Lab16- IMVic test

IMViC is a series of tests including the following tests:

Indole

Methyl Red (MR)

Voges- Proskauer (VP)

Citrate.

All of the series were designed to differentiate among ***Enterobacteriaceae*** though because many microbiology classes do not or cannot perform all of the bacterial tests, the IMViC tests are often used somewhat inappropriately to differentiate among the other families.

Indole test

Principle:

Indole production is the I portion of the test IMViC tests used in enteric bacteria identification, the amino acid tryptophan could be degraded via the enzyme tryptophanase to yield ammonia (NH3), indole, and pyruvic acid, so this enzyme will differentiate between ***E.coli*** from ***Enterobacter aerugenes*** *and* ***Klebsiella pneumoniae*** whose don t have this enzyme.

Method:

1- Inoculate one tube of peptone wate with bacterial isolate under test.
2- Incubate for 48 h. at 37°C. Sometimes a period of 96 h. at 37°C may be required foroptimum accumulation of indole.
3- Add 0.5 ml Kovac's reagent and shake gently.

A red color in the alcohol layer indicates a positive reaction. Yellow color layer of Kovac'sreagent indicates negative test

Shows indole [tryptophane production] positive test result with red ring regarding a growth of *E.coli* (right), and negative result regarding *Klebsiella pneumoniae*. (left).

Methyl Red (MR) test

Purpose: to see if bacteria can perform mixed acid fermentation of glucose.

The methyl red test is the "M" portion of the IMViC tests used to characterize enteric bacteria. The methyl red test is used to identify enteric bacteria based on the pattern of **glucose metabolism**. All enterics initially produce **pyruvic acid** from glucose metabolism.

Some enterics subsequently use the mixed acid pathway to metabolize pyruvic acid toother acids, such as lactic, acetic, and formic acids. These bacteria are called methyl red **positive** and include ***Escherichia coli Salmonella, Proteus, Klebsiella ozoenae.***

Other enterics subsequently use the butylene glycol pathway to metabolize pyruvic acid to **neutral end products**. These bacteria are methyl red **negative** and include ***Enterobacter aerogenes*** and ***Serratia marcescens***.

Voges-Proskauer (VP)

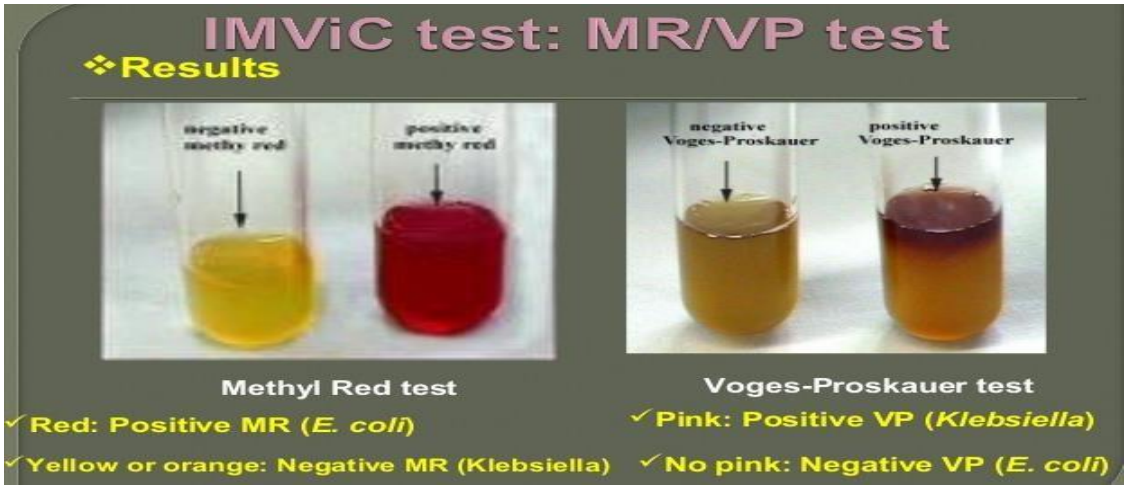
Purpose: the VP test identifies bacteria that produce **acetoin** (acetylmethylcarbinaol) from fermenting glucose. Glucose is fermented first to pyruvic acid. Some organisms in the presence of oxygen will convert pyruvic acid to acetoin and then to 2,3-butanediol.

The addition of the VP reagents to acetoin produces a **red color** (which is scored as a positive for the test). However, a **copper color** may result a side reaction and is negative for VP. Carefully distinguished copper from a red as the two are similar. No color change or a copper color are negative for the VP test.

REAGENT FORMULA

Voges-Proskauer Reagent A:	
Alpha-Naphthol, 5%	50.0gm
Absolute Ethanol	1000.0ml

Voges-Proskauer Reagent B:	
Potassium Hydroxide	400.0gm
Deionized Water	1000.0ml



Methyl Red (MR) test positive test result of *E.coli* and *Klebsiella pneumoniae*.

Citrate Utilization

Citrate utilization is the C portion of the IMViC test, which is used in enteric bacteria haracterization; citrate is an organic molecule that utilized by bacteria capable to produce the enzyme citrase. ***Klebsiella pneumoniae*** produce this enzyme but ***Escherichia coli*** not produce it.

Principle:

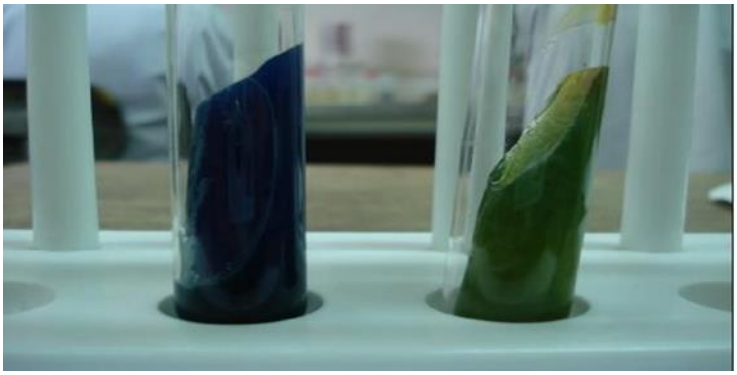
This test is used to determine the **ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source**. Bacteria that can grow on this medium **turn the bromthymol blue indicator from green to blue**.

Method:

Inoculate Simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is **18 to 24 hours old.**

Note: there is **no need to stab into the butt of the tube**. Do not inoculate from a broth culture, because the inoculums will be too heavy.

Incubate at 35°C to 37°C for up to **24-48 hours (longer incubation "up to 7 days" may be required).**



Shows citrate utilization **positive** test result with blue color regarding *Klebsiella* spp. (left) **negative result** with green color regarding *E.coli* (right).