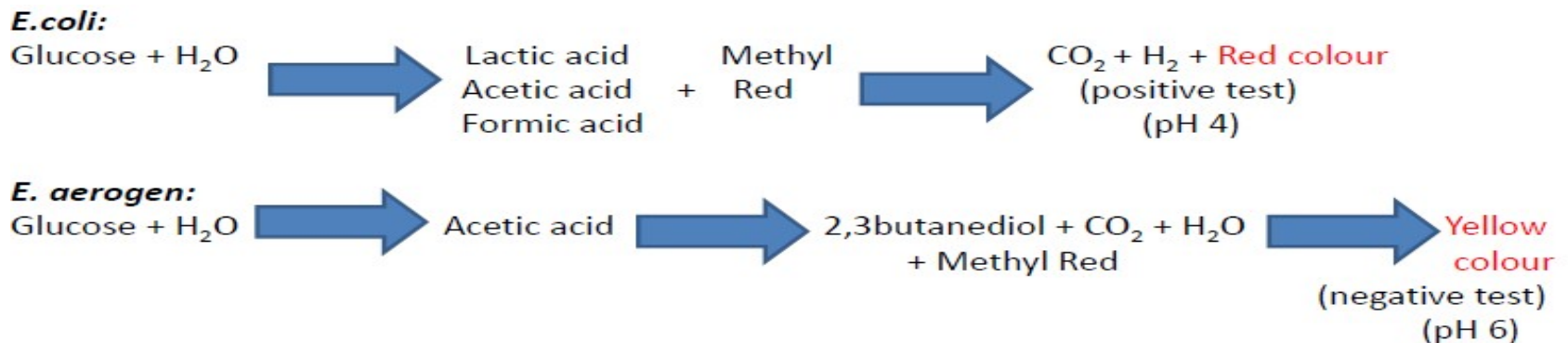
# Interpretation:

- Development of cherry red colour at the interface of the reagent and the broth, within seconds after adding the Kovac's reagent indicates the presence of indole and the test is positive.
- If no colour change is observed, then the test is negative and so organisms are not capable of producing indole.

# Methyl red (MR) Test

- Used to detect the production of acid during fermentation of glucose.
- Mainly used to differentiate between *E.coli* and *E. aerogen*
- By production of acid, pH of the medium falls and **maintained below 4.5**
- Methyl red is a pH indicator (red at pH less than 4.4 and yellow at a pH greater than 6).

## Principle Reactions:



# Procedure

- Inoculate the test microorganisms in MR-VP broth and incubate at **37°C for 48 hrs.**
- Then add five drops **of 0.04% solution of methyl red**, mix and observe the result in the form of change in color.

## MR-VP Broth

Peptone – 0.7 gm

Dextrose – 0.5 gm

Dipotassium Phosphate – 0.5 gm

Distilled water – 100 ml

# Interpretation

- A red colour indicates that glucose has been oxidized.
- Methyl red positive tube on the right (Acid production)- *E.coli*
- Methyl red negative tube on the left (No acid production)- *E. aerogen*



# Voges-Proskauer Test

- Used to determine the ability of microbes to produce **non-acidic or neutral end products** (acetyl methyl corbinol- acetoin, 2,3-butanediol and Diacetyl).
- **MR-VP broth** is a combined medium used for two tests—**Methyl Red and Voges-Proskauer**.
- This test characterizes *E.aerogen*
- **Procedure**  
Inoculate the test organism into MR-VP broth and incubate at 37 ° C for 48 hrs.
- Then add 1ml of 40% potassium hydroxide and 3ml of 5% solution of  $\alpha$ -naphthol in absolute alcohol. **Barritt's Reagent**
- **Stand for 5-10mins for colour development**



# Interpretation

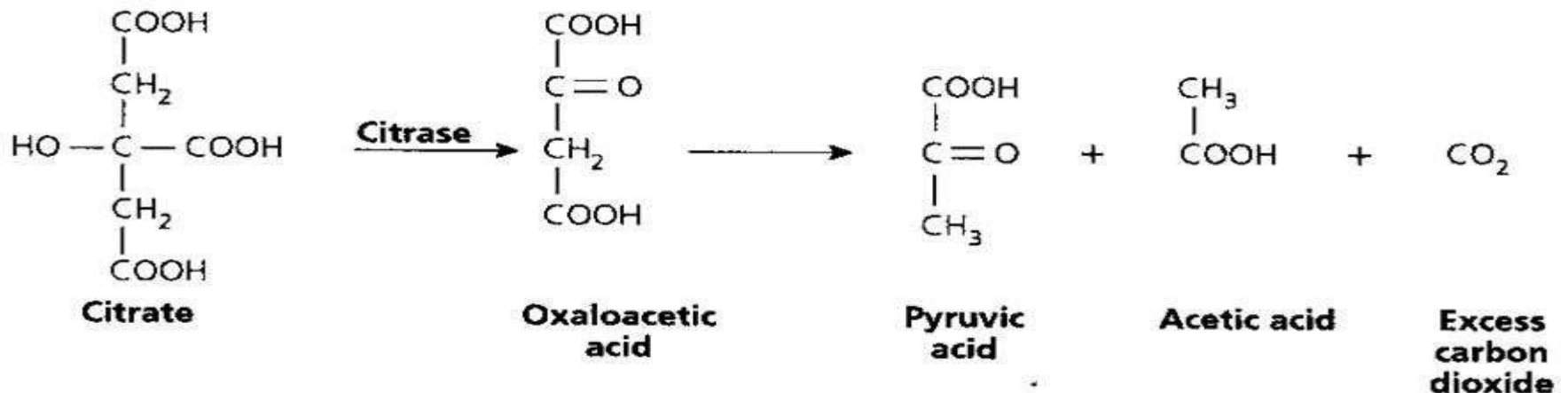
- Development of **crimson red** colour indicates **positive test for *E.aerogen***.
- And no colour change indicates **negative test**.



# Citrate Utilization Test

- Used to determine if an organism is capable of fermenting citrate as the **sole source of carbon for growth**.
- **It indicated by the production of turbidity in the medium.**
- The ability of an organism to utilize citrate occurs via the **enzyme citrase**.

## Principle Reaction:



# Citrate Utilization Test

## Simmon's citrate agar medium

Magnesium sulphate	- 0.02 gm
Ammonium dihydrogen phosphate	- 0.1 gm
Dipotassium phosphate	- 0.1 gm
Sodium citrate	- 0.2 gm
Sodium chloride	- 0.5 gm
Bromothymol blue	- 0.008 gm
Agar	- 1.5 gm
Distilled water	- 100 ml

# Citrate Utilization Test

## Procedure

- Prepare simmon's citrate agar slants aseptically
- Incubate the slants by stabbing to the base of slant. Thereafter streak the surface (Stab and streak inoculation)
- Incubate the slants at 37 °C for 48 hrs.

# Citrate Utilization Test

- When citric acid is metabolized the  $\text{CO}_2$  generated, combined with sodium and water to form sodium carbonate (An Alkaline product)
- which changes the color of the indicator from green to blue as in positive test
- The indicator is bromothymol blue. It is green when acidic pH (6.8 or below) and blue when alkaline (PH 7.6 or higher)
- The complete green in color (Negative)
- Turns to blue (Positive), when the test organism is capable of utilizing citrate. (Salmonella sp.)

