Biochemical Tests of Bacteria

Part Two

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Lab training

- Oxidase Test
- Indole Test
- Methyl Red Test
- Voges-Proskauer (MR/VP) Test
- Citrate Utilization Test
- Urease test

Oxidase Test

Principle

Oxidase test can define as a biochemical test that differentiates organism into **oxidase-positive** and **oxidase-negative** microorganisms based on the existence of **cytochrome oxidase enzyme** in their electron transport chain system. The living-organisms which are having cytochrome oxidases can **oxidize** the **TMPD reagent** into a **blue coloured** complex refers as "**Indophenol**". Those organisms which lack such enzyme cannot oxidize the reagent and remains in a reduced form (appears **colourless**).

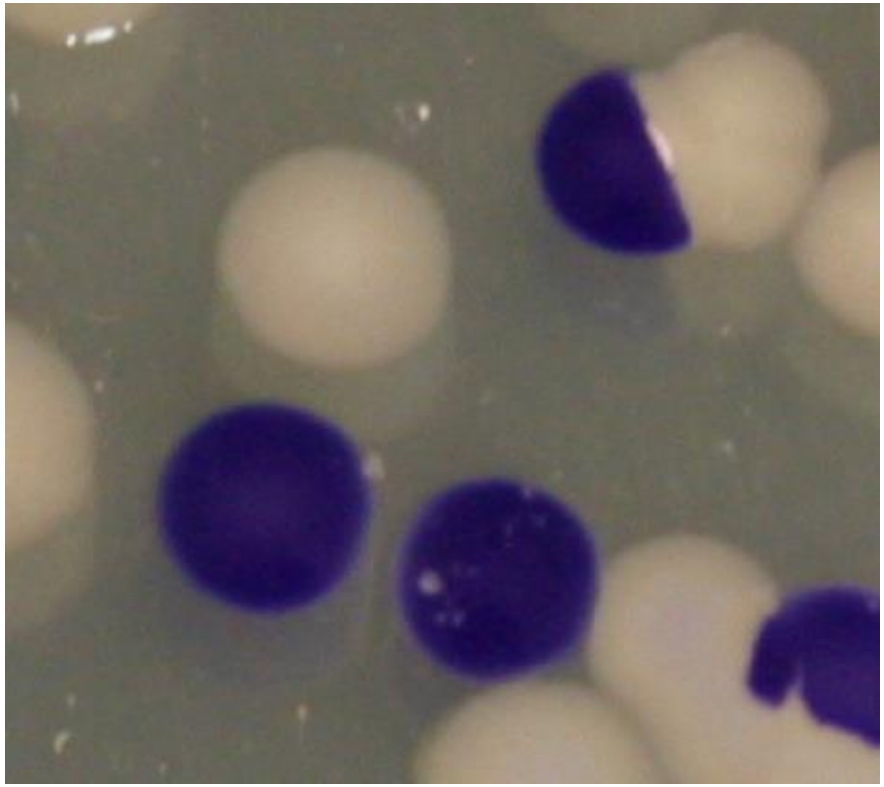


Figure . This is a mixed culture of oxidase-negative *Escherichia coli and* oxidase-positive *Vibrio cholerae* showing how the direct oxidase test differentiates between the two organisms

Procedure of Oxidase Test

There are many method variations to the oxidase test.

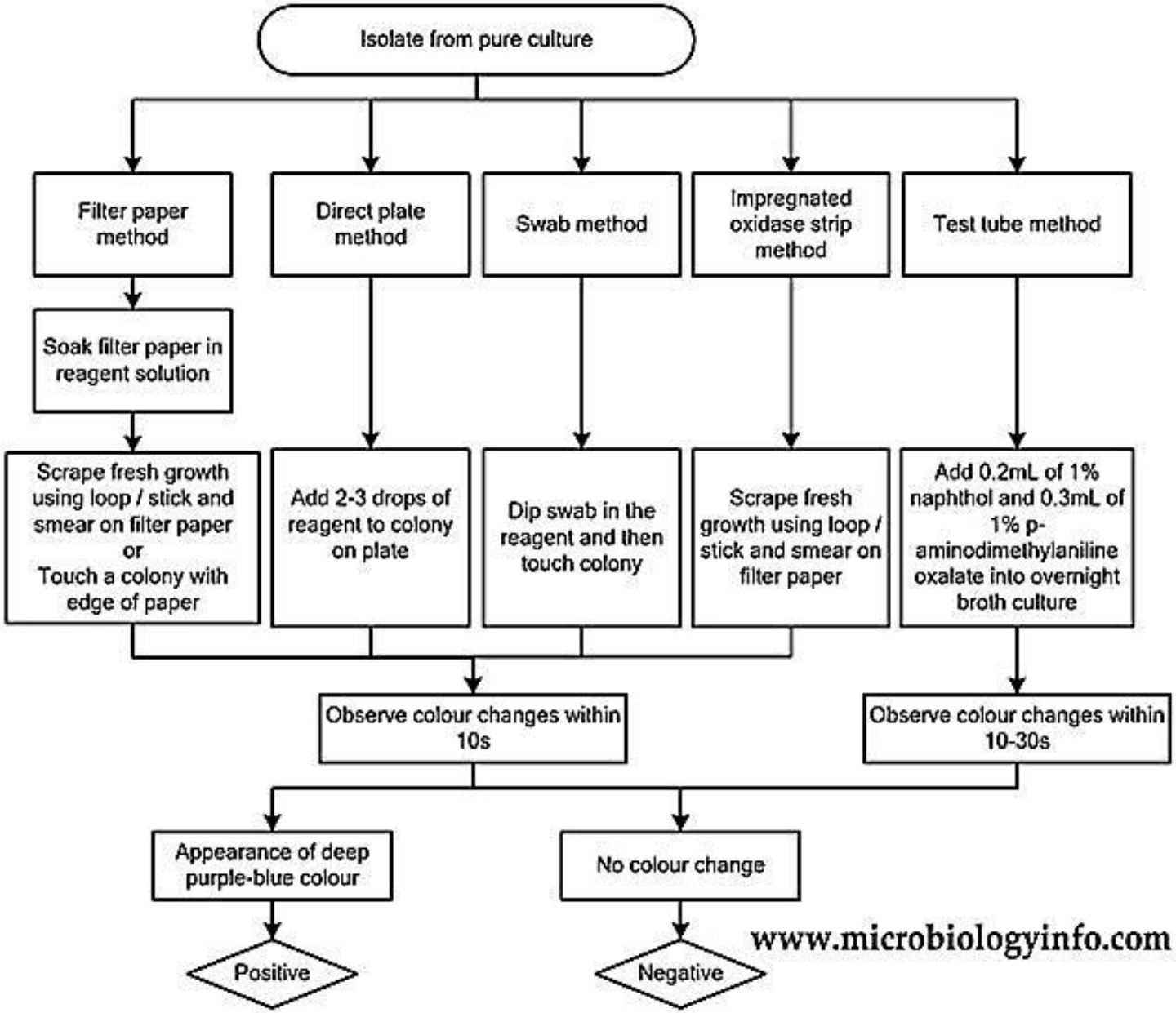
These include the filter paper test, direct plate method,

swab method, impregnated oxidase test strip method and

test tube method.

Tetramethyl-p-phenylenediamine dihydrochloride (TMPD oxidase reagent)

Flow chart of Procedures of Oxidase Test



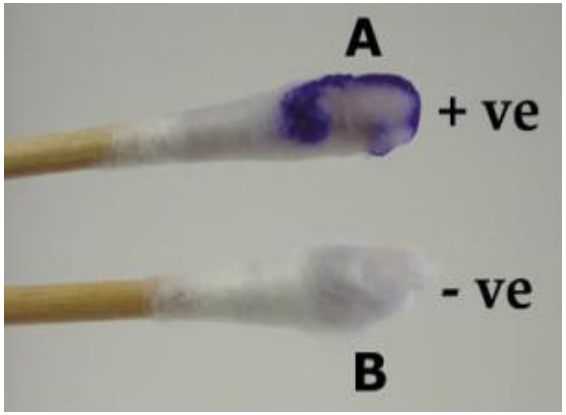
Expected results of Oxidase test

Positive: Development of dark purple color (**indophenols**) within 10 seconds

Negative: Absence of color

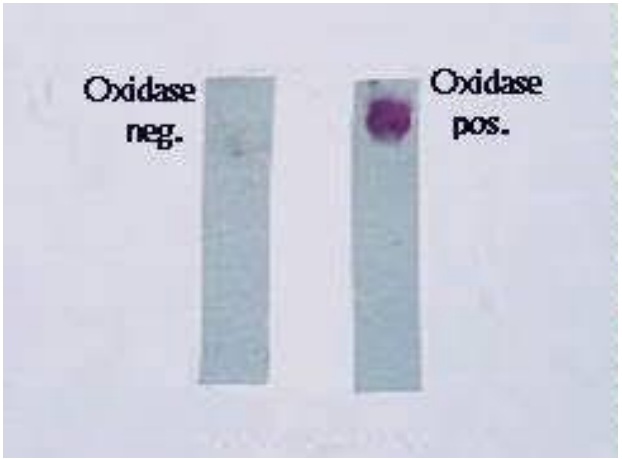
Filter Paper Method

Swab Method



Direct plate method

Impregnated oxidase test strip



Test tube method

Positive

- *Pseudomonas* spp.
- *Vibrio* spp.
- *Neisseria* spp.
- *Haemophilus* spp.

Negative

- Enterobacteriaceae
- *Acenitobacter* spp.

Indole Test

This test demonstrate the ability of certain bacteria to decompose the amino acid **tryptophane** to **indole**, which accumulates in the medium.

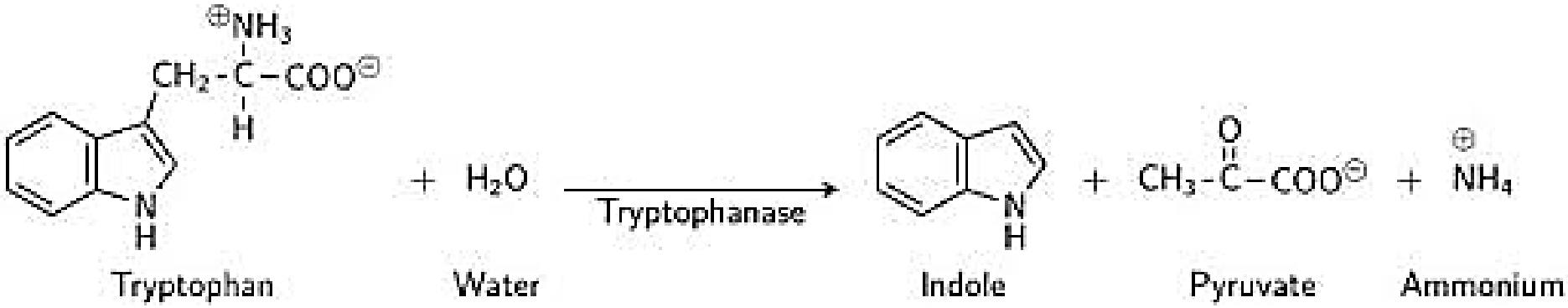
Indole production test is important in the identification of Enterobacteria.

E. coli and *P.* ***vulgaris*** break down the amino acid tryptophan with the release of indole. This is performed by **tryptophanase**.

It is used as part of the **IMViC** procedures,a tests designed to distinguish among members of the family Enterobacteriaceae

Principle of Indole Test

Tryptophan is an amino acid that can undergo deamination and hydrolysis by bacteria that express tryptophanase enzyme. **Tryptophanase** catalyzes the deamination reaction, during which the **amine (-NH2)** group of the **tryptophan** molecule is removed. Final products of the reaction are **indole, pyruvic acid, ammonium** (NH4+) and **energy**.



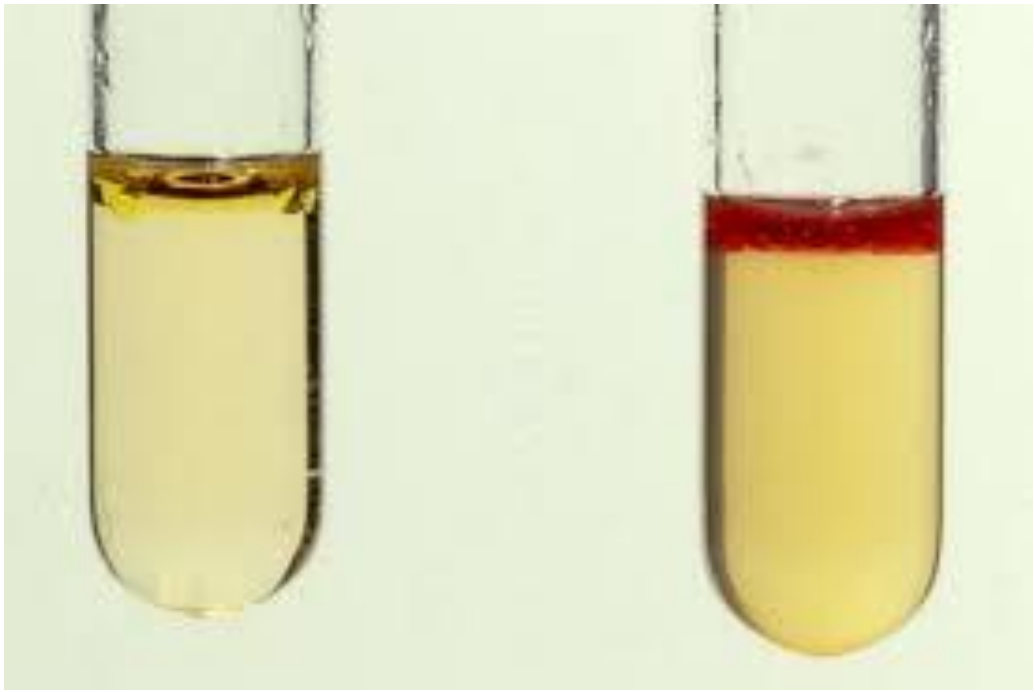
PROTOCOL

Inoculate the tube of **tryptone broth** with a small amount of a pure culture. Incubate at 37°C for 24 to 48 hours. To test for indole production, add 5 drops of **Kovács reagent** directly to the tube.

When **indole** is combined with **Kovac's Reagent** (which contains hydrochloric acid and p-dimethylaminobenzaldehyde in amyl alcohol) the solution turns from yellow to **cherry red**. Because amyl alcohol is not water soluble, the red coloration will form in an **oily layer at the top of the broth**.

Results

A positive indole test is indicated by the formation of a pink to red color ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent. If a culture is indole negative, the reagent layer will remain yellow.



Salmonella sp.
Klebsiella sp.
Neisseria sp
Proteus mirabilis

Escherichia coli,
Enterococcus faecalis,
Vibrio sp.
Proteus vulgaris

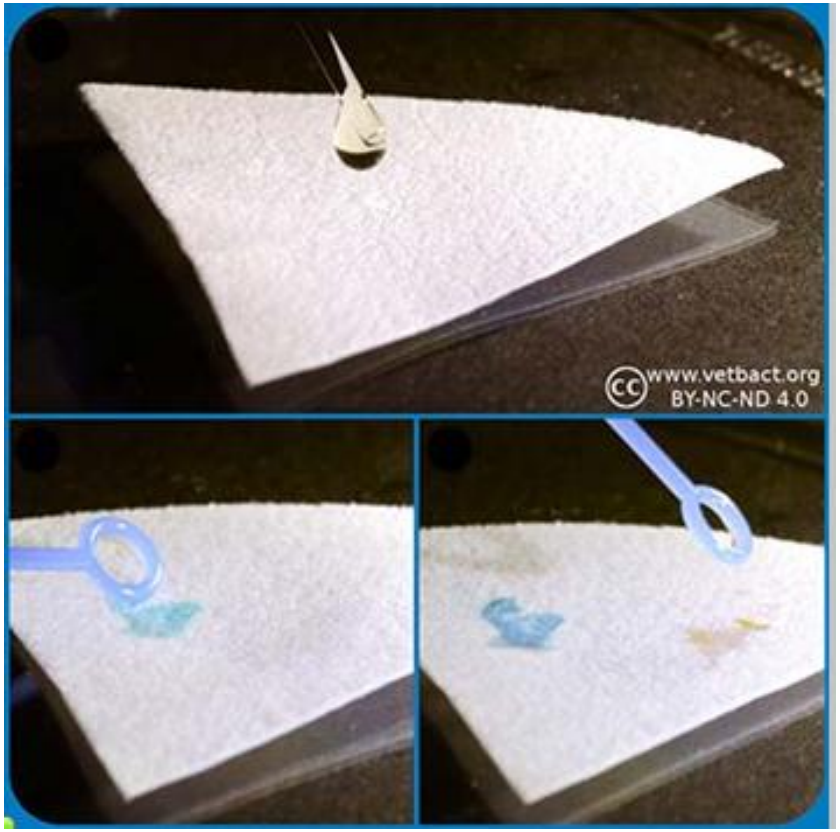
Spot indole test

Indole Spot Reagent (DMACA) Procedure

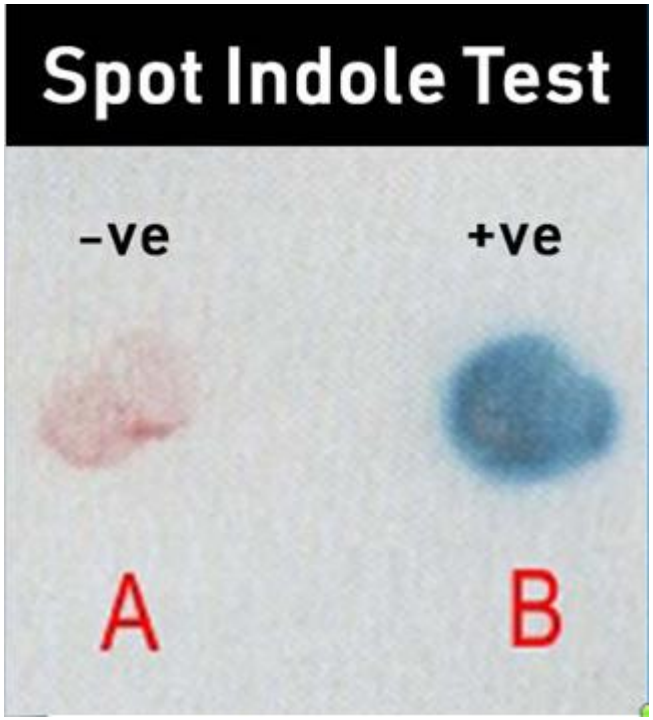
-Place several drops of Indole Spot Reagent (p Dimethylaminocinnamaldehyde (DMACA) ,Hydrochloric Acid, Deionized Water) on a piece of filter paper.

With an inoculating loop, pick a portion of an isolated colony and rub it onto the reagent saturated area of the filter paper.

Examine immediately



In the spot test, indole combines, in the filter paper matrix, at an acid pH with **p-Dimethylaminocinnamaldehyde (DMACA)** to produce a **blue to blue-green compound.**



Positive test: Development of a blue color within 1 to 3 minutes
Negative test: No color development or slightly pink color

Uses of Indole Spot Test

To differentiate *Proteus mirabilis* (indole negative) from *Proteus vulgaris* (indole positive).

To differentiate *Klebssiella pneumoniae* (indole negative) from *Klebsiella oxytoca* (indole positive).

Methyl Red (MR) Test

Principle of Methyl Red (MR) Test

The methyl red (MR) test detects the production of sufficient acid during the fermentation of glucose

Some bacteria have the ability to utilize glucose and convert it to a stable acid.

These bacteria initially metabolise **glucose to pyruvic acid**, which is further metabolized to produce the **stable acid**. The type of acid produced differs from species to species and depends on the specific enzymatic pathways present in the bacteria.

The acid so produced decreases the pH to 4.5 or below, which is indicated by a change in the color of **methyl red** from **yellow to red**.

In the methyl red test (MR test), the test bacteria is grown in a broth medium containing **glucose**. If the bacteria has the ability to utilize **glucose** with production of a **stable acid**, the color of the methyl red changes from **yellow to red**, when added into the broth culture.

Procedure of Methyl Red (MR) Test

Inoculate the medium (**MRVP broth (pH 6.9)** with bacteria

Incubate aerobically at 37 ^{o}C for 24 hours

Add 2 to 3 drops of **methyl red indicator** to aliquot.

Observe for red color immediately.

Result Interpretation of Methyl Red (MR) test

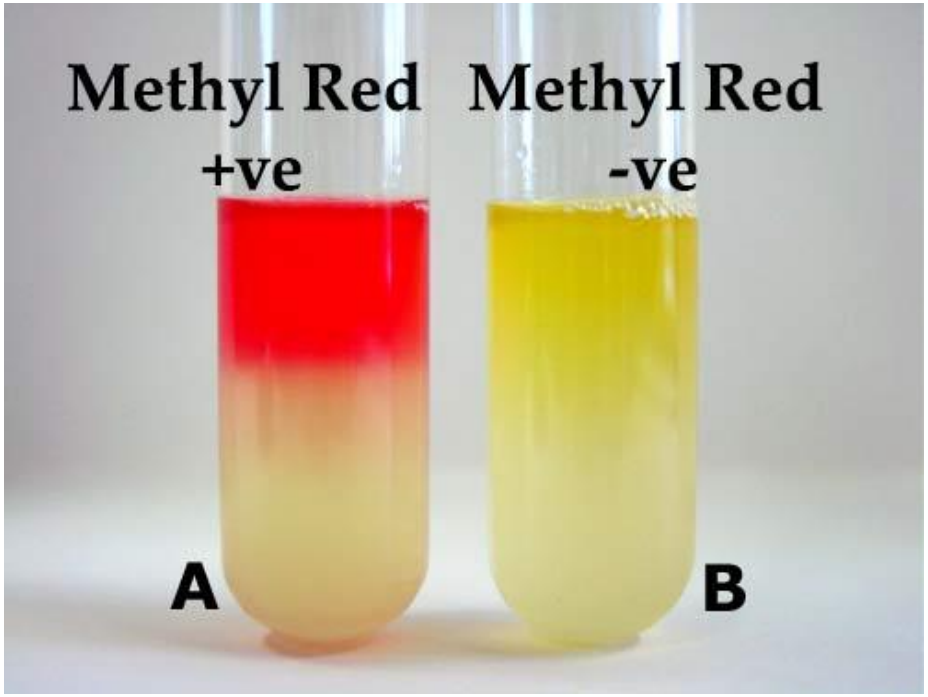
Positive Reaction: A distinct red color **(A)**

Examples: *E. coli, Yersinia* spp,

Negative Reaction: A yellow color **(B)**

Examples: *Enterobacter aerogenes,*

Klebsiella pneumoniae



Voges–Proskauer (VP) Test

Principle of Voges–Proskauer (VP) Test

The Voges-Proskauer (VP) test is used to determine if an organism produces **acetylmethyl carbinol** from glucose fermentation.

If present, **acetylmethyl carbinol** is converted to **diacetyl** in the presence of **∝- naphthol**, strong alkali (**40% KOH**), and atmospheric oxygen.

The **diacetyl** and **quanidine**-containing compounds found in the **peptones** of the broth then condense to form a **pinkish red polymer**..

Media and Reagents used in Voges–Proskauer (VP) Test

Media: MRVP broth (pH 6.9)

Voges-Proskauer Reagent A: Barritt's reagent A(Alpha-Naphthol, Absolute Ethanol).

Voges-Proskauer Reagent B: Barritt's reagent B(Potassium Hydroxide, Deionized Water

Procedure of Voges–Proskauer (VP) Test

Inoculate the **MRVP broth** medium with bacteria

Incubate aerobically at 37 degrees C. for 24 hours.

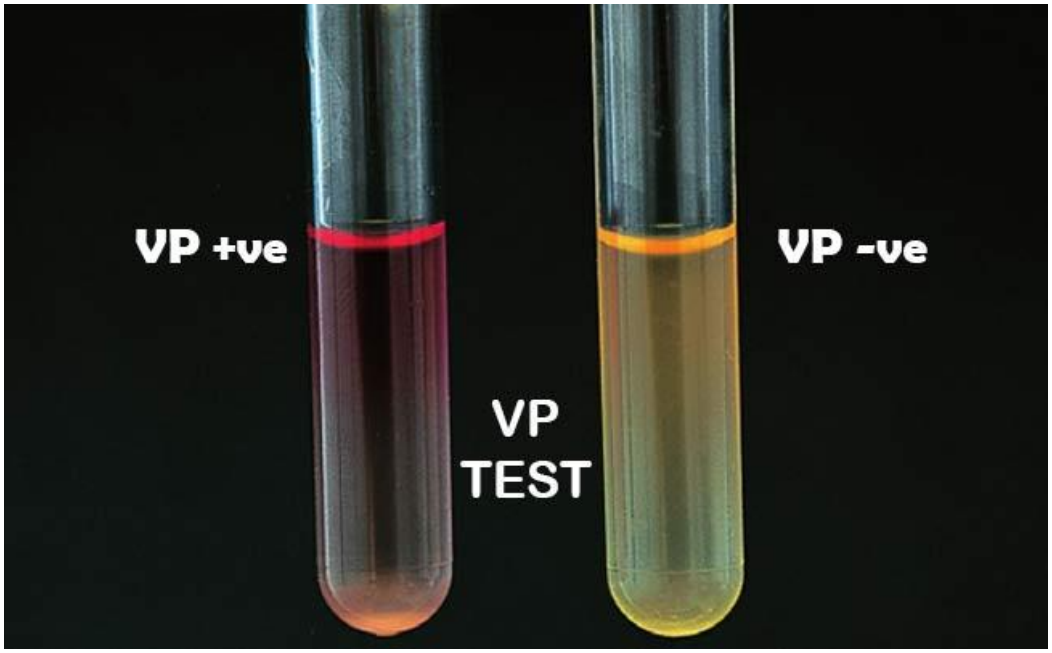
Add **6 drops of 5% alpha-naphthol,** and mix well .

Add **2 drops of 40% potassium hydroxide,** and mix well.

Observe for a **pink-red color** at the surface within **30 min**.

Positive Reaction: A pink-red color at the surface
Examples: *Listeria, Enterobacter, Klebsiella, Serratia marcescens*

Negative Reaction: A lack of a pink-red color
Examples: *Shigella, Salmonella,* and *Vibrio parahaemolyticus*



Citrate Utilization Test

Principle of Citrate Utilization Test

Citrate agar is used to test an organism's ability to utilize citrate as a source of energy. The medium contains **citrate** as the sole **carbon source** and **inorganic ammonium salts (NH4H2PO4)** as the sole source of **nitrogen**.

The **enzyme citrase** hydrolyzes **citrate** into **oxaoloacetic acid** and **acetic acid**. The **oxaloacetic acid** is then hydrolyzed into **pyruvic acid** and **CO_2**. If CO_2 is produced, it reacts with components of the medium to produce **an alkaline compound (e.g. Na_2CO_3).** The **alkaline** pH turns the pH indicator (**bromthymol blue**) from green to **blue**.

Procedure of Citrate Utilization Test

Streak the slant back and forth with a light inoculum picked from a well-isolated colony.

Incubate aerobically at 37℃ for up to 4-7 days.

Observe a color change from green to blue along the slant

Positive Reaction: Growth with color change from green to intense blue along the slant.

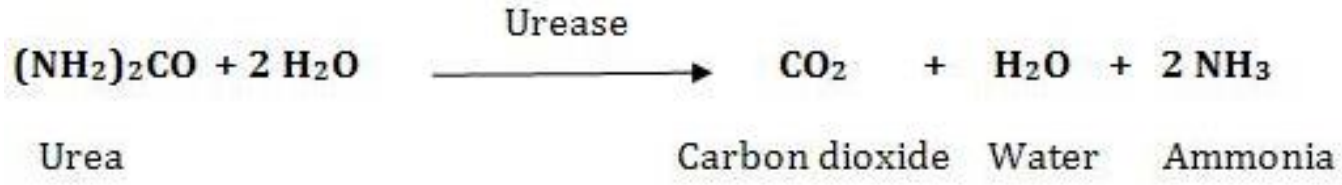
Klebsiella pneumoniae and *Proteus mirabilis* are examples of citrate positive organisms.

Negative Reaction: No growth and No color change; Slant remains green

Escherichia coli and *Shigella dysenteria*e are citrate negative.

Urease test

This test is used to identify bacteria capable of hydrolyzing **urea** using the enzyme **urease**. It is commonly used to distinguish the genus *Proteus* from other enteric bacteria. The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and the pH indicator, **phenol red**, turns from **yellow to pink**.



Procedure of urease Test

Streak the surface of a urea agar slant with a portion of a well-isolated colony.

Incubate the tube at 37°C for 48 hours to 7 days.

Examine for the development of a pink color

Positive Reaction: Development of a pink color

Example: ***Proteus mirabilis***

Negative Reaction: No color change.

Examples: *Escherichia, Shigella, Salmonella*

Thanks