Biochemical tests

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Objectives

- Definition of biochemical test
- Principle of the following tests:

-Kliger’s iron agar(KIA)

-IMViC test

-Oxidase test

-Catalase test

-Urease test

-Coagulase test

-Analytical profile index(API)test

Definition of biochemical test

Biochemical tests are tests that identify the bacteria on the basis of the presence of certain enzymes and differences in the biochemical activities of different bacteria . They are among the most important methods for microbial identification.

Biochemical Tests:

1. **Kliger's Iron Agar (KIA)**
2. **IMViC test**
3. **Oxidase test**
4. **Catalase test**
5. **Urease test**
6. **Coagulase test**
7. **API test**

1.Kliger’s Iron Agar(KIA)

is a differential slope medium used to identification of enteric bacteria, it indicate the ability of the bacteria ferment either glucose(0.1%) or lactose (1%)with or without gas and also indicate the presence of H2S.

Results:

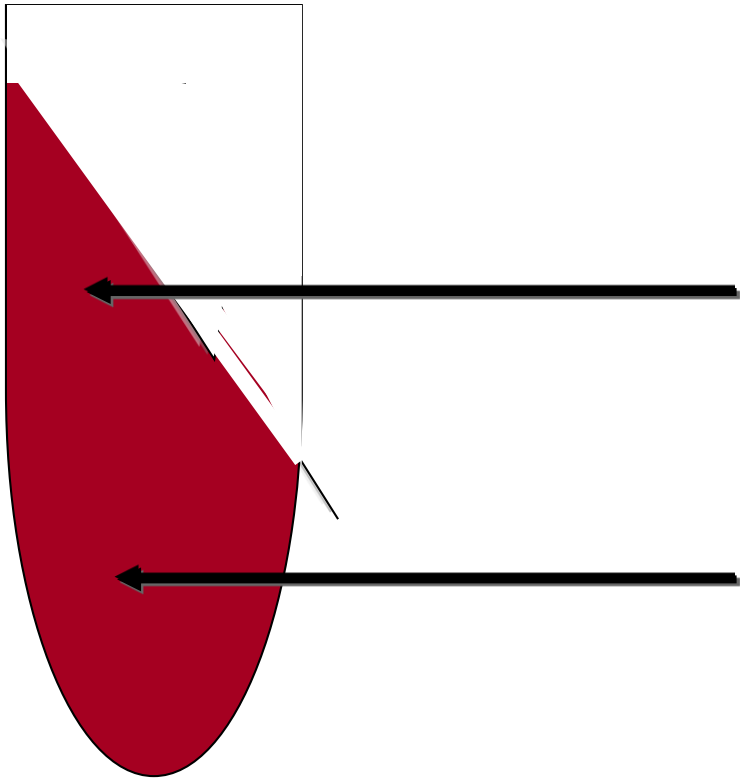
A yellow slant: the organism ferments sucrose or lactose.

A yellow butt: the organism fermentd glucose.

Black precipate in the butt indicates H2S

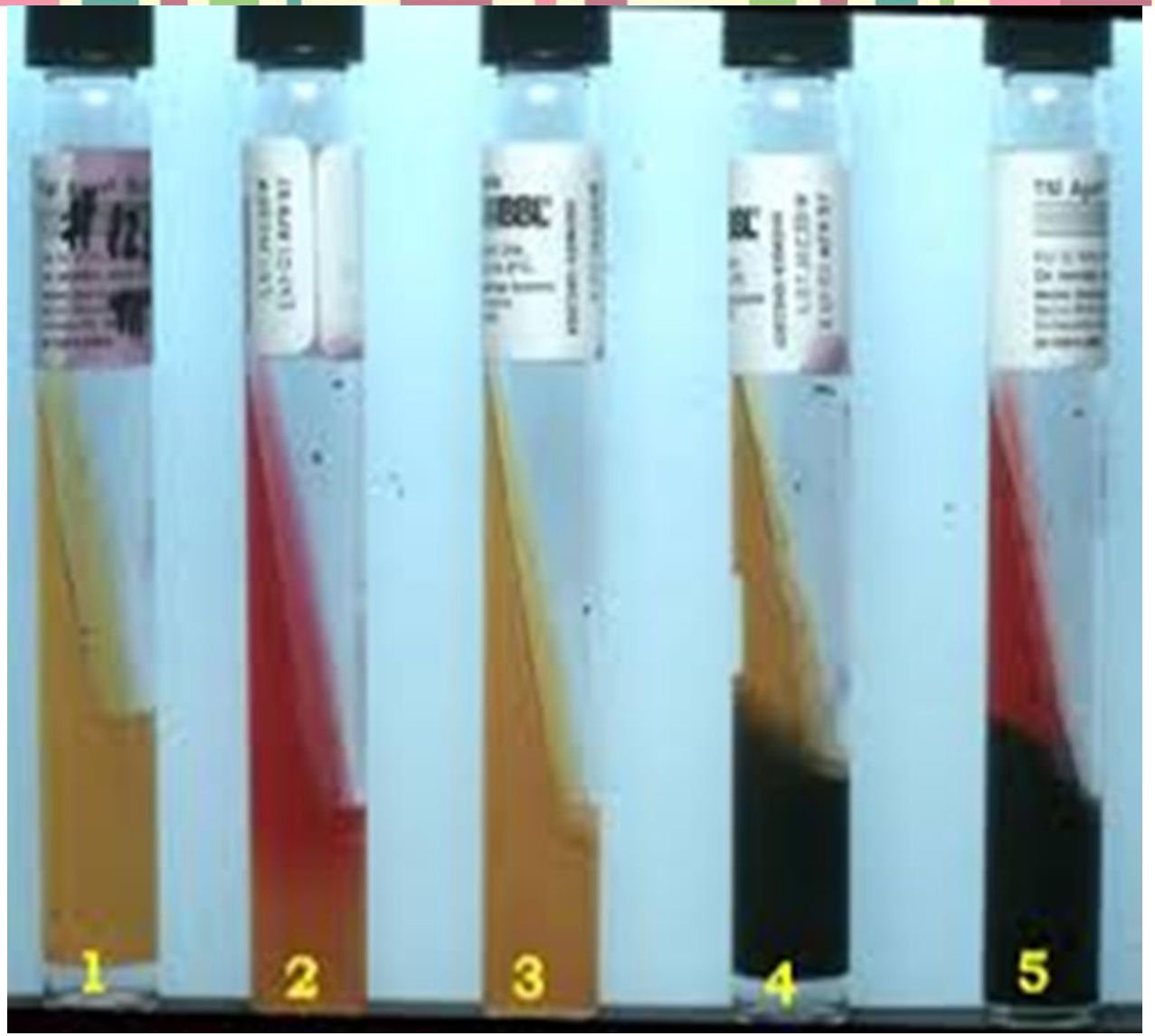
Gas is indicated either by cracks or bubbles in the media.

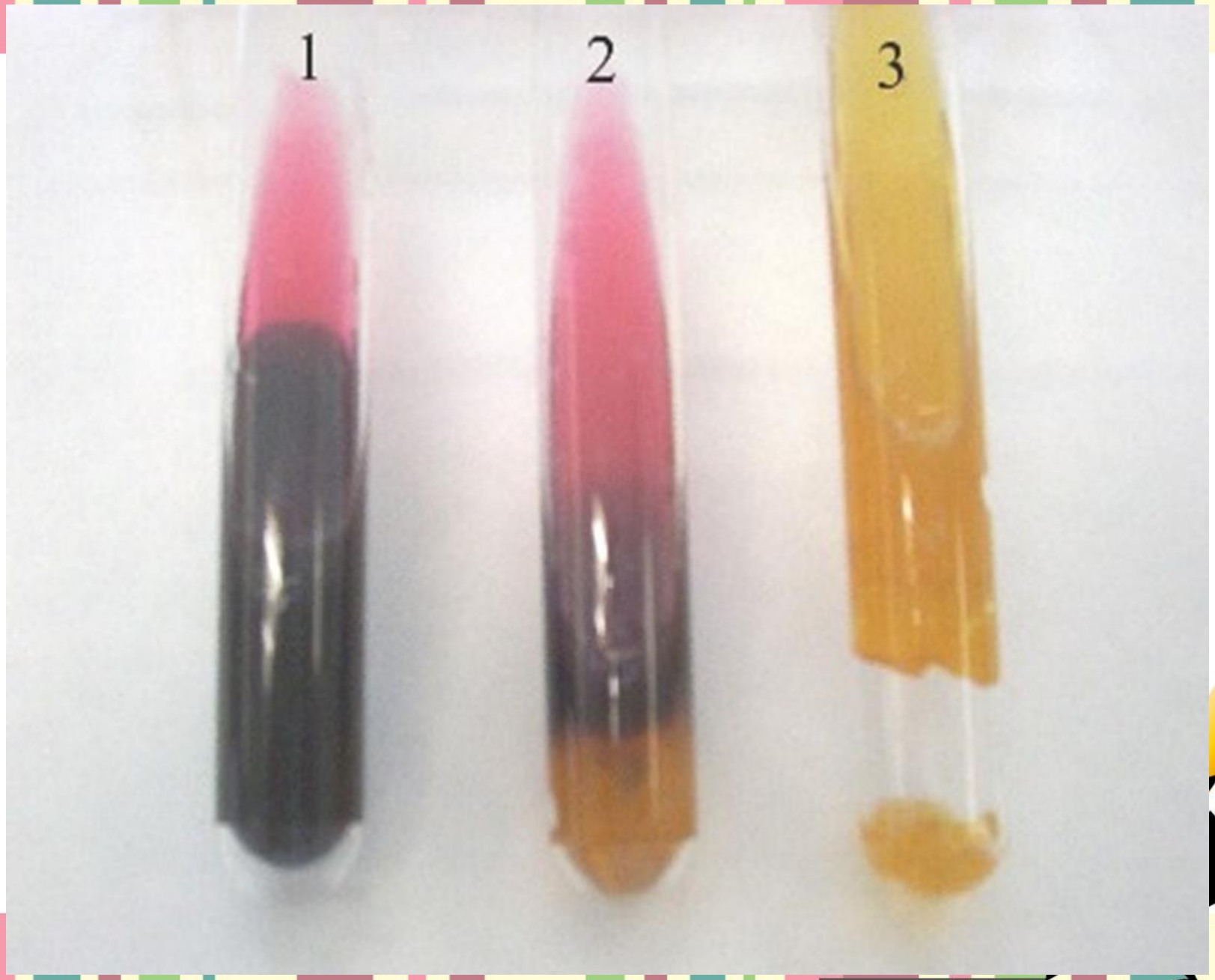
The Principle of KIA



Slant
(for lactose fermentation)

Butt
(for glucose fermentation)





2- IMViC Tests:

IMViC Test include the following tests:-

- Indole test
- Methyl Red
- Voges – Proskauer test
- Citrate Test

Indole test:

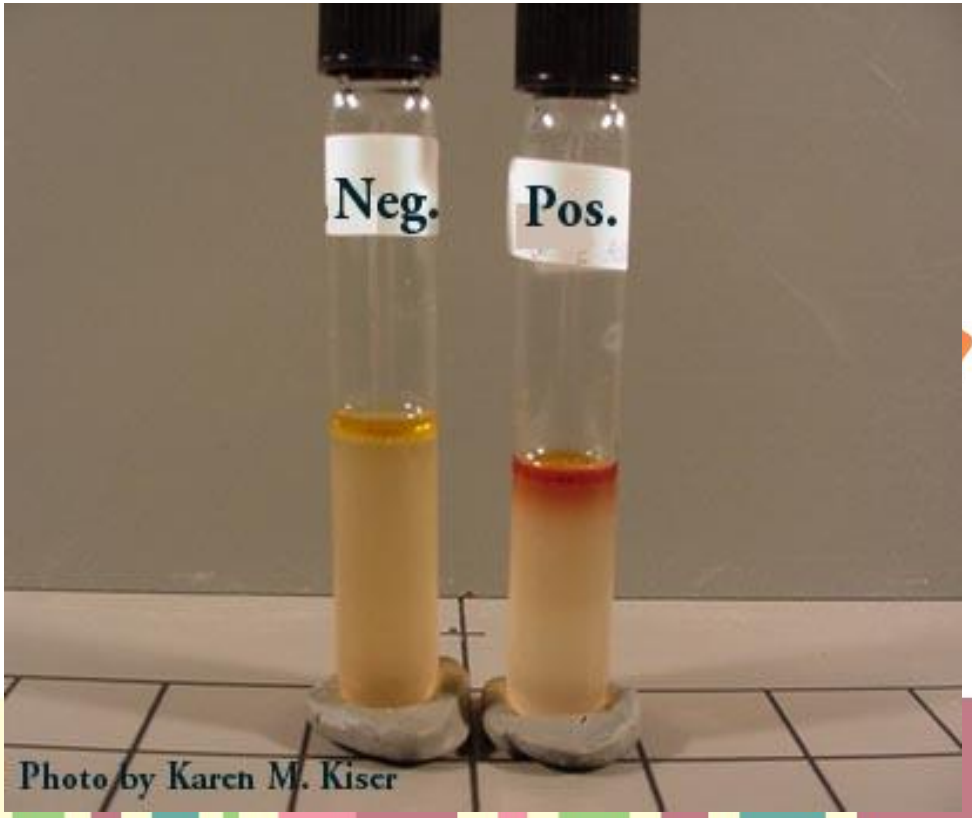
Principle: Identifies bacteria capability of producing indole, Some bacteria are capable of converting tryptophan (an amino acid present in peptone water) to indole and pyruvic acid by using the enzyme tryptophanase, after adding the Kovac's Reagent.

The Result:

Positive Test
Red ring
(Indole production)

/

Negative Test
Yellow ring
(no Indole production)



Methyle Red test:

Principle:

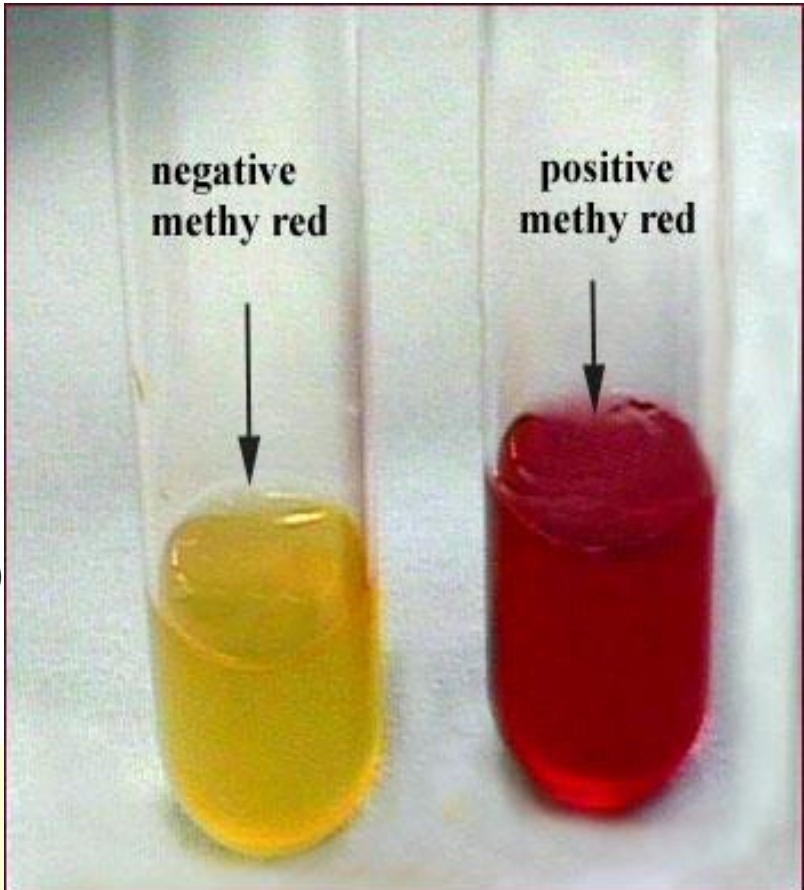
Used to determine the ability of a bacteria to oxidize glucose and produce stable acid end products.

Medium:

glucose phosphate broth (GPB)

Result:

positive test= **Bright red color** *(E.coli)*
negative test= **Yellow-orange color***(klebsiella spp)*



Voges-Proskauer Test:

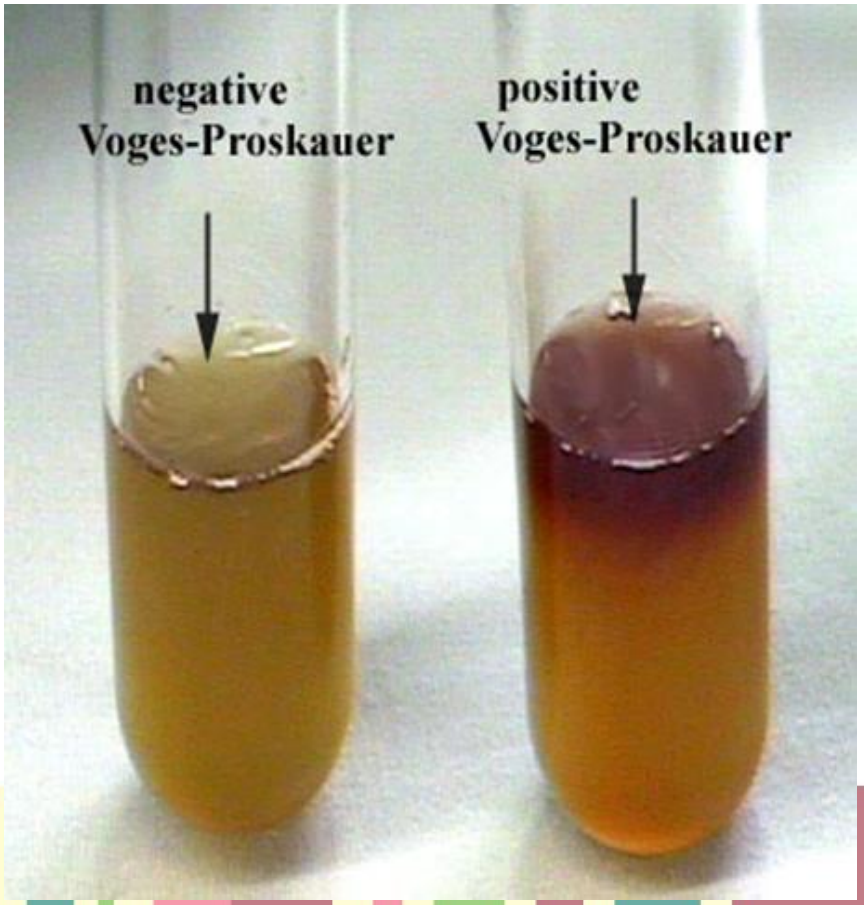
Principle:

To determine the ability of bacteria to produce acetoin from pyruvic acid.

Medium: GPB

Result:

positive test=(**red color)** ***(klebsiella spp.)***
negative test= **Yellow-orange color*(E. coli)***



Simmon's Citrate test:

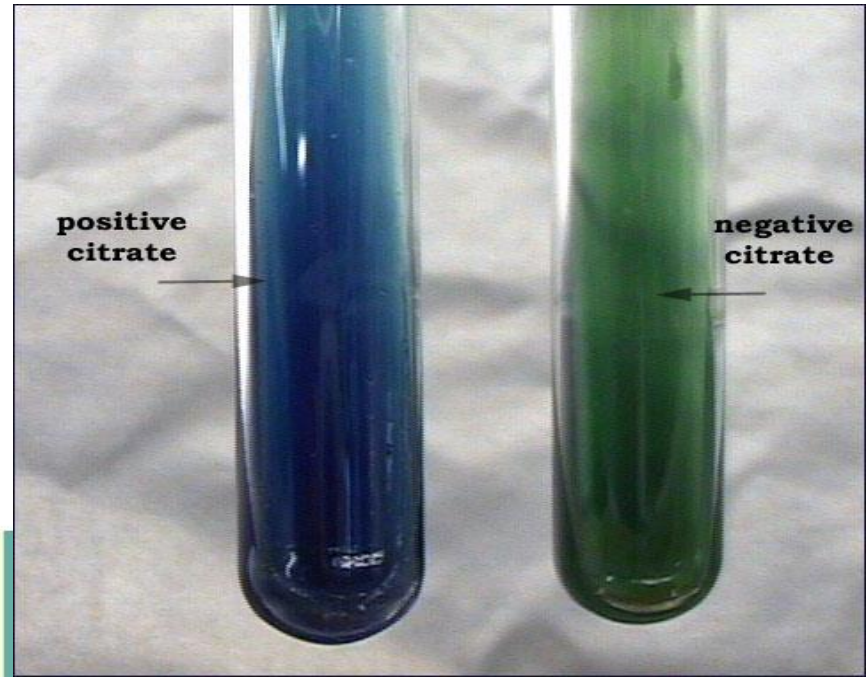
Principle:

To determine the ability of bacteria to utilize citrate as the sole source of carbon for its growth.

Medium: Simmon citrate medium which contain bromothymol blue as indicator

Result:

+ve test change color
from green to blue (*Klebsiella sp*).
-ve test no change color (*E. coli*).



3. Oxidase test

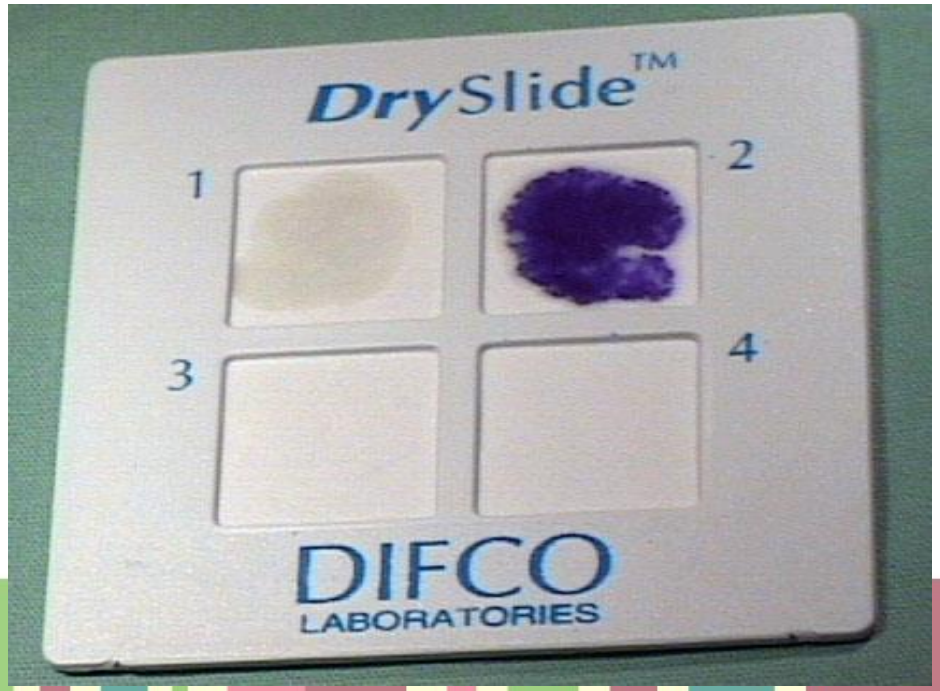
To determine the ability of bacteria to praduce an enzyme cytochrome oxidase.

Result: Positive test

dark purple

negative test

no color



4. Catalase test:

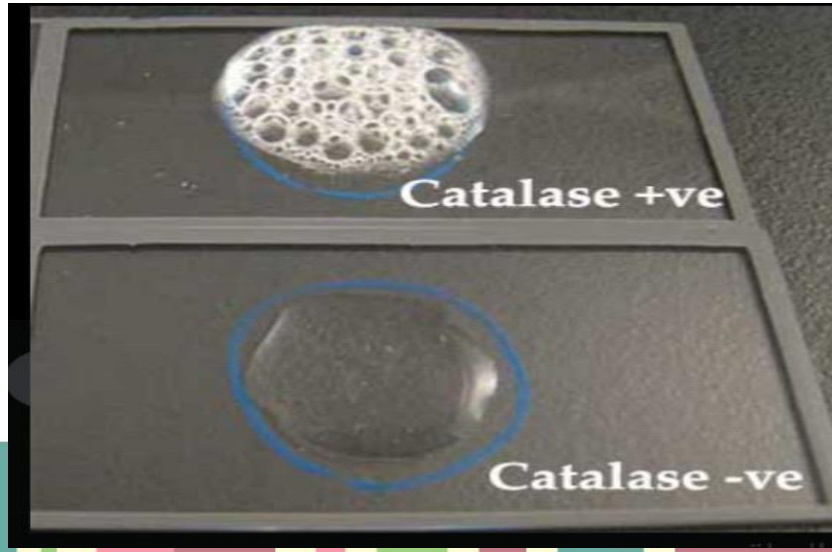
Principle:

Catalase is produced by certain bacteria, which acts as a catalyst in breakdown of hydrogen peroxide into water and oxygen.

Results:

Active bubbling=positive catalase test.

No bubbles=negative catalase test.



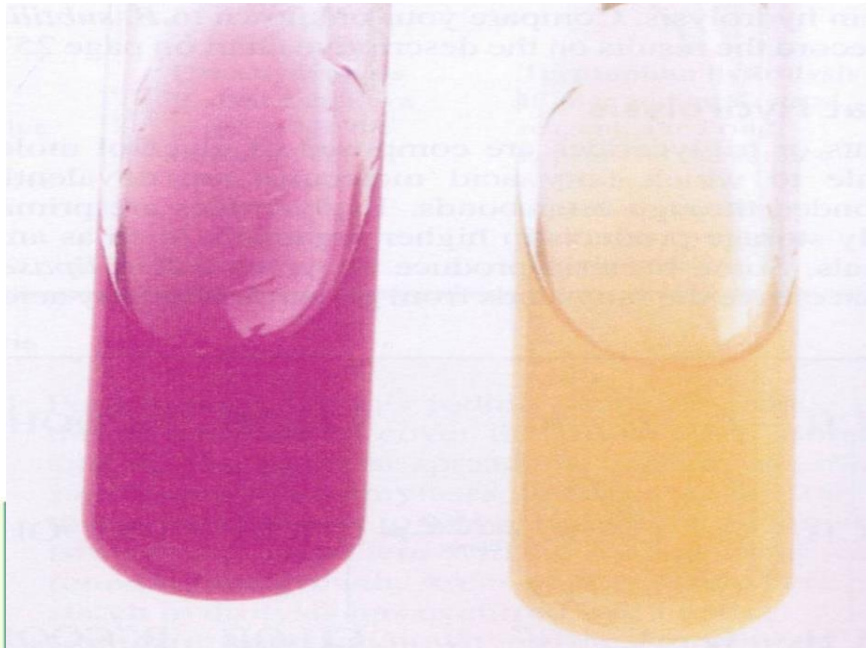
5. Urease test:

Principle:

Many organisms especially those that infect the urinary tract have a urease enzyme that is able to split urea in the presence of water to release ammonia and carbone dioxide

Result:

Urease positive organisms such as *proteus spp* will turn the medium deep pink.



6.Coagulase test:

This test is used to identify Staph. aureus, which produces coagulase enzyme.

Principle:

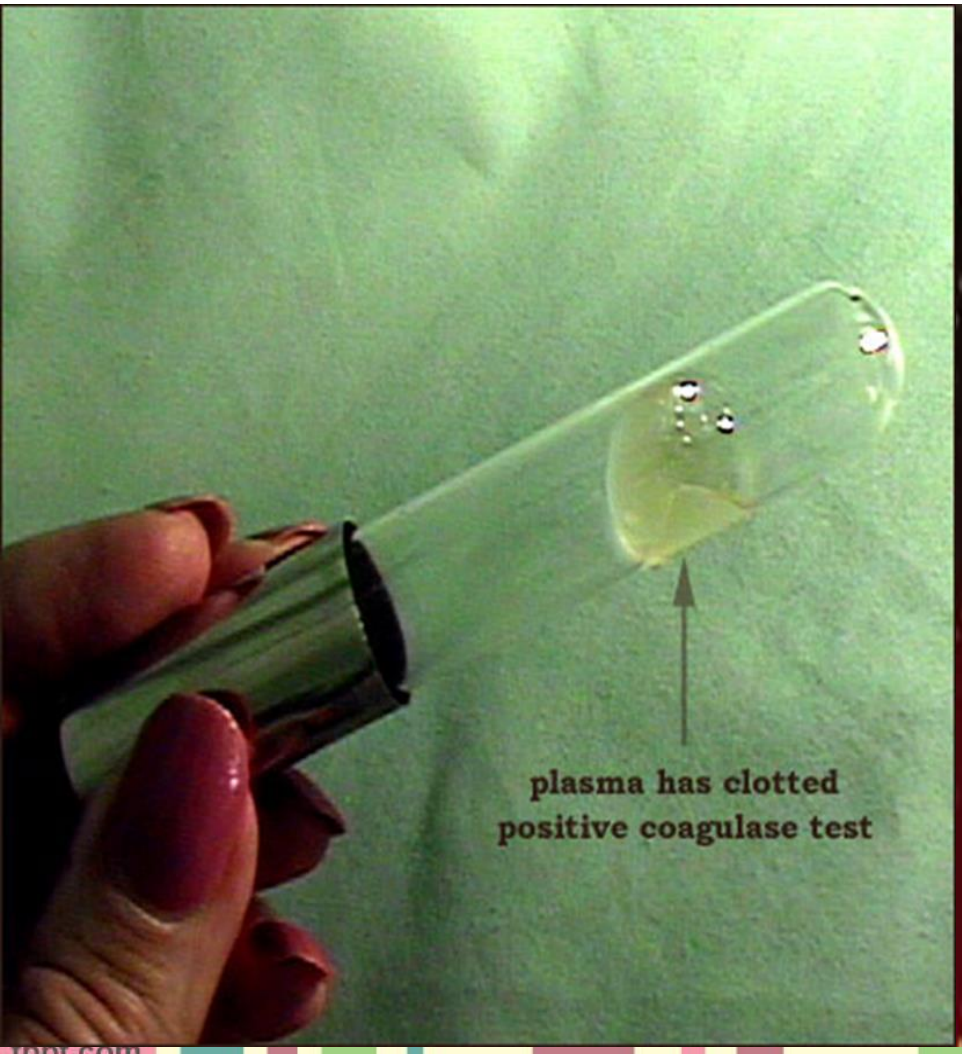
Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *Staph. aureus*:

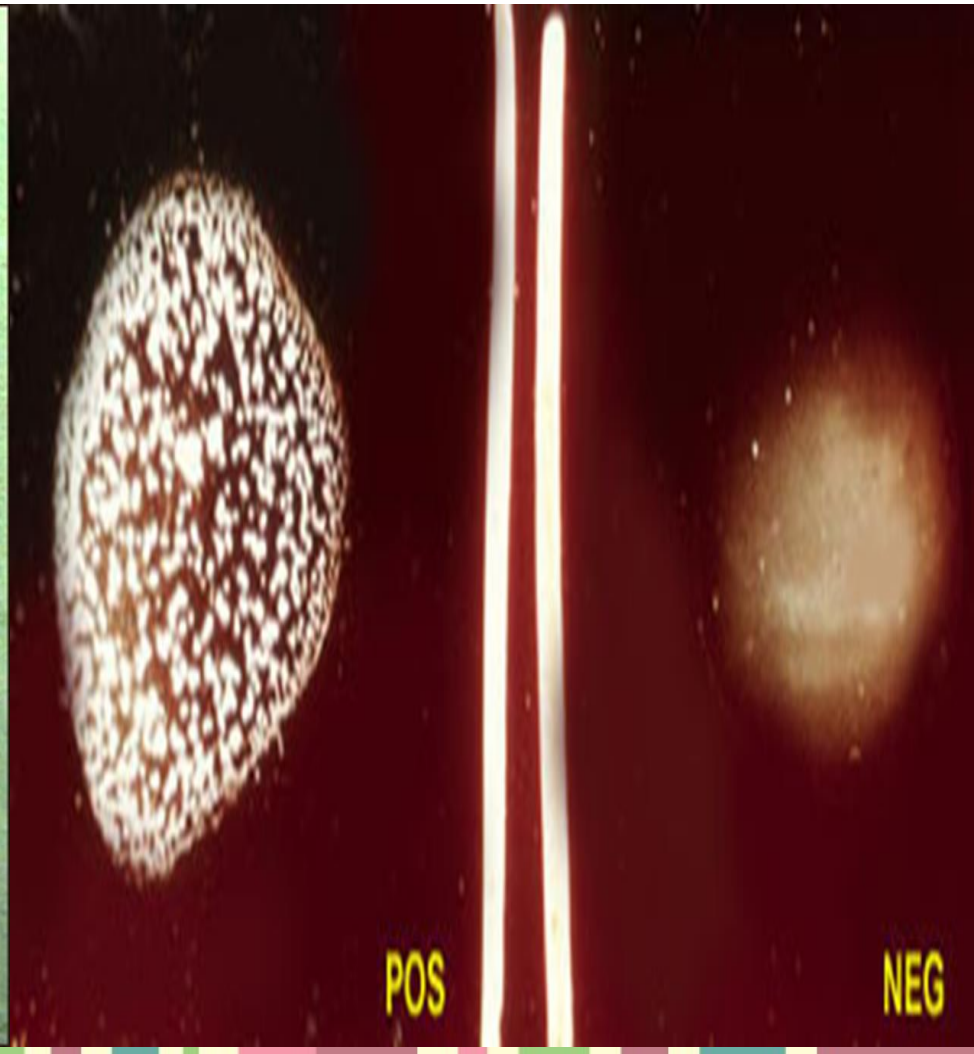
Free coagulase

Bound coagulase(clumping factor).

Tube method

Slide method





Analytical Profile Index (API) system:

Consist of series of microcupules on a plastic strip that contain dehydrated substrates for the demonstration of enzymatic activity or the fermentation of carbohydrates. Depending on the type of microorganism and the API strip utilized (eg. API 20E for identification of *Enterobacteriaceae*,API STAPH for staphylococcus(identification) .

Proteus vulgaris

Proteus mirabilis

Escherichia coli

Providencia alcalifaciens