Biochemical Tests

Indole test

Objective:

to detect the ability of organism to produce enzyme tryptophanase.

Principle:

Indole test is a biochemical test which differentiates the coliform from other members of Enterobacteriacee by detecting their ability to produce the enzyme tryptophanase. This enzyme hydrolyses the amino acid tryptophan into indole, pyruvic acid and ammonia. It is the intracellular enzyme (endoenzyme).

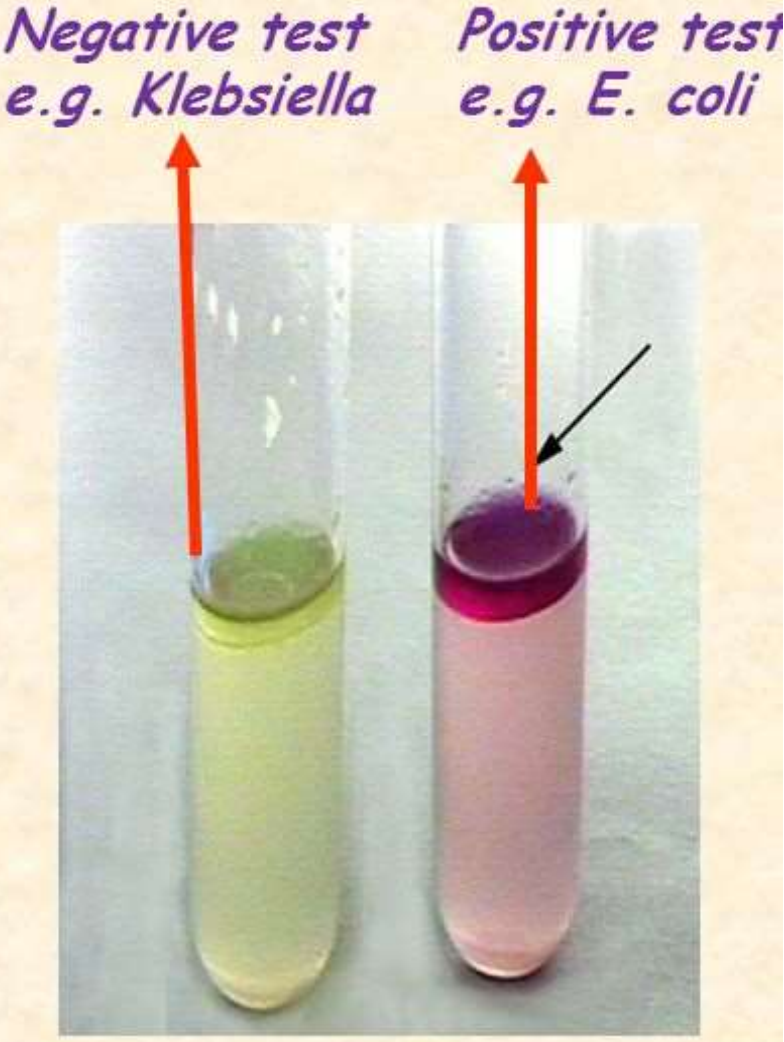
Tryptophan + H_2O——————tryptophanase enzyme——> Indole + Pyruvic acid + Ammonia

Pyruvic acid can then be used by the organism in the Kreb's cycle or it can enter glycolysis and be used to synthesize other compounds necessary for the cell. The media that is used for indole test is SIM (sulphide, indole, motility) medium or nutrient peptone, both of those media provides sufficient amino acid, tryptophan which acts as substrate for the above reaction. Hence, the organism that are able to produce the pyruvic acid as main product and indole, ammonia as the byproduct. The reagent used for this test is kovac's reagent; (HCI and dimenthyl aminobenzaldehyde dissolved in amyl alcohol) which reacts with the side product of the tryptophan catabolism reaction i.e indole to form Rosindole dye which is cherry red in colour. Hence the formation of cherry red colour of Rosindole dye indicates positive indole test, otherwise not Escherichia coli is positive to indole test while klebsiella is negative to it.

IMViC: Indole test

- **Result:**
  - A bright pink color in the top layer indicates the presence of indole
  - The absence of color means that indole was not produced i.e. indole is negative

- **Special Features:**
  - Used in the differentiation of genera and species. e.g. *E. coli* (+) from *Klebsiella* (-).

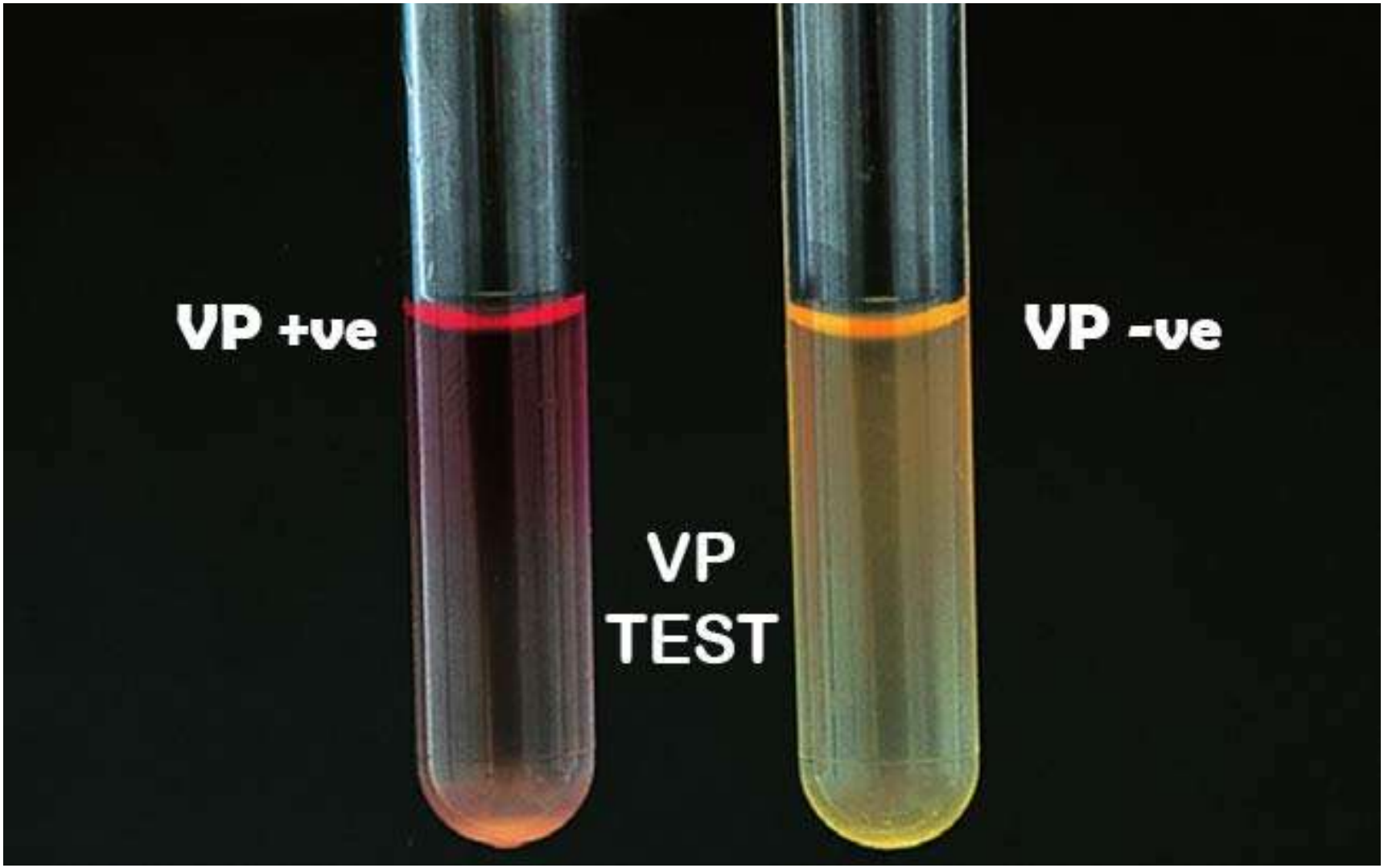


Voges–Proskauer (VP) Test

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 **∝-naphthol** was not part of the original procedure but was found to act as a color intensifier by Barritt and must be added first.
The **diacetyl** and **quanidine**-containing compounds found in the **peptones** of the broth then condense to form a **pinkish red polymer**.

2 pyruvate = acetoin + $2CO_2$

acetoin + NADH + H^+ = 2,3-butanediol + NAD^+


VP +ve
VP -ve
VP
TEST

Citrate utilization test

Objective

•to detect the ability of organisms to produce citrase enzyme.

Principle of citrate utilization test:

The basic principle of this test is to detect the ability of an organism which can utilize citrate as a sole source of carbon for their metabolism with resulting alkalinity. The ***citrase enzyme*** hydrolyses the citrate to form oxaloacetic acid and acetic acid.

Reaction:

Step I:

Citrate ———citrase enzyme—-> Oxaloacetic acid + acetic acid

Step II:

Oxaloacetic acid———-> pyruvic acid + CO2

The test organism is cultured in a medium which contains sodium citrate and indicator bromothymol blue. Change in color of indicator from light green to blue due to alkaline reaction is indication of citrate utilization by the test orgaism.

All coliforms metabolize citrate when the molecule is generated inside the bacterial cell. But not all coliforms produce transport enzyme that bring citrate from environment across the cytoplasmic membrane and into the cell. In bacteria, that utilize citrate, the cleavage of citrate involves an enzyme system without the intervention of coenzyme. This enzyme system requires a divalent cation for its activity which is supplied by the Mg++ or Mn++ ion. The product obtained from the citrate metabolism depends on the pH of the medium. In alkaline medium, mostly acetic and formic acid are produced.

Pyruvic acid———–> Formoc acid + Acetic acid + CO_2

In acidic medium, acetoin and lactic acid are produced

Pyruvic acid———–> acetoin + Lactic acid + CO_2

In both cases, there is production of CO_2 which then combines with sodium present in medium to form Sodium carbonate, an alkaline product.

$Na + CO_2 \longrightarrow Na_2CO_3$

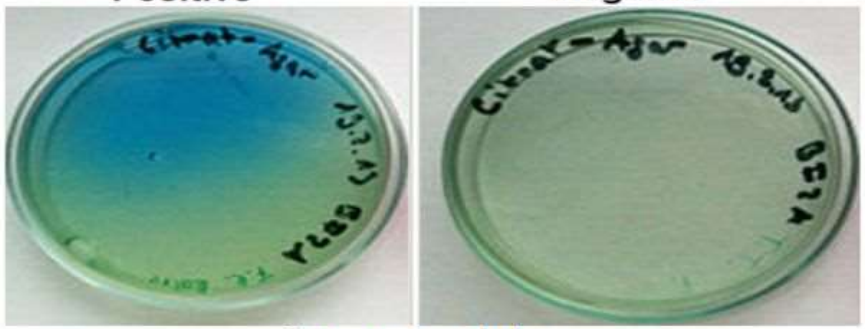
The organisms capable of utilizing citrate as sole source of carbon can also utilize ammonium salts as sole source of Nitrogen. When ammonium salts are used by microorganisms, it breakdown into NH_3 which increases the pH of medium.

The Citrate medium contains ammonium salts as sole source of Nitrogen and Citrate as sole source of carbon. It also contains a pH indicator bromothymol blue, which is green as neutral pH and changes to deep prussian blue at alkaline pH above 7.5. Henc, the formation of green color ie. no change in color indicates negative citrate test while the formation of blue color indicates positive citrate test.

Citrate Utilization Test

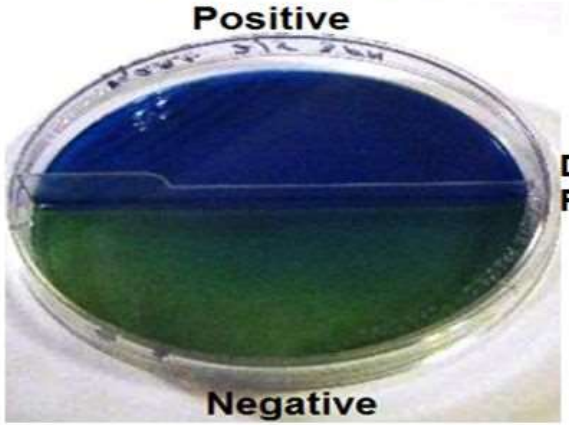
Positive

Negative



Separate plates

Positive



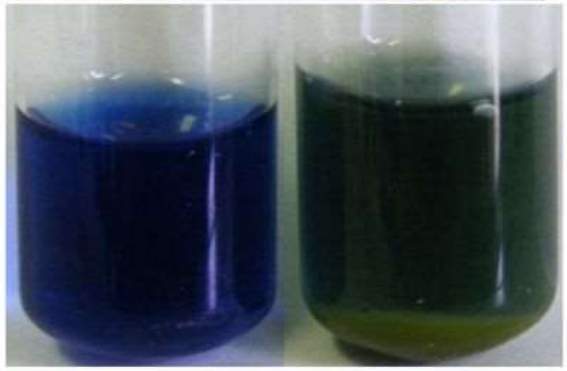
Negative

Divided Plate

Positive

Negative

Slant



Butt

Coagulase Test

Coagulase test is used to differentiate *Staphylococcus aureus* (positive) which produce the enzyme coagulase, from *S. epidermis* and *S. saprophyticus* (negative) which do not produce coagulase. i.e Coagulase Negative *Staphylococcus* (CONS).

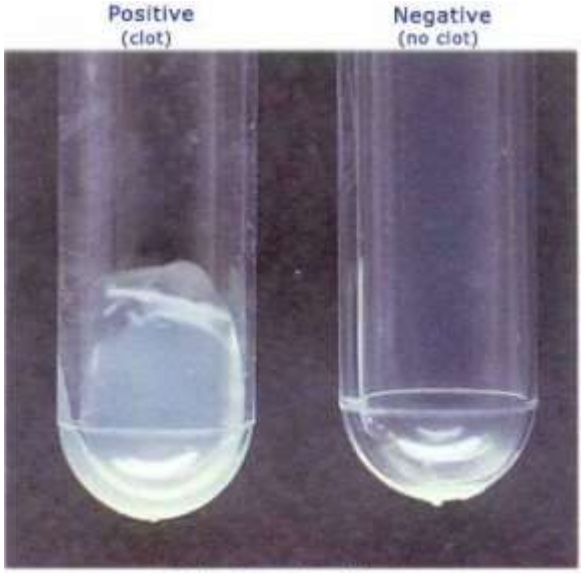
Principle of Coagulase Test
Coagulase is an enzyme-like protein and causes plasma to clot by converting fibrinogen to fibrin. *Staphylococcus aureus* produces two forms of coagulase: bound and free.

Bound coagulase (clumping factor) is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in an alternation of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma. This doesn't require coagulase-reacting factor.

Free coagulase involves the activation of plasma coagulase-reacting factor (CRP), which is a modified or derived thrombin molecule, to from a coagulase-CRP complex. This complex in turn reacts with fibrinogen to produce the fibrin clot.

Interpretation of Coagulase Test

Slide Coagulase Test



Tube Coagulase Test

Clumping in both drops of slides indicates that the organism auto agglutinates and is unsuitable for the slide coagulase test. All the negative slide test must be confirmed using the tube test.

During slide test, there may be chance to false positive results in case of citrate utilizing bacteria (*Enterococcus* and *Pseudomonas*). In this case also, tube test should be performed and confirmed.

Hydrogen Sulfide (H2S) Production Test

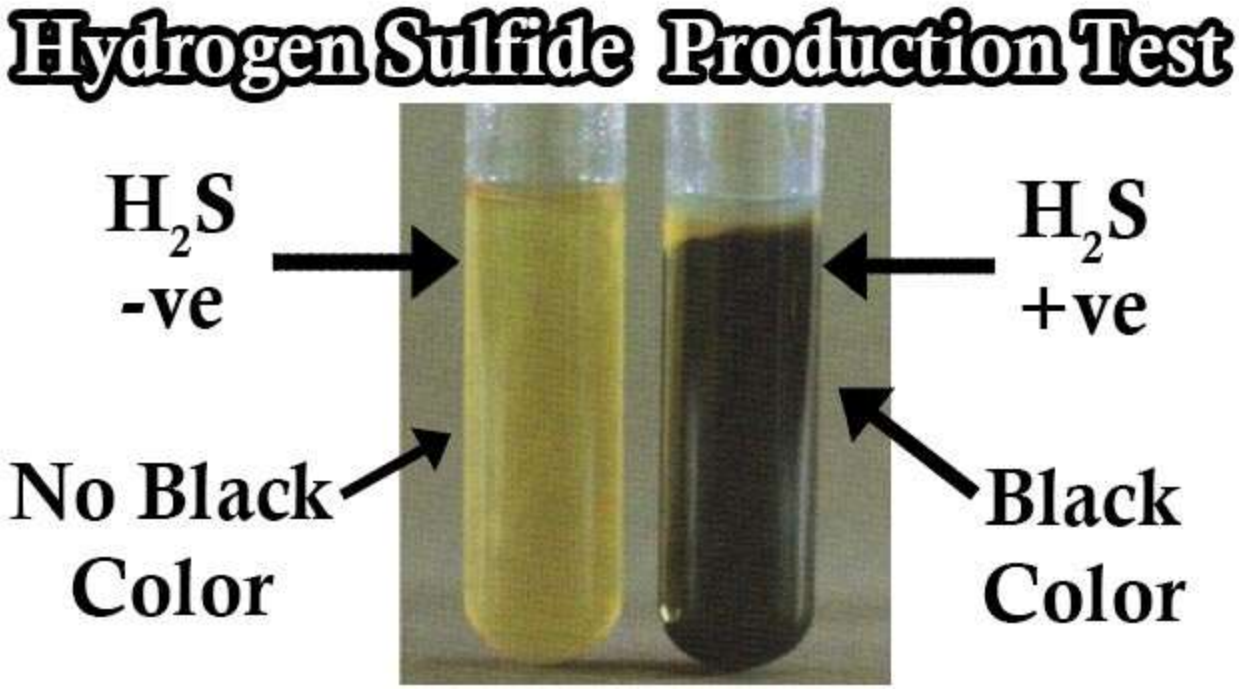
Objective of Hydrogen Sulfide (H2S) Production Test

To determine the ability of the organism to produce hydrogen sulphide.

Principle of Hydrogen Sulfide (H2S) Production Test

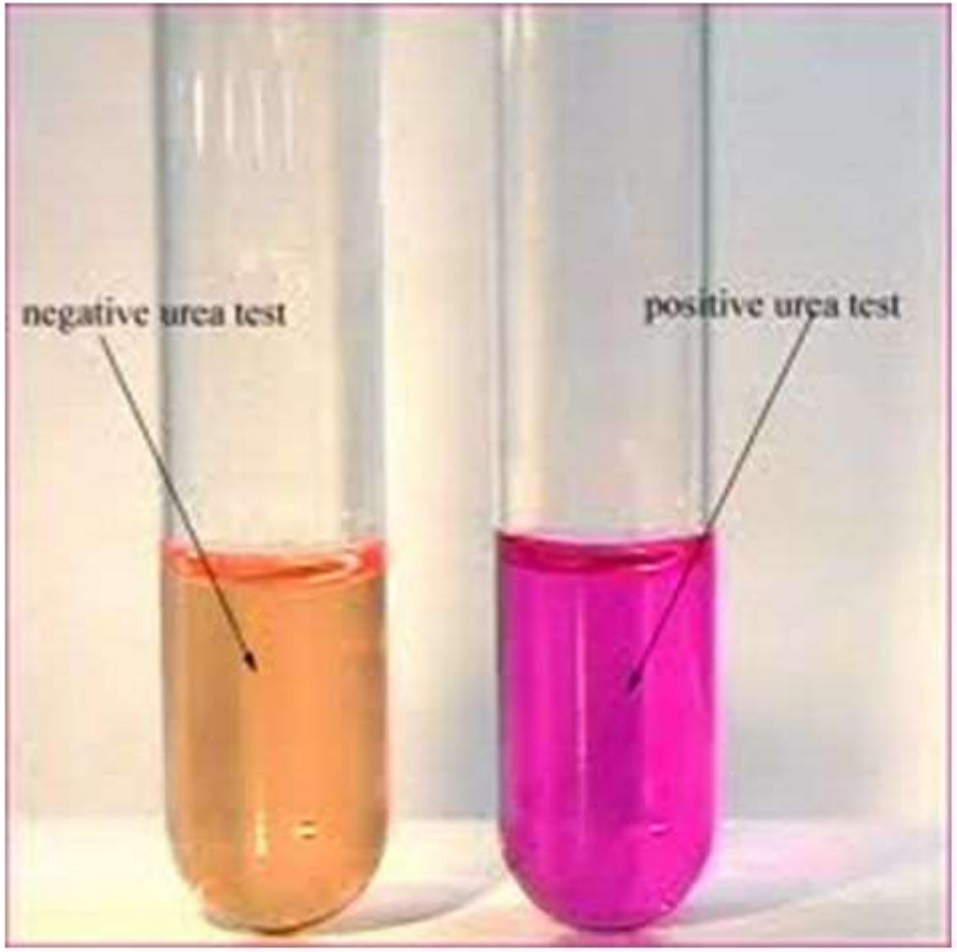
Hydrogen sulphide (H_2S) production test is used for the detection of hydrogen sulphide (H_2S) gas produced by an organism. It is used mainly to assist in the identification of members of family Enterobacteriaceae. H2S is produced by certain bacteria through reduction of sulphur containing amino acids like cystine, methionine or through the reduction of inorganic sulphur compounds such as thiosulfates, sulfates or sulfites. The hydrogen sulphide production can be detected by incorporating a heavy metal salt containing iron or lead as H2S indicator to a nutrient culture medium containing cystine and sodium thiosulfates as the sulfur substrates. Hydrogen sulphide, a colorless gas, if produced reacts with the metal salt forming visible insoluble black precipitate of ferrous sulphide.

Result Interpretation of Hydrogen Sulfide (H2S) Production Test



Positive result: blackening on the medium
Negative result: no blackening on the medium

Urease Test



The test organism is cultured in a medium which contains urea and the indicator phenol red.

When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

Urease test

Objective

to check whether the organism can produce urease enzyme for the degradation of urea or not.

Principle of urease test:

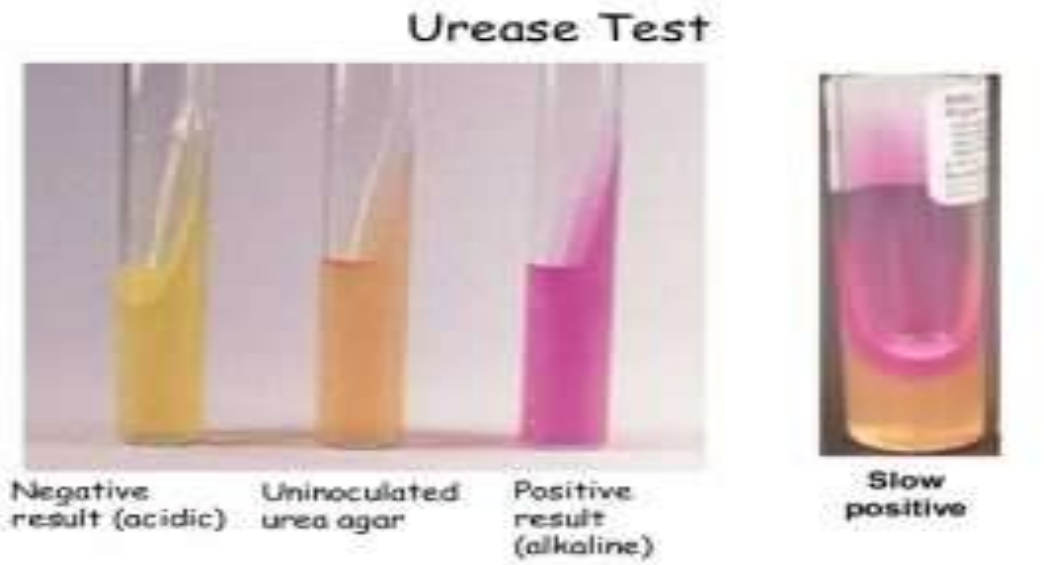
Urea is common metabolic waste product of protein digestion in most vertebrates that is toxic to most living organism. Urease catalyses the breakdown of urea into ammonia and carbondioxide. The test organism is cultured in a medium containing urea and the indicator phenol red. If the bacterial strain is urease-producing, the enzyme will hydrolyse the urea to give ammonia and carbondioxide. With the release of ammonia, the medium become alkaline shown by change in color of indicator to reddish pink.

Urea + H2O————urease enzyme———>Ammonia + CO2

This test is done to determine the ability of certain micro organism to produce enzyme urease. It is one of the useful diagnostics for identifying bacteria, particularly *Proteus* from other gram negative bacteria.

The basic principle of this test is to check the color change of indicator phenol red used in the media caused due to the degradation of urea and formation of basic product i.e NH3. The change in the color of the medium from orange to pink red due to the rise in pH indicates the positive test, while the unchanged color of media indicates negative test.

Result:



Urease test Positive: Red-pink color (*Proteus vulgaris)*

Urease test Negative: No pink color (*E. coli*)

Urease Producig bacteria

Proteus spp
Klebsiella spp
Morganella spp
Providentia spp
Serratia spp
Staphylococcus saprophyticus
Bacillus spp
Mycoplasma
Ureaplasma urealyticum
Nocardia
Corynebacterium urealyticum
Helicobacter pylori

Phenylalanine Deaminase Test

Objectives of Phenylalanine Deaminase Test

To determine the ability of an organism to oxidatively deaminate phenylalanine to phenylpyruvic acid.
To differentiate enteric Gram-negative bacilli on the basis of their ability to produce phenylpyruvic acid from phenylalanine.

Phenylalanine deaminase medium tests the ability of an organism to produce the enzyme deaminase. Microorganisms capable of producing phenylalanine deaminase remove the amine (NH_2) from phenylalanine and releases the amine group as free ammonia. The deamination of phenylalanine by oxidative enzymes results in the formation of phenylpyruvic acid.

Media Used in Phenylalanine Deaminase Test

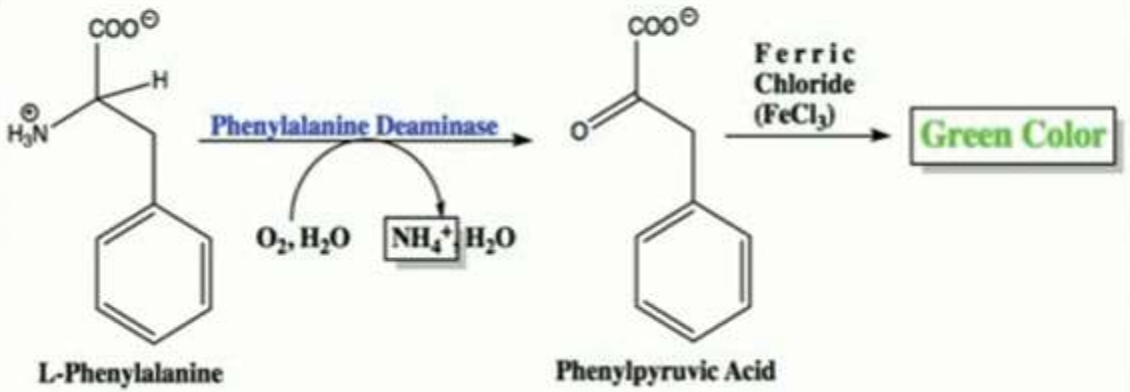
Phenylalanine agar

Result Interpretation of Phenylalanine Deaminase Test

Positive test: development of a light to dark green color within 1-5 minutes after applying ferric chloride reagent.

Negative test: absence of a green color reaction or yellow coloration due to the color of the ferric chloride.

Phenylalanine Deaminase Test



Phenylalanine Deaminase → Green Color

NO Phenylalanine Deaminase → No Color Change