IMViC Reactions

I = *Indole* production from tryptophan

M = *methyl red* test in which acidification of glucose broth (pH<4.4) due to formation of mixed carboxylic acids (lactic, acetic, formic) from pyruvate results in pH indicator methyl red turning red

Vi = positive *Voges-Proskauer* test due to formation of acetoin from pyruvate in glucose broth

C = ability to utilize *citrate* as single carbon source

Indole Test

Enterobacteriaceae that possess tryptophanase can utilize tryptophan by deamination and hydrolytic removal of the indole side chain.

Free indole is detected by *p*-dimethylamino- benzaldehyde, whose aldehyde group reacts with indole forming a red-colored complex.

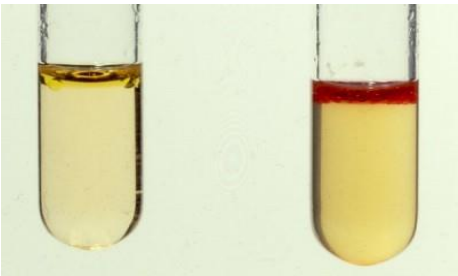
Production of indole from tryptophan is an important biochemical property of *Escherichia coli*, many strains of group A, B, and C *Shigella*, *Edwardsiella tarda*, *Klebsiella oxytoca*, and *Proteus vulgaris*.

How to Perform Test: Inoculate Tryptone broth with inoculating loop.

Property it tests for: This test is performed to help differentiate species of the family *Enterobacteriaceae.* It tests for the bacteria species' ability to produce indole. Bacteria use an enzyme, tryptophanase to break down the amino acid, tryptophan, which makes by-products, of which, indole is one.

Media and Reagents Used: Tryptone broth contains tryptophan. Kovac's reagent—contains hydrochloric acid, dimethylaminobenzaldehyde, and amyl alcohol—yellow in color.

Reading Results: Kovac's reagent reacts with indole and creates a red color at the top part of the test tube.



Methyl Red/Voges Proskauer (MR/VP)

How to Perform Tests: Inoculate 2 glucose broths with inoculating loop. After 48 hours of incubation, add a few drops of MR to one tube, and VP reagents to the other tube.

Properties they test for: Both tests are used to help differentiate species of the family *Enterobacteriaceae.*

MR—tests for acid end products from glucose fermentation.

VP—tests for acetoin production from glucose fermentation.

Media and Reagents Used:

Glucose Broth

Methyl Red indicator for acid

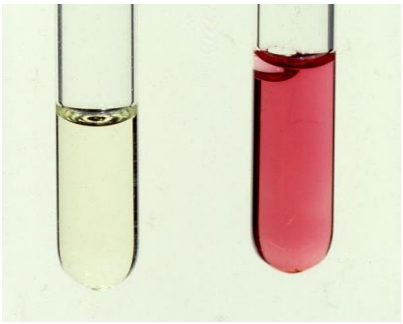
Voges Proskauer reagente—A: 5% Alpha-Naphthol, B: Potassium Hydroxide.

The production of acetoin and butylene glycol by glucose fermentation is an important biochemical property used for the identification of *Klebsiella*, *Enterobacter*, and *Serratia*.

Reading Results:

MR— a + result is red (indicating pH below 6) and a – result is yellow (indicating no acid production)

VP— a + result is red after VP reagents are added (indicating the presence of acetoin) and a – result is no color change.



Citrate Test

Citrate is utilized by several of the *Enterobacteriaceae* as a single carbon source. To test this ability bacteria are incubated in medium that contains only citrate as a source of carbon.

Ammonium phosphate is available as a nitrogen source.

How to Perform Test: Inoculate slant with inoculating loop.

Property it tests for: This test is used to help differentiate species of the family *Enterobacteriaceae.* It is selective for bacteria that has the ability to consume citrate as its sole source of carbon and ammonium as sole nitrogen source.

Media and Reagents Used: Simmon's Citrate Agar contains sodium citrate (carbon source),

Reading Results:

a + result is blue (meaning the bacteria metabolised citrate and produced an acid end product).

a – result remains green.