

MEDICAL MICROBIOLOGY

LAB 6

Assays for specific metabolic activities



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Voges Proskauer Test

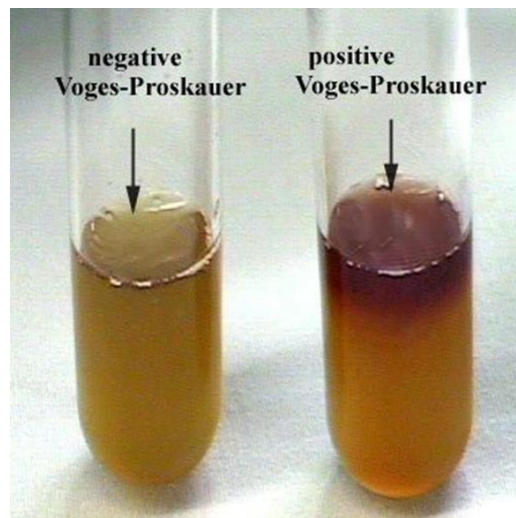
The **Voges –Proskauer Test (VP)** is a component of the **IMViC** battery of tests (Indole , Methyl Red , Voges –Proskauer, and Citrate) used to distinguish between members of the family Enterobacteriaceae and differentiate them from other Gram –negative rods . **MR-VP broth** is a medium used for Methyl Red (MR) and Voges – Proskauer test . It is containing peptone , glucose , and phosphate buffer

. Organisms ferment glucose , but convert their acid products to **acetoin**. Inoculate pure culture of the test organism into MR/VP broth and incubate for 24 hours at 37 ° C . Reagents A and B are added to MR/ VP broth after incubation .

A.5% Alpha Naphthol

B.Potassium Hydroxide

Results : red color (indicating the presence of acetoin) = positive
No color change = negative



Methyl Red Test

It identifies bacteria ability to produce stable acid end products by means of a mixed –acid fermentation of glucose .

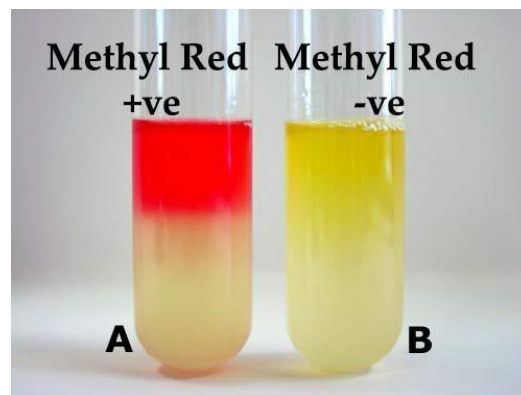
MR-VP broth used for Methyl Red test .

Inoculate pure culture of the test organism into MR/VP broth and incubate for 48 hours at 37 ° C.

Indicator : Methyl Red

Results : Red color = positive

Yellow color = negative



Indole Test

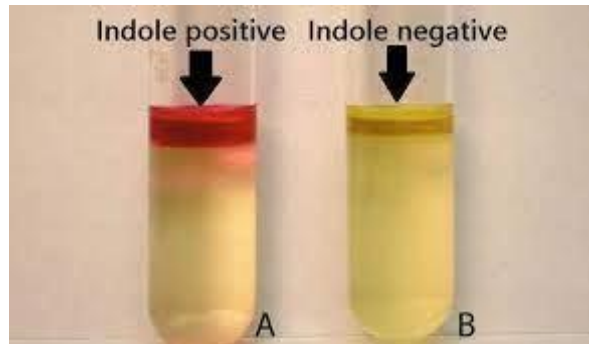
The Indole test identifies bacteria capable of producing indole using the enzyme tryptophanase . Bacteria possessing the enzyme tryptophanase can hydrolyze tryptophan to indole and other compounds .

Inoculate tryptone broth with the organisms and incubate for 24 hours at 37°C .

Reagent : Kovac's (add 15 drops)

Results : red color at the top part of the test tube positive

No color : negative

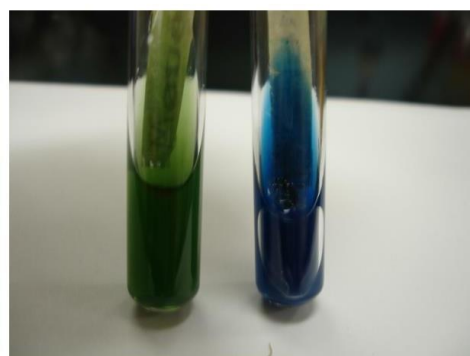


Citrate test

Some bacteria are capable of using citrate as a sole carbon source . Citrate test is one part of a test series referred to as the IMViC . Simmons Citrate Agar is a defined medium that contains sodium citrate as the sole carbon source and ammonium phosphate as the sole nitrogen source .

Results : blue color (indicating the bacteria consume citrate)positive
Green color = negative

Citrate test



Negative

positive

KM

Fermentation Tests

Carbohydrate fermentation is a metabolic process, any carbohydrate can be used, but glucose, lactose, sucrose are common choices. Fermentation of glucose begins with the production of pyruvate. The end products of pyruvate fermentation include a variety of acids, alcohols, and H₂ or CO₂ gas.

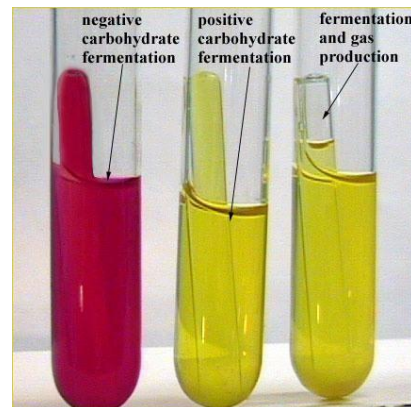
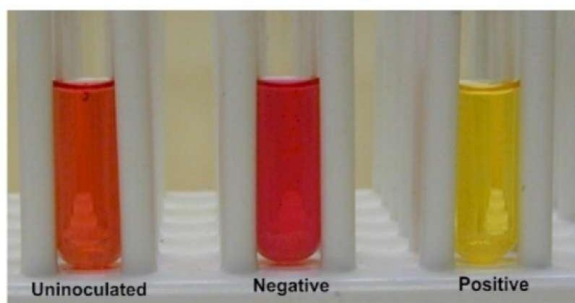
Indicator: phenol red

An inverted Durham tube is placed in each tube as an indicator of gas production.

Results: yellow color (indicating carbohydrate fermentation)
positive, Red color = negative

Carbohydrate Fermentation Tests

Sucrose Fermentation

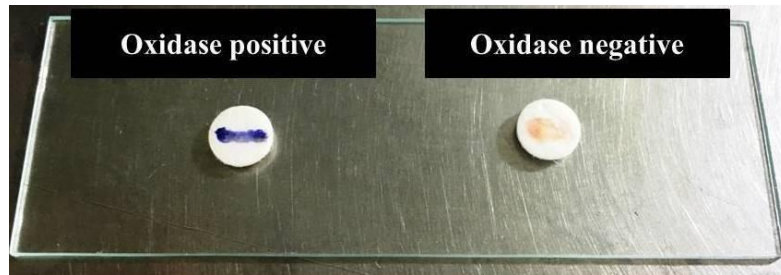


Oxidase test

The oxidase test assays for the presence of cytochrome oxidase, an enzyme in the electron transport chain. The oxidase test will differentiate most species of oxidase-positive *Pseudomonas* from the *Enterobacteriaceae*, which are oxidase-negative.

A positive culture will cause the reagent to turn from yellow to purple (in the oxidized stage) in 10 to 30 seconds.

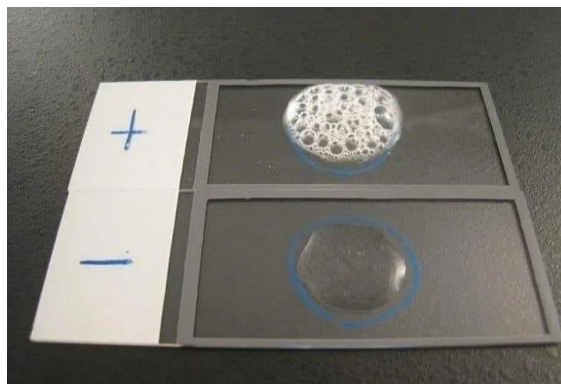
A change after 30 seconds is considered a negative reaction (in the reduced stage) .



Catalase test

When aerobic bacteria grow by respiration , they use oxygen as a terminal electron acceptor , converting it to water . However , they also produce hydrogen peroxide as a by-product of this reaction . Hydrogen peroxide is a highly reactive oxidizing agent that can damage enzymes , nucleic acids , and other essential molecules in the bacterial cell . To avoid this damage , aerobes produce the enzyme catalase , which degrades hydrogen peroxide into harmless oxygen and water .

To determine if catalase is produced , a small amount of growth is transferred from a plate or slant to a clean microscope slide . A couple of drops of 3% hydrogen peroxide are added to the cells on the slide . If catalase is produced , there will be vigorous bubbling due to the breakdown of hydrogen peroxide and the production of oxygen gas.



Triple Sugar Iron Agar (TSIA)

TSIA is a rich medium designed to differentiate bacteria on the basis of glucose fermentation , lactose fermentation , sucrose fermentation , and sulfur reduction . phenol red is the pH indicator . The medium is prepared as a shallow agar slant with a deep butt . It is inoculated by a stab in the agar butt followed by a streak of the slant . The incubation period is 18-24 hours .

Results :

1- Yellow slant / yellow butt

Glucose and lactose and / or sucrose fermentation with acid accumulation in slant and butt .

2- Red slant / yellow butt

Glucose fermentation with acid production .

3- Red slant / red butt

No fermentation

4- No change in slant / no change in butt

Organism is growing slowly or not at all .

5- Black precipitate in the agar

Sulfur reduction (H_2S formation)

