AL-Mustaqbal University Collage.
Department of Pathological Analysis Technique.
Subject:- Advanced laboratory techniques.
Lecture-No. 9.
IMViC REACTIONS
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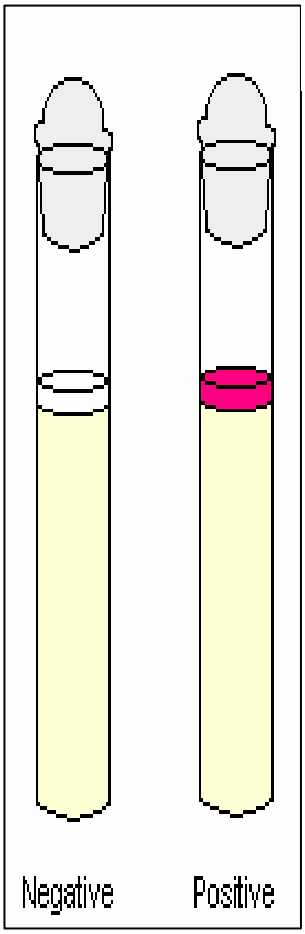
IMViC REACTIONS

IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae. The four reactions are: Indole test, **Methyl Red test**, **Voges Proskauer test** and **Citrate utilization** test. The letter "i" is only for rhyming purpose.

INDOLE TEST:

Principle: Some bacteria can produce indole from amino acid tryptophan using the enzyme typtophanase.



$$\text{Tryptophan} \xrightarrow[H_2O]{\text{tryptophanase}} \text{Indole + pyruvic acid + } NH_3$$

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: Bacterium to be tested is inoculated in peptone water, which contains amino acid tryptophan and incubated overnight at 37^{O}C. Following incubation few drops of Kovac's reagent are added. Kovac's reagent consists of para-dimethyl aminobenzaldehyde, isoamyl alcohol and con. HCl. Ehrlich's reagent is more sensitive in detecting indole production in anaerobes and non-fermenters. Formation of a red or pink colored ring at the top is taken as positive.

Example: *Escherichia coli*: Positive;

Klebsiella pneumoniae: Negative

METHYL RED (MR) TEST:

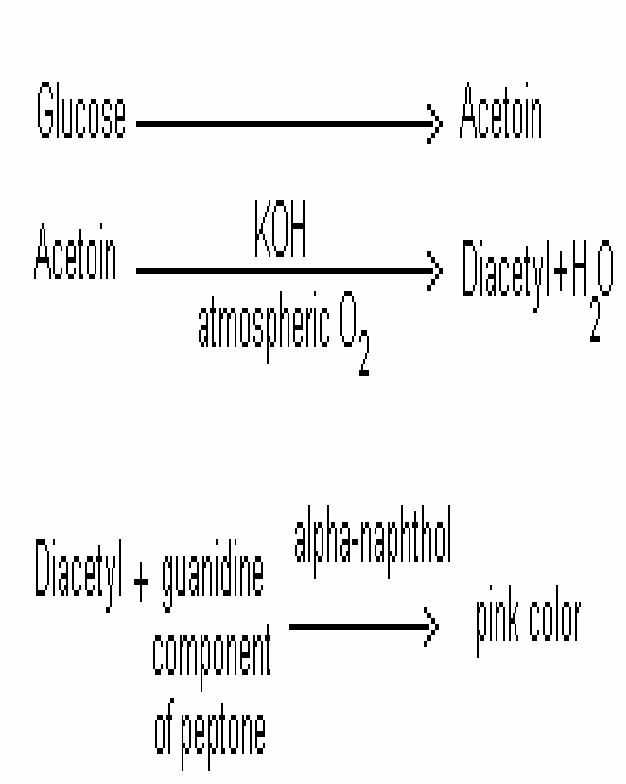
Principle: This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation that they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in color at a pH of 4.4 or less.

Procedure: the bacterium to be tested in inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at $37^{o}C$ for 48 hours. Over the 48 hours the mixed-acid producing organism must produce sufficient acid to overcome the phosphate buffer and remain acid. The pH of the medium is tested by the addition of 5 drops of MR reagent. Development of red color is taken as positive. MR negative organism produce yellow color.

Example: *Eschericihia coli*: Positive; *Klebsiella pneumoniae*: Negative

VOGES PROSKAUER (VP) TEST:

Principle: While MR test is useful in detecting mixed acid producers, VP test detects butylene glycol producers.



Acetyl-methyl carbinol (acetoin) is an intermediate in the production of butylene glycol. In this test two reagents, 40% KOH and alpha-naphthol are added to test broth after incubation and exposed to atmospheric oxygen. If acetoin is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alpha- naphthol to produce red color. Role of alpha-naphthol is that of a catalyst and a color intensifier.

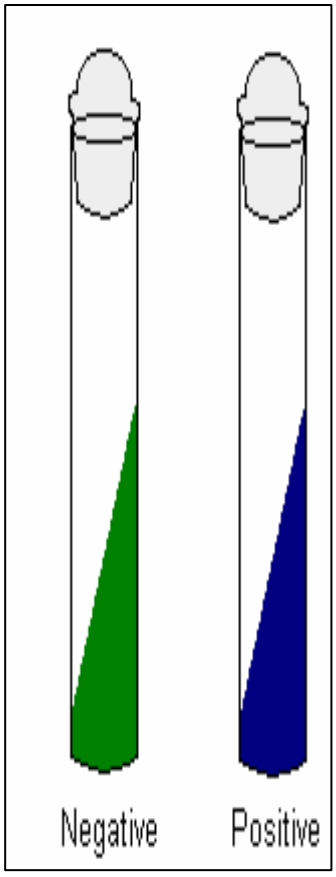
Procedure: Bacterium to be tested is inoculated into glucose phosphate broth and incubated for at least 48 hours.

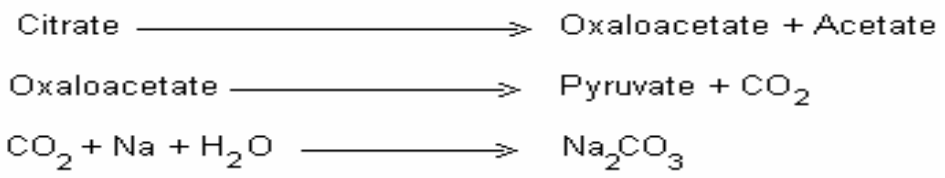
0.6 ml of alpha-naphthol is added to the test broth and shaken. 0.2 ml of

40% KOH is added to the broth and shaken. The tube is allowed to stand for 15 minutes. Appearance of red color is taken as a positive test. The negative tubes must be held for one hour, since maximum color development occurs within one hour after addition of reagents.
Examples: *Escherichia coli*: Negative; *Klebsiella pneumoniae*: Positive.

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

Bacterium	Indole	MR	VP	Citrate
E.coli	**+ (98%)**	**+ (99%)**	**- (1%)**	**- (1%)**
K.pneumoniae	**- (0%)**	**- (10%)**	**+ (98%)**	**+ (98%)**
Enterobacter aerogenes	**- (0%)**	**- (5%)**	**+ (98%)**	**+ (95%)**
Proteus mirabilis	**- (2%)**	**+ (97%)**	**± (50%)**	**± (65%)**