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IMViC REACTIONS

IMViC Reactions Overview

IMViC reactions are essential tests used for identifying and differentiating members of the **Enterobacteriaceae** family, particularly **Escherichia** and **Enterobacter**. These tests are designed to study the physiological characteristics of Gram-negative intestinal bacilli, which are biochemically and genetically related.

The IMViC tests consist of four distinct biochemical tests, each denoted by a letter in "IMViC":

- **I** - Indole Test
- **M** - Methyl Red Test
- **V** - Voges-Proskauer Test
- **C** - Citrate Utilization Test

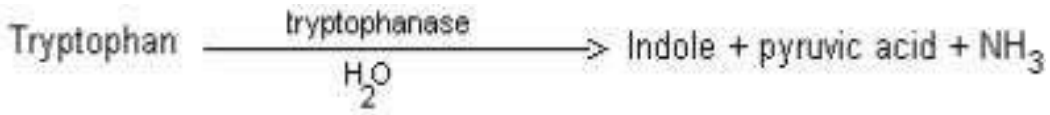
The letter "i" in IMViC is for rhyming purposes only and does not correspond to an actual test.

❖ INDOLE TEST

Principle:

The Indole Test assesses an organism's ability to produce indole from the amino acid tryptophan using the enzyme tryptophanase.

Biochemical Reaction: Tryptophanase catalyzes the hydrolysis of tryptophan to produce indole, ammonia, and pyruvate. The chemical equation for this reaction is:



Detection: Production of indole is detected using Ehrlich's reagent or Kovac's reagent. Indole reacts with the aldehyde in the reagent to give a red color.

An alcoholic layer concentrates the red color as a ring at the top.

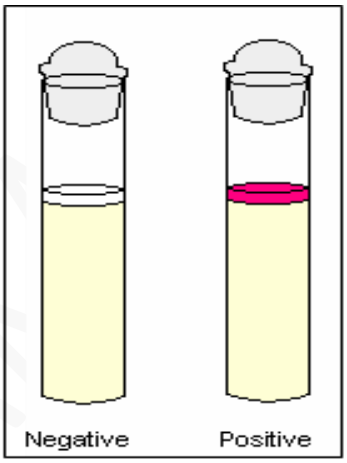
Procedure:

1. **Preparation:**
    - Inoculate the test bacterium into peptone water containing tryptophan.
    - Incubate at 37°C overnight.
    - Prepare a 1% tryptophan broth for the test.
2. **Controls:**
    - Include a test tube with the test organism (e.g., **E. coli**) as the positive control.
    - Include a negative control with peptone water but without inoculation.
3. **Addition of Reagents:**
    - Add a few drops of Kovac's reagent to the test and control tubes.
4. **Observation:**
    - Shake the tubes gently and allow them to stand for 2 minutes.
    - The reagent will rise to the top, where it forms a red or pink ring if indole is present.
5. **Ehrlich's Reagent:**

- Note that Ehrlich's reagent can also be used, and it is more sensitive in detecting indole production in anaerobes and non-fermenters.

Results:

- **Positive:** Formation of a red or pink ring at the top of the solution.
- **Negative:** No colour change or a yellow ring.



Examples:

- **Escherichia coli:** Positive
- **Klebsiella pneumoniae:** Negative

❖ METHYL RED TEST:

Principle: The Methyl Red (MR) test determines the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria ferment glucose to produce large amounts of mixed acids, which can overcome the buffering capacity of the medium, resulting in a low pH. Methyl Red is a pH indicator that turns red at a pH of 4.4 or below.

Glucose → Pyruvic acid → Mixed acid fermentation (pH 4.4)
↓
Red color with methyl indicator

Procedure:

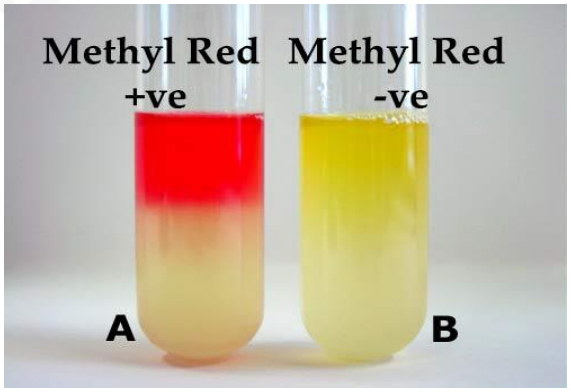
1. **Inoculation:** Inoculate the bacterium to be tested into a glucose phosphate broth, which contains glucose and a phosphate buffer.
2. **Incubation:** Incubate the broth at 37°C for 48 hours.
3. **Testing:** After incubation, add 5 drops of Methyl Red reagent to the broth.
4. **Observation:** Examine the colour change.

Interpretation:

- **Positive Result:** Development of a red colour indicates that the organism produces sufficient acids to lower the pH below 4.4, demonstrating mixed-acid fermentation**.**
- **Negative Result:** No colour change or a yellow colour indicates that the organism does not produce sufficient acids, and the pH is above 6.0.

Examples:

- **Escherichia coli:** Positive (Red colour)
- **Klebsiella pneumoniae:** Negative (Yellow colour)



❖ VOGES-PROSKAUER TEST

Principle: The Voges-Proskauer (VP) test detects the production of acetoin (acetyl-methyl carbinol) and butylene glycol during glucose fermentation. Acetoin is an intermediate in the production of butylene glycol. After incubation, two reagents are added to the test broth. Acetoin, in the presence of atmospheric oxygen and potassium hydroxide (KOH), is oxidized to diacetyl. Diacetyl then

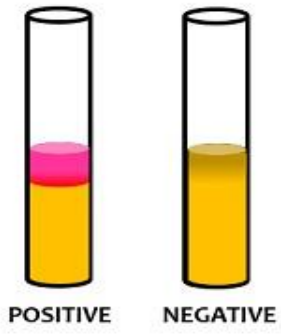
reacts with guanidine components of peptone in the presence of alpha-naphthol to produce a red color. Alpha-naphthol serves as a catalyst and color intensifier.

Procedure:

1. **Inoculation**: Inoculate the bacterial culture into glucose phosphate broth and incubate at 37°C for at least 48 hours.
2. **Reagent Addition**:
   - Add 0.6 ml of alpha-naphthol to the test broth and mix thoroughly.
   - Add 0.2 ml of 40% KOH to the broth and mix thoroughly.
   - Allow the tube to stand for 15 minutes.
3. **Observation**: Examine the colour of the broth.

Interpretation:

- **Positive Result**: A red colour indicates the presence of acetoin, demonstrating butylene glycol production.
- **Negative Result**: No colour change or a copper colour indicates the absence of acetoin. Maximum colour development occurs within one hour after the addition of reagents.



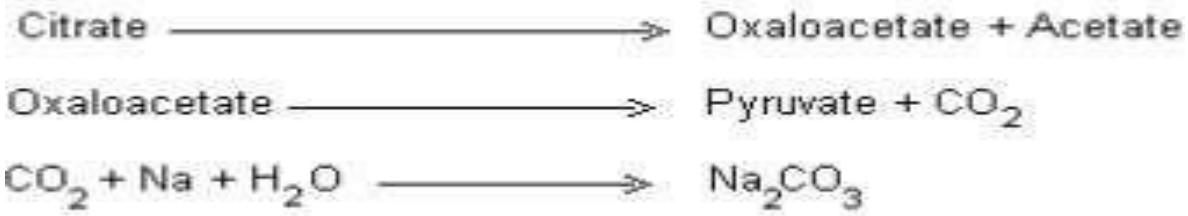
Examples:

- **Escherichia coli**: Negative (No red colour)
- **Klebsiella pneumoniae**: Positive (Red colour)

❖ CITRATE UTILIZATION TEST

Principle: This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator bromothymol

blue. The medium also contains inorganic ammonium salts, which is utilized as sole source of nitrogen.

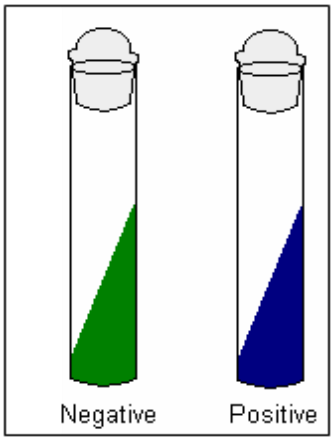


Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and CO2. Production of Na2CO3 as well as NH3 from utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in change of medium's color from green to blue.

Procedure:

1. Bacterial colonies are picked up from a straight wire and inoculated into slope of Simmon's citrate agar and incubated overnight at 37°C.
2. If the organism has the ability to utilize citrate, the medium changes its color from green to blue.

Observation- If colour of the medium change to blue it is citrate Positive E. coli is citrate Positive.



Examples: *Escherichia coli*: Negative;

Klebsiella pneumoniae: Positive

Differentiation of enteric bacteria by IMViC tests:

Genus	Indole	M.R.	V.P.	Citrate
Escherichia	+	+	-	-
Enterobacter	-	-	+	+
Klebsiella	-	-	+	+
Salmonella	-	+	-	+
Proteus	+	+	-	+