4. Urease test

Many organisms especially those that infect the urinary tract, have an urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water from ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.

► Procedure for urease test

1. The broth medium is inoculated with a loopful of a pure culture of the test organism; the surface of the agar slant is streaked with the test organism.

2. Incubate the test tube at 35 °C for 18 to 24 hours.

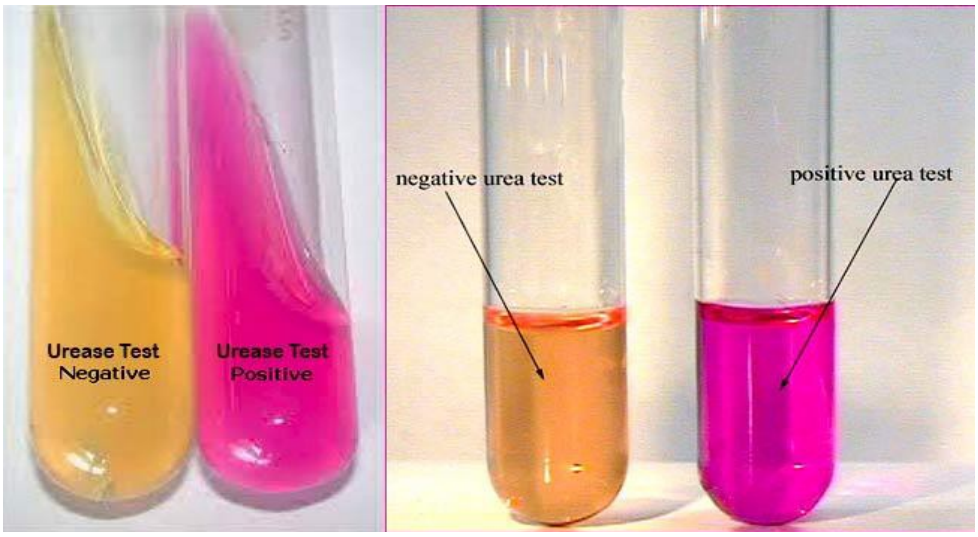
► Results

Positive: If organism produces urease enzyme, the color of the slant changes from light orange to pink.

Examples: Proteus spp., Cryptococcus spp., Helicobacter pylori, Yersinia spp., Brucella spp.

Negative: If organism do not produce urease the agar slant and butt remain light orange (medium retains original color).

Examples: Escherichia coli.



Urease Test Results

5. Triple Sugar Iron Agar (TSI) and H2S production test

Whenever you see the name of this test i.e. Triple Sugar Iron Agar, you have to remember that it's a test which has three sugar (Lactose, Sucrose and Glucose) and also iron; and it contains Agar Agar as solidifying agent (TSI is a semi solid media having slant and butt).

► Composition of Triple Sugar Iron Agar (TSI)

● 0.1% Glucose: if only glucose is fermented, only enough acid is produced to turn the butt yellow. The slant will remain red.

● 1.0% lactose /1.0% sucrose: a large amount of acid turns both butt and slant yellow, thus indicating the ability of the culture to ferment either lactose or sucrose.

● Iron: (Ferrous sulfate), indicator of H2S formation

● Phenol red: indicator of acidification (it is yellow in acidic condition and red under alkaline conditions).

● It also contains Peptone which acts as source of nitrogen. (Remember that whenever peptone is utilized under aerobic condition ammonia is produced).

► Procedure

1. With a sterilized straight inoculation needle touch the top of a well-isolated colony.

2. Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.

3. Leave the cap on loosely and incubate the tube at 35 °C in ambient air for 18 to 24 hours.

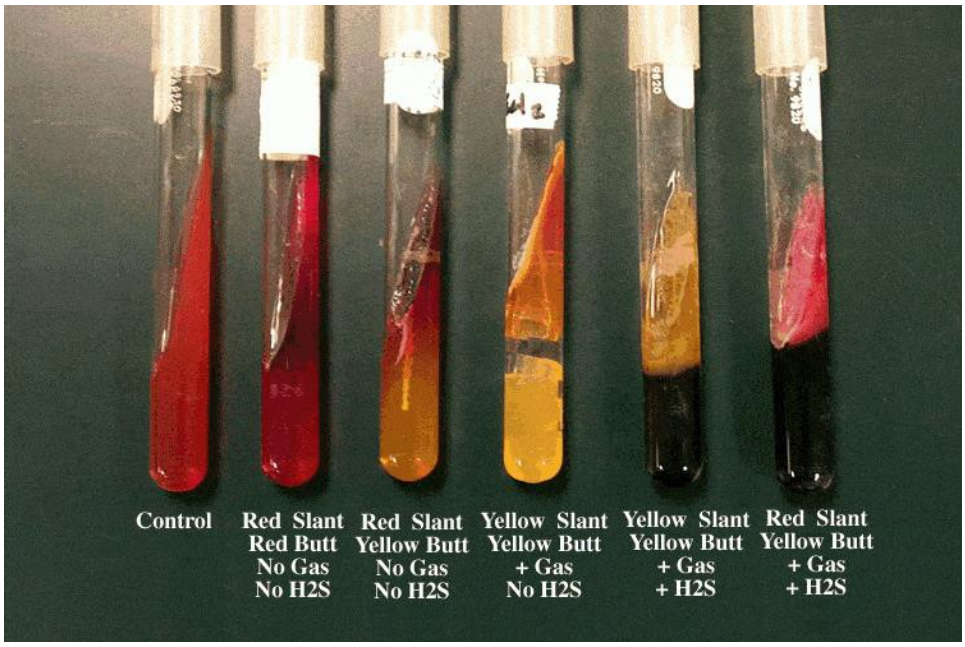
► Results

1. If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both the butt and the slant. Some organisms generate gases, which produces bubbles/cracks on the medium.

2. If lactose is not fermented but the small amount of glucose is, the oxygen deficient butt will be yellow (remember that butt comparatively have more glucose compared to slant i.e. more media more glucose), but on the slant the acid (less acid as media in slant is very less) will be oxidized to carbon dioxide and water by the organism and the slant will be red (alkaline or neutral pH.

3. If neither lactose/sucrose nor glucose is fermented, both the butt and the slant will be red. The slant can because a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids (remember peptone is a major constituents of TSI Agar).

4. If H_2S is produced, the black color of ferrous sulfide is seen.



6. IMViC Tests

Each of the letters in "IMViC" stands for one of these tests. "I" is for indole; "M" is for methyl red; "V" is for Voges-Proskauer, and "C" is for citrate, lowercase "i" is added for ease of pronunciation. IMViC is an acronym that stands for four different tests:

a. Indol test

b. Methyl red test

c. Voges-proskauer test

d. Citrate utilization test

To obtain the results of these four tests, three test tubes are inoculated: tryptone broth (indole test), methyl red – Voges Proskauer broth (MR-VP broth), and citrate. IMViC tests are employed in the identification / differentiation of members of family enterobacteriaceae.

► Procedure

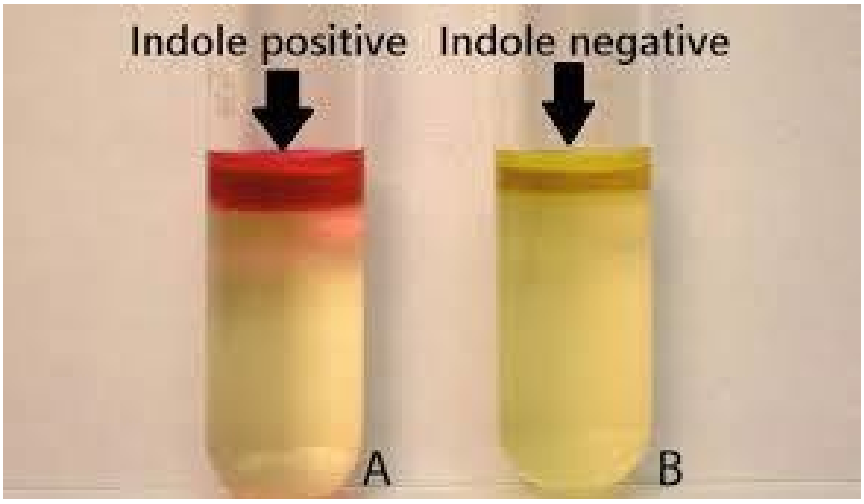
Cultures of any members of enterobacteriaceae have to grow for 24 to 48 hours at 37 °C and the respective tests can be performed:

a. Indole test

It is performed on sulfide-indole-motility (SIM) medium or in tryptophan broth. Result is read after adding Kovac's reagent.

1. The positive result is indicated by the red layer at the top of the tube after the addition of Kovac's reagent.

2. A negative result is indicated by the lack of color change at the top of the tube after the addition of Kovac's reagent.



Indole test results: positive-development of Red-ring

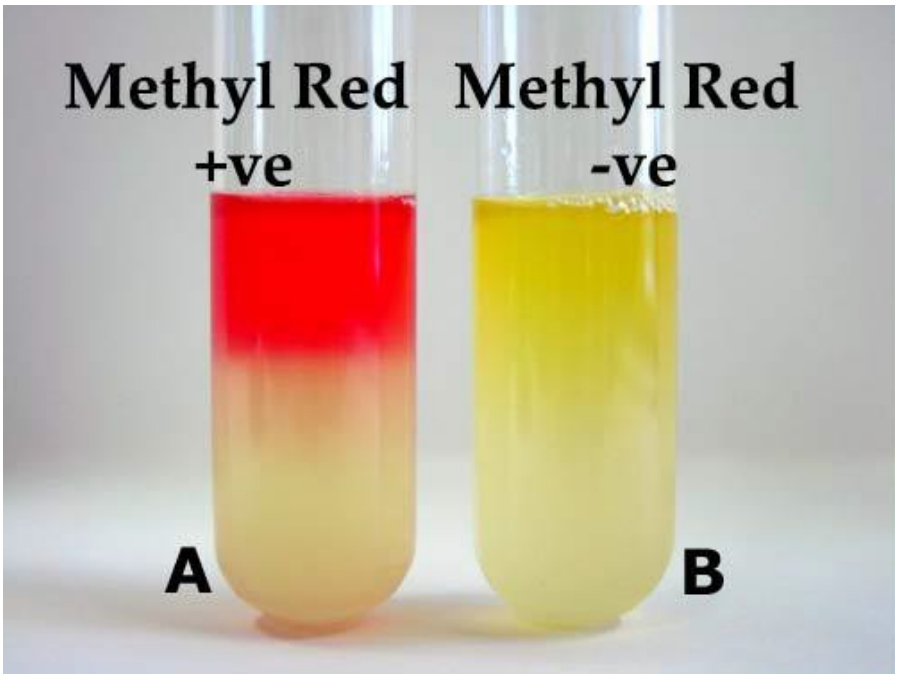
b. Methyl red test and Voges-Proskauer test

both are done in methyl red Voges-Proskauer (MR-VP) broth, but the reagents that we added differs

* Methyl Red (MR) test:

● Positive methyl red test are indicated by the development of the red color after the addition of methyl red reagent.

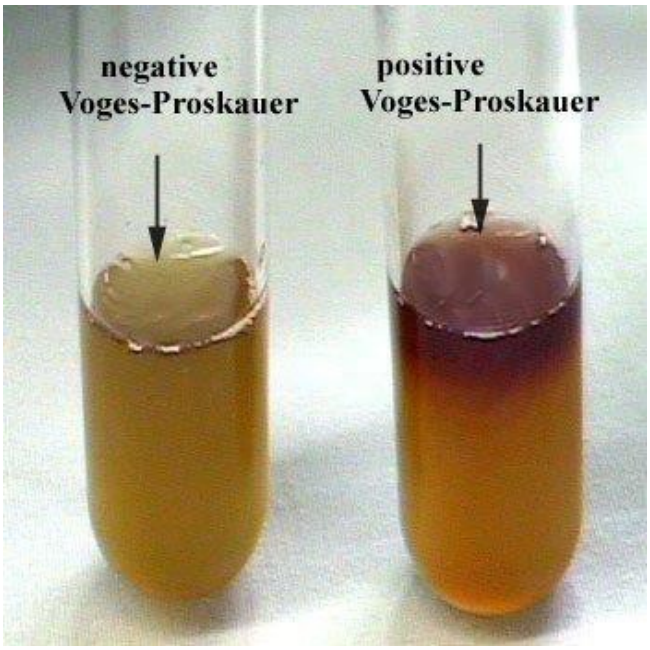
● A negative methyl red test is indicated by no color change after the addition of methyl red reagent.



*** Voges-Proskauer (VP) test:**

● Negative test is indicated by lack of color change after the addition of Barritt's A and Barritt's B reagents.

● A positive Voges-Proskauer test is indicated by the development of red-brown color after the addition of Barritt's A and Barritt's B reagents.



c. Citrate utilization test

The test performed on Simmons citrate agar:

● Negative citrate utilization test is indicated by the lack of growth and color change in the tube

● Positive citrate result as indicated by growth and a blue color change.

University of AL-Mustansiriyah
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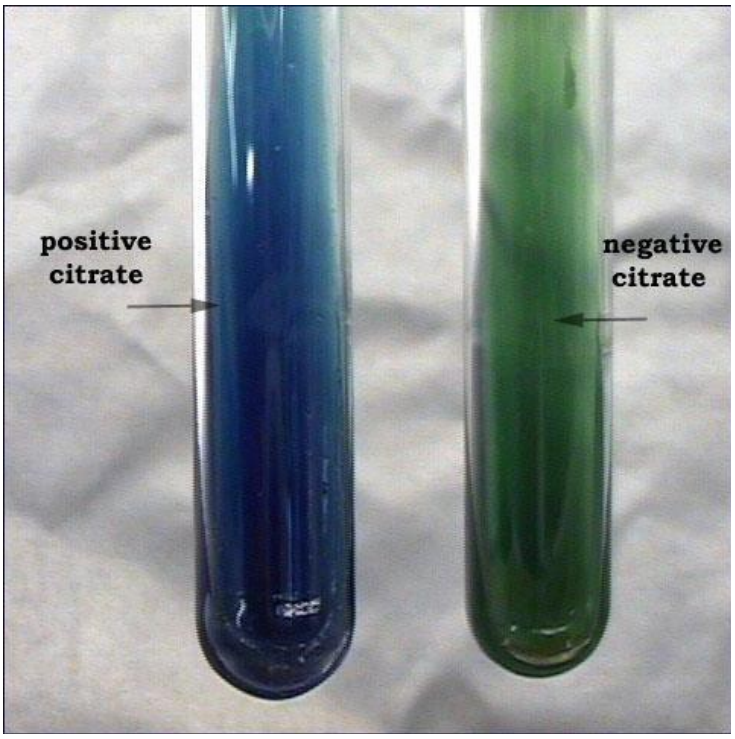
College of Sciences
Biology Department

Microbiology 2 Lab 8



► IMViC Test results of some Genera of Enterobacteriaceae:

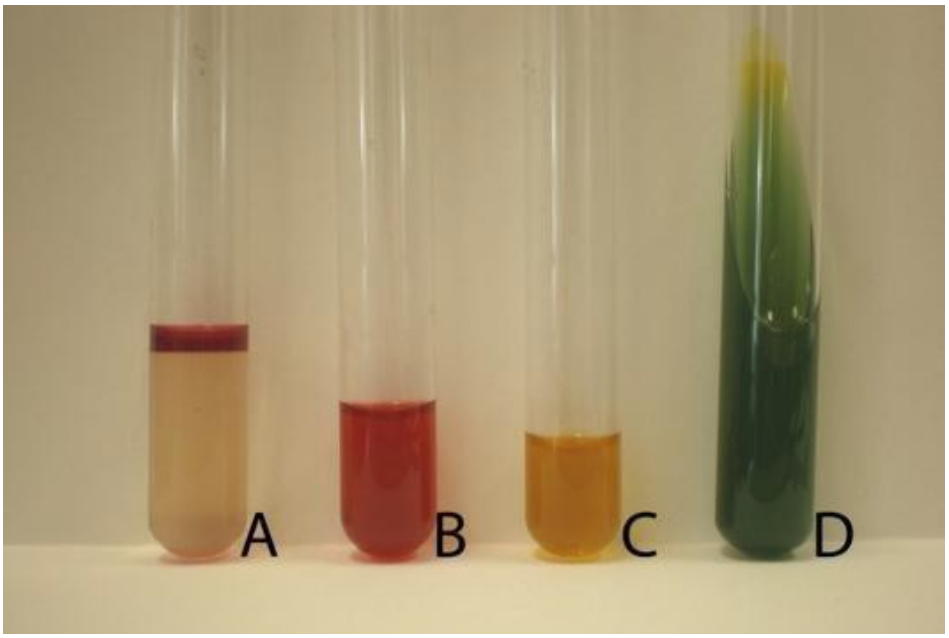
1. IMViC tests of Escherichia coli

1. Indole: positive

2. Methyl-Red: positive

3. Voges-Proskauer test: Negative

4. Citrate test: Negative



IMViC Test of E. coli: (++ - -)