Bacterial taxonomy Lab:6

Differential biochemical tests

Indole , Methyl Red , Voges-Proskauer and Citrate utilization tests (IMViC).

Indole Test

The indole test determines a bacterium's ability to produce the enzyme tryptophanase, which breaks down the amino acid tryptophan into indole. It is a key test in identifying Gram-negative bacteria, particularly in the IMViC series (Indole, Methyl red, Voges-Proskauer, Citrate) to differentiate Enterobacteriaceae. A positive result is indicated by a red ring after adding Kovac's reagent, while a negative result remains yellow.

Principle and Procedure

- **Principle:** Bacteria possessing the enzyme **tryptophanase** break down tryptophan into **indole**, **ammonia**, and **pyruvic acid**.
- **Reagent:** **Kovac's reagent** (containing p-dimethyl amino benzaldehyde) is added, which reacts with indole to form a red dye (rosin dole).
- **Procedure (Tube Method):**
  1. Inoculate tryptophan or peptone broth with the bacteria.
  2. Incubate for 24–48 hours at 35–37°C.
  3. Add 5 drops of Kovac's reagent.
  4. Observe for a red layer at the top of the broth within minutes.
- **Rapid/Spot Method:** Rub a colony onto filter paper saturated with dimethylamino-cinnamaldehyde (DMACA) reagent. A blue-green color indicates a positive result.

Results

- **Positive (+):** Formation of a red (Kovac's) or blue (spot test) color.
- **Negative (-):** The reagent layer remains yellow or colorless.

Common Bacteria Tested

Indole Positive:

***Escherichia coli*,**

***Klebsiella oxytoca*, *Proteus vulgaris*,**

***Morganella morganii*, *Vibrio cholerae*.**

Indole Negative:

Klebsiella pneumoniae

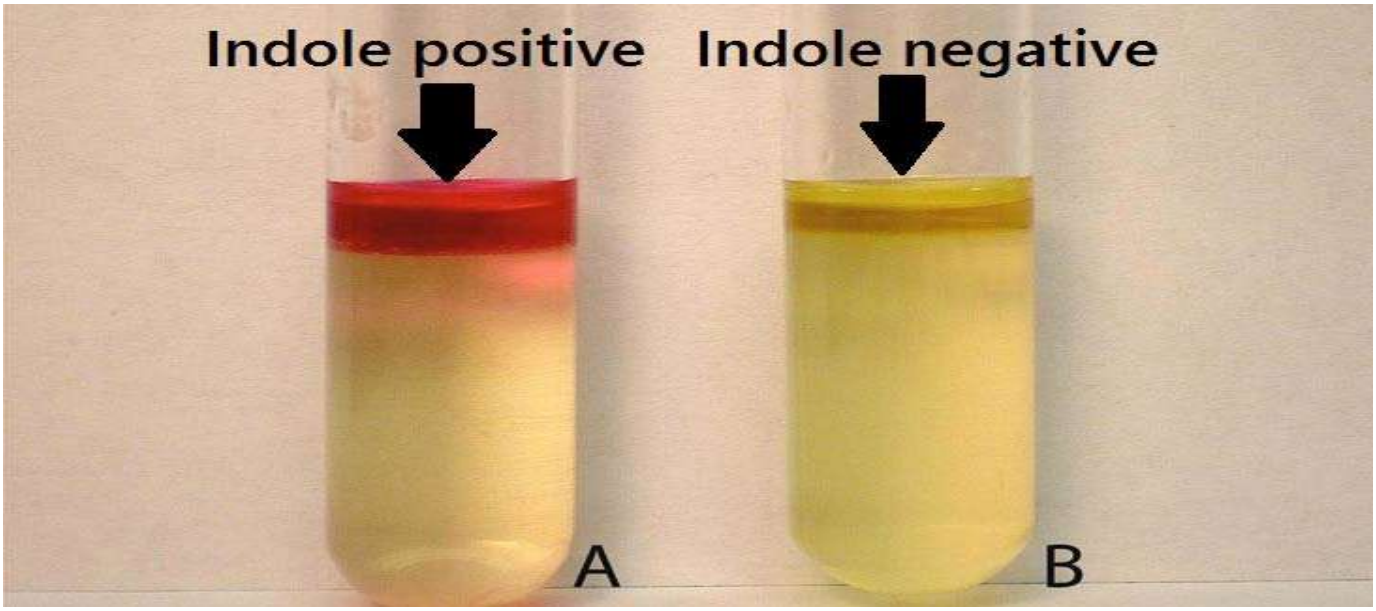
Proteus mirabilis

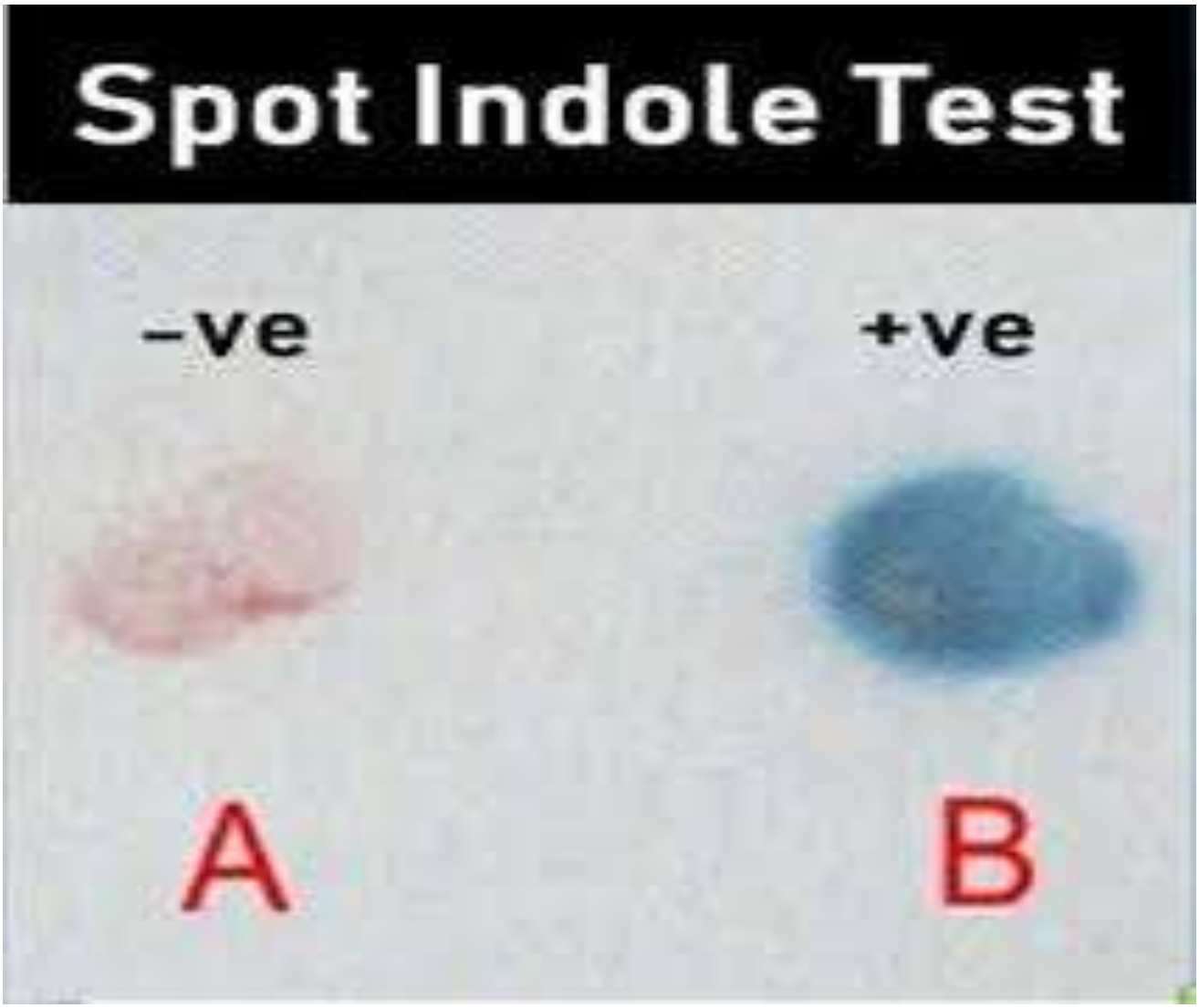
***Salmonella* spp**

***Pseudomonas aeruginosa*.**

Limitations

- **Media Requirements: The medium must be rich in tryptophan (e.g., tryptone or peptone broth).**
- **Medium Interference: Do not use indole tests on colonies from MacConkey agar as the medium color can interfere with results.**
- **Anaerobes: For anaerobes, Ehrlich's reagent is preferred over Kovac's reagent**




Spot Indole Test
-ve
+ve
A
B

Methyl Red test

The Methyl Red test (MR test) identifies bacteria that perform mixed acid fermentation of glucose, producing stable, acidic end-products, by using the pH indicator methyl red which turns red in acidic conditions (pH below 4.4) and yellow at higher pH. Used with the Voges-Proskauer test (VP) and Indole test (part of IMViC), it helps differentiate Enterobacteriaceae, like E. coli (positive) from Enterobacter (negative). A red color indicates a positive result, while yellow signifies a negative-result.

Principle

- **Bacteria ferment glucose, producing pyruvic acid.**
- **Positive (Mixed Acid Pathway):**

  Some bacteria further convert pyruvic acid into significant amounts of stable acids (lactic, acetic, formic, succinic), drastically lowering the pH below 4.4.

- **Negative (Butylene Glycol Pathway):**

  Other bacteria convert pyruvic acid to neutral products (like acetoin), resulting in less acidic conditions.

- **Methyl red indicator is added; red means positive (low pH), yellow means negative (higher pH).**

Procedure

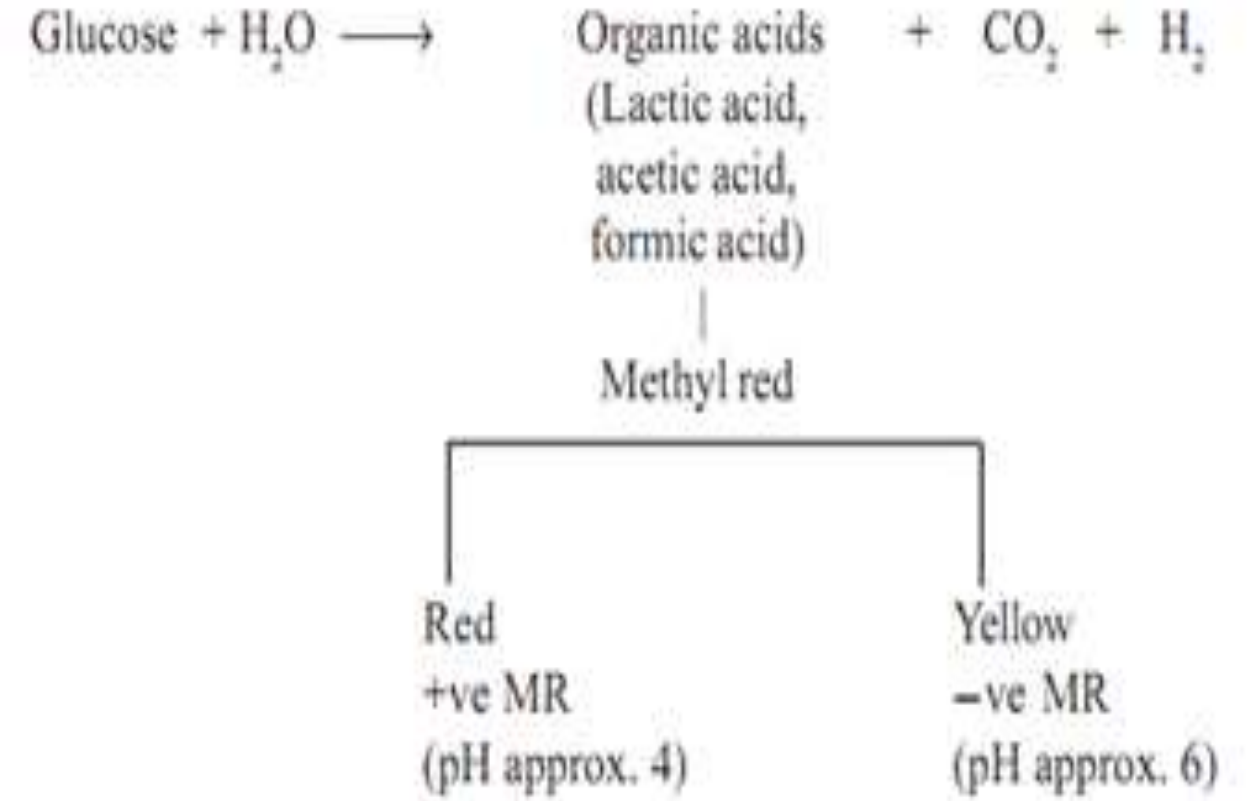
1. **Inoculate MR-VP broth (containing glucose, peptone, buffer) with the test organism.**
2. **Incubate aerobically at 37°C for 24-48 hours.**
3. **Add 2-3 drops of methyl red indicator to a portion of the broth.**
4. **Observe the color change immediately.**

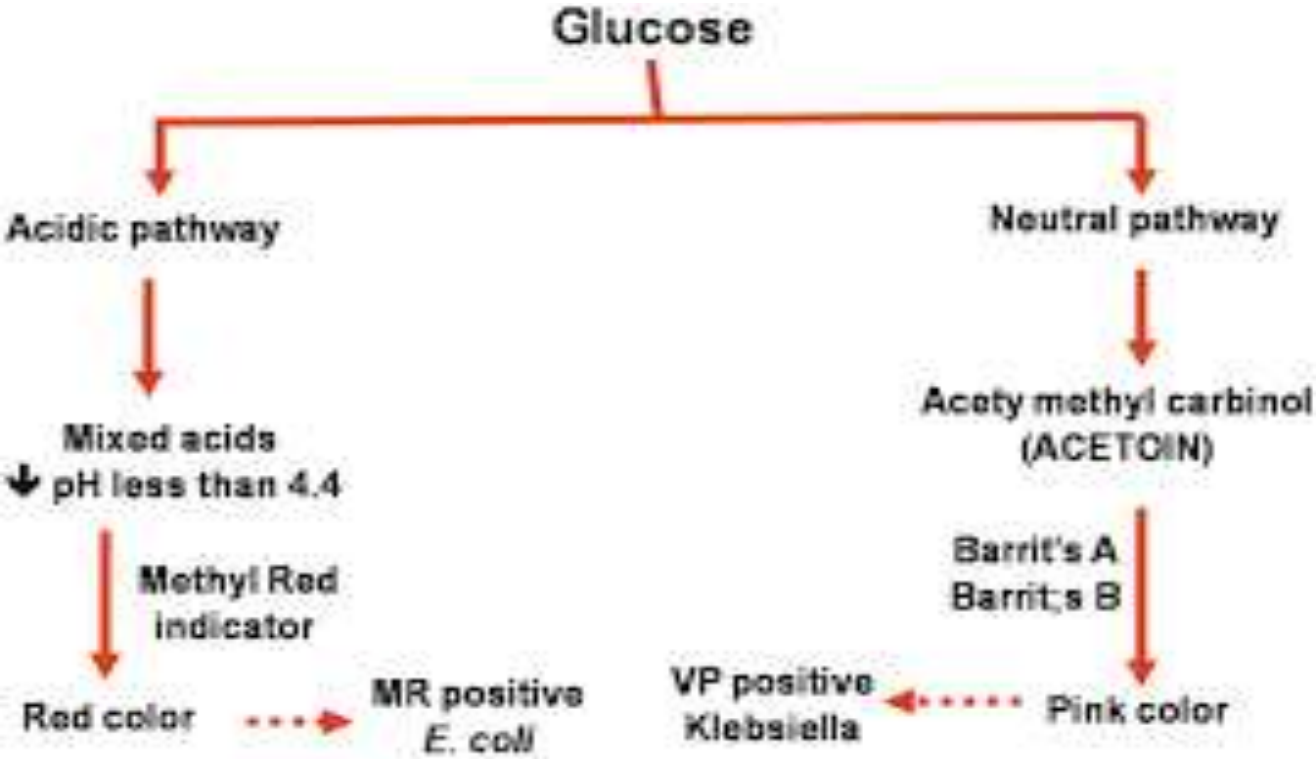
Results

- **Positive: A stable red color.**
- **Negative: A yellow color (or no change from the initial yellow/orange of the broth).**

Common Uses

- **Differentiating bacteria within the Enterobacteriaceae family, such as Escherichia coli (MR positive) from Enterobacter aerogenes (MR negative).**
- **Microbiology identification schemes (IMViC tests).**


Glucose + H_2O ⟶
Organic acids
(Lactic acid,
acetic acid,
formic acid)
+ CO_2 + H_2
Methyl red
Red
+ve MR
(pH approx. 4)
Yellow
–ve MR
(pH approx. 6)


Glucose
Acidic pathway
Neutral pathway
Mixed acids
pH less than 4.4
Methyl Red
indicator
Red color
MR positive
E. coli
Acety methyl carbinol
(ACETOIN)
Barrit's A
Barrit's B
VP positive
Klebsiella
Pink color

Voges Proskauer test

The Voges-Proskauer (VP) test is a biochemical test used to determine if a bacterium produces acetoin (acetyl methyl carbinol) as a neutral end product of glucose fermentation. It is a key test in the IMViC series.

primarily used to differentiate members of the family *Enterobacteriaceae*, such as *Escherichia coli*(VP= -)

from *Klebsiella* and *Enterobacter* species VP=+).

Principle

Bacteria that use the butanediol fermentation pathway metabolize pyruvic acid (a product of glucose metabolism) into acetoin, a neutral intermediate product. The VP test works by detecting this intermediate. When the VP reagents (alpha-naphthol and potassium hydroxide) are added, the acetoin in the medium is oxidized to diacetyl in the presence of atmospheric oxygen. Diacetyl then reacts with the guanidine compounds in the peptone broth to form a pinkish-red polymer, indicating a positive result. Alpha-naphthol serves as a color intensifier in this reaction.

Procedure

1. **Inoculation: A pure culture of the bacterium is aseptically inoculated into a tube containing MR-VP broth (Methyl Red-Voges-Proskauer broth), a buffered peptone-glucose medium.**
2. **Incubation: The inoculated tube is incubated at 35-37°C for 24 to 48 hours.**
3. **Reagent Addition: After incubation, the following reagents are added to an aliquot of the culture:**

1. **5% alpha-naphthol (VP Reagent A) is added first.**
2. **40% potassium hydroxide (KOH) (VP Reagent B) is then added.**
3. **The order of reagent addition is critical to prevent false results.**

4. **Observation: The tube is gently shaken to expose the medium to atmospheric oxygen and observed for color development for up to one hour.**

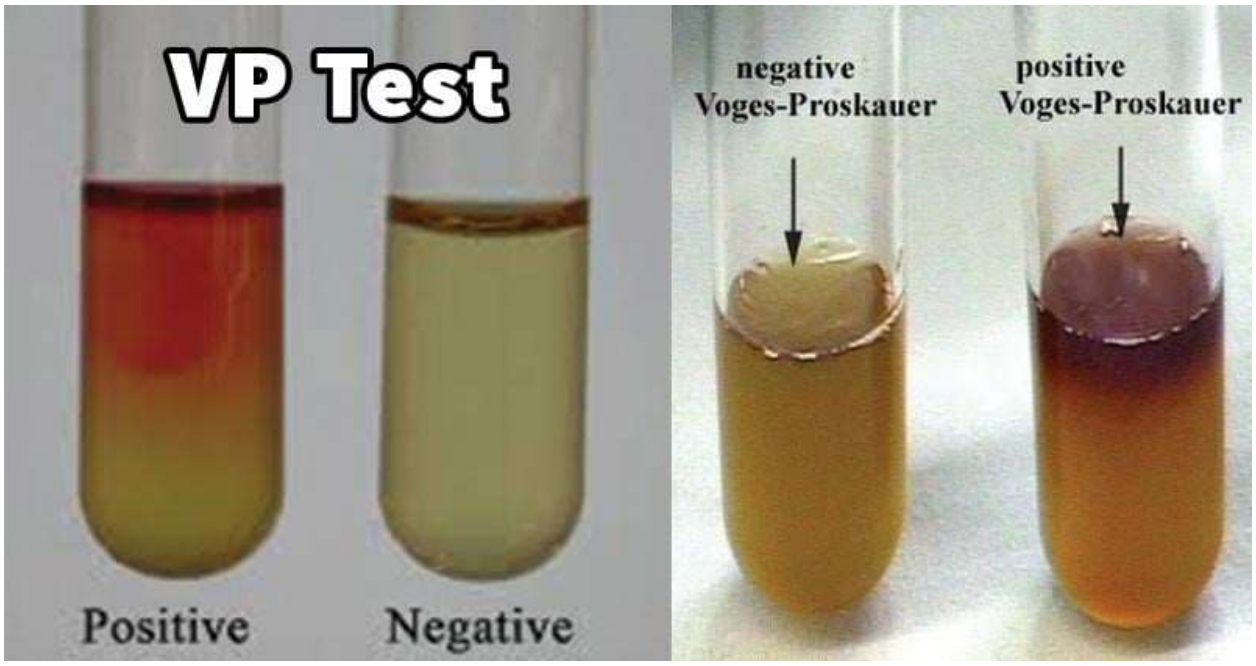
Results and Interpretation

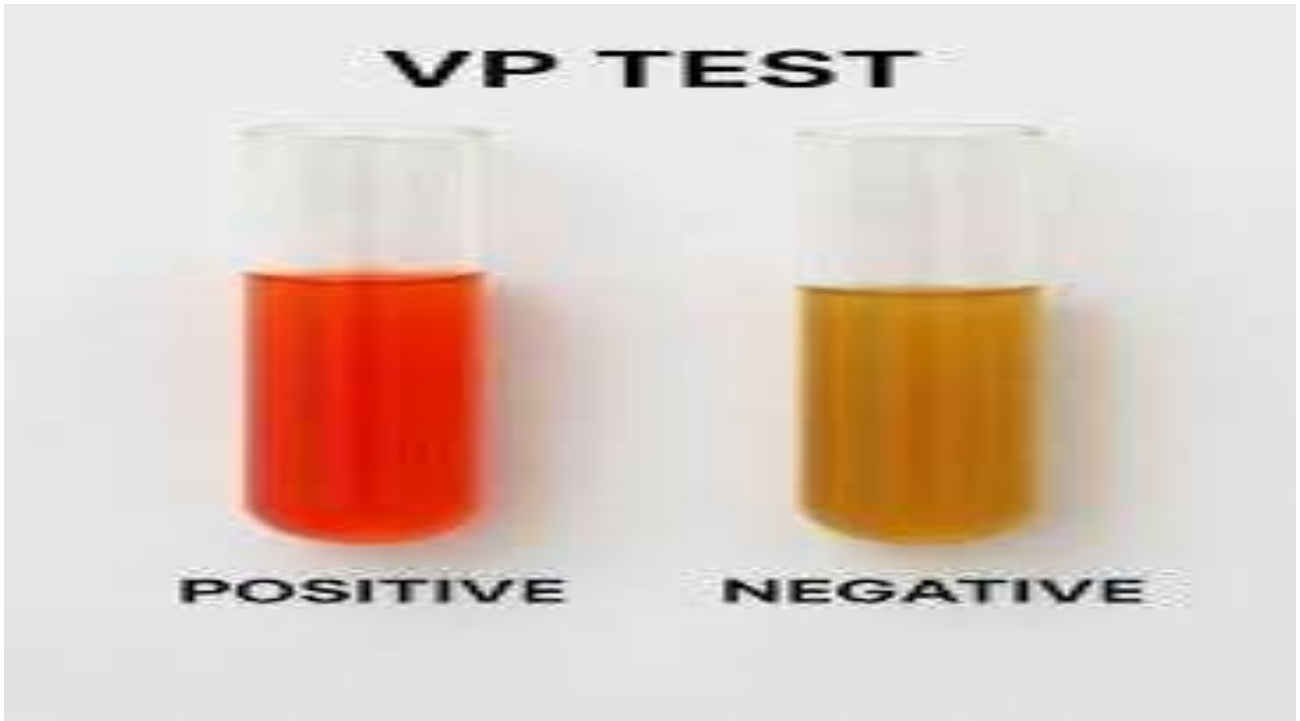
- **VP Positive:** A pink-red or cherry red color develops on the surface of the medium within 15 minutes to an hour, indicating the presence of acetoin.
- **VP Negative:** The medium remains yellow or yellow-brown (or may develop a copper color) after the addition of reagents, indicating the absence of acetoin.

VP Positive Organisms

Specific bacteria are known to be VP positive, including:

- *Klebsiella* species
- *Enterobacter* species
- *Hafnia* species
- *Serratia* species
- *Staphylococcus aureus*
- Most Viridans streptococci (except for some species like *S. mitis*)


VP Test
Positive
Negative
negative
Voges-Proskauer
positive
Voges-Proskauer


VP TEST
POSITIVE
NEGATIVE

Citrate Utilization Test

The citrate utilization test determines if an organism can use sodium citrate as its sole carbon source and ammonium salts as a nitrogen source, typically using Simmons Citrate Agar. A positive result, indicating the enzyme citrase is present, turns the green medium blue (pH > 7.6) due to alkaline byproducts.

common for identifying *Enterobacteriaceae*.

Purpose:

Differentiates gram-negative bacteria based on their ability to metabolize citrate.

Medium: Simmons Citrate Agar

(contains sodium citrate, ammonium salt, and bromothymol blue).

Procedure: A streak-stab technique is used to inoculate a slant, which is incubated at35-37°C for 24–48 hours.

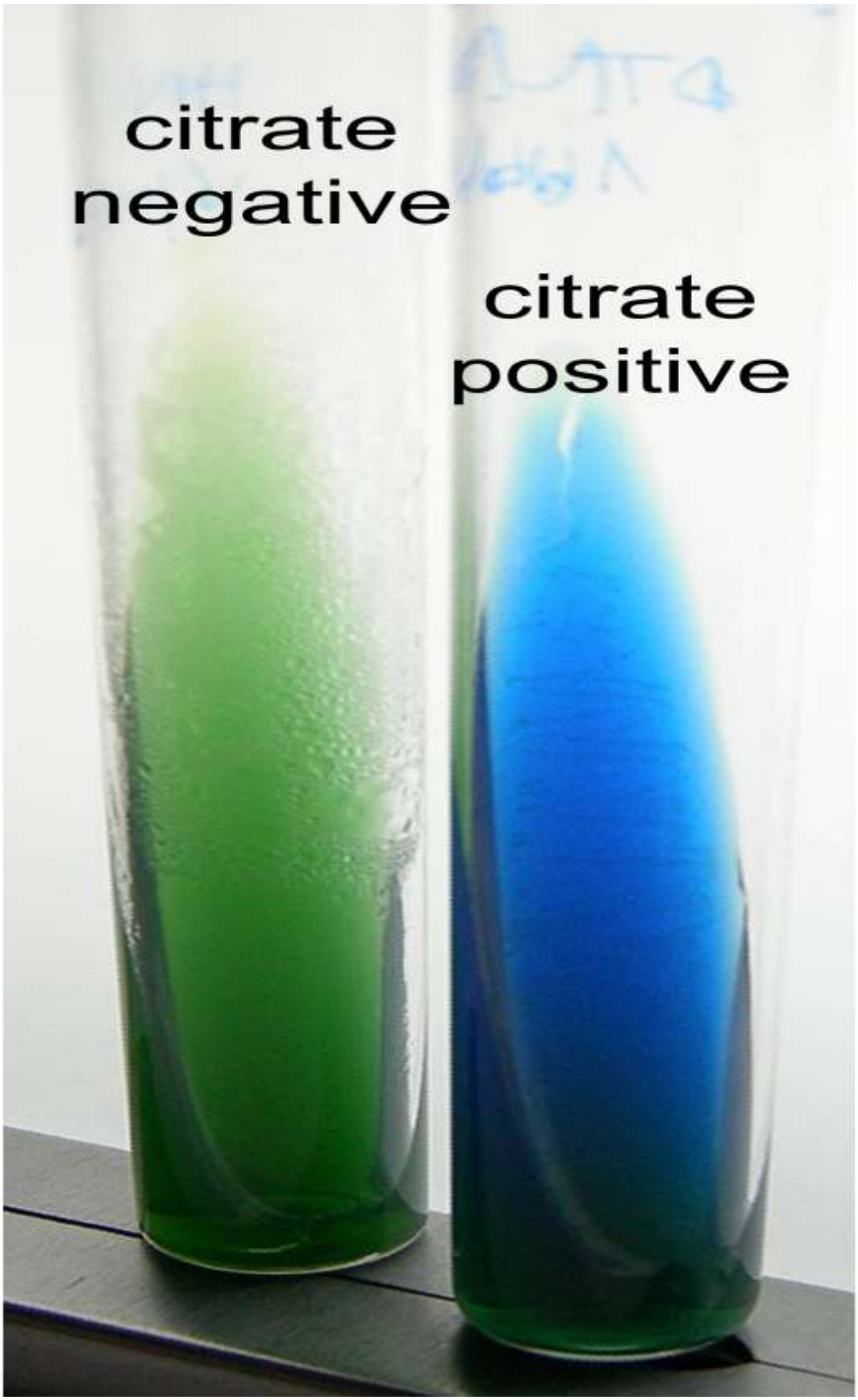
Interpretation:

- **Positive (+): Growth on the slant and a color change from green to deep blue.**
- **Negative (-): No growth, medium remains green.**

Examples:

***Klebsiella pneumoniae* and *Enterobacter aerogenes* are positive**

while *Escherichia coli* is negative.


citrate
negative
citrate
positive