Isolation of enteric bacteria

Practical Microbiology

3rd grade

Second course

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Enteric bacteria

- These are Gram Negative bacilli bacterial group, some are motile and some are not.
- This group of bacteria is found in the intestinal tract of humans and animals, some are harmless while others are opportunistic pathogens.
- These are found in the sewage and pollutes the water supplies, food etc. The pathogens may be endemic among the animals or occur in carrier or persisted in humans, such as *Escherichia, Salmonella, Shigella, Vibrio, Enterobacter, Proteus* & *Klebsiella.*
- Some of them has been isolated from oral cavity associates with other infectious bacteria.
- There are many sources of enteric bacterial transmission into the oral cavity, such as contaminated water, Food, Dairy products and hospital environment.

Isolation of enteric bacteria

Procedure;

1- Swab from stool (**in case gastroenteritis**), urine (**in case urinary tract infection**) or blood (**in case of enteric fever and septicemia**).

2- Roll the swab near the edge of blood agar plate and macConky agar .and eosin methyline blue agar (**EMB**)

3- Streak with sterile loop, label the plates.

4- Incubate the plate at 37ºC for 24-48 hr.

5- Observe the growth on blood agar and macConky agar (lactose fermenter or non-lactose fermenter) and EMB

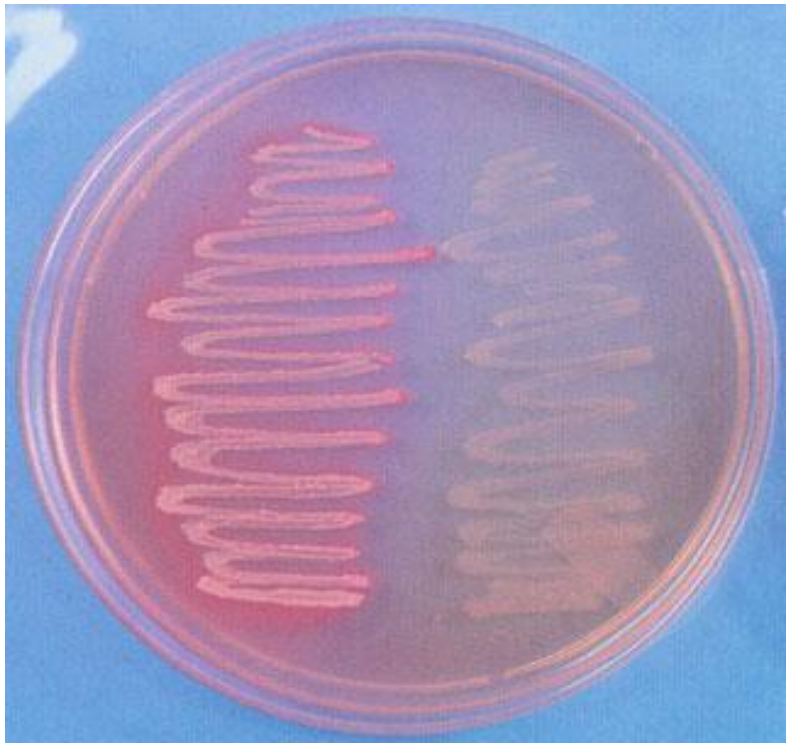
6- Prepare slides from different colonies for gram stain observe the gram reaction (**short bacilli**).

7- Do the biochemical tests (**IMVIC**); **TSI**, sugar fermentation and other test

8- Do the serological test for determining the serotype.

MacConkey agar

- It is designed to **selectively** isolate Gram-negative and enteric (normally found in the intestinal tract) bacteria and differentiate them based on lactose fermentation.
- it is **Selective** because it contains:
  - crystal violet (which inhibits G+ve bacteria).
  - Bile salts (which inhibits G-ve bacteria except the bile salt resistant)..
- Lactose fermenters turn red or pink on MacConkey agar, and non-fermenters do not change color.
- The media detects lactose fermentation by enteric bacteria with the pH indicator neutral red.



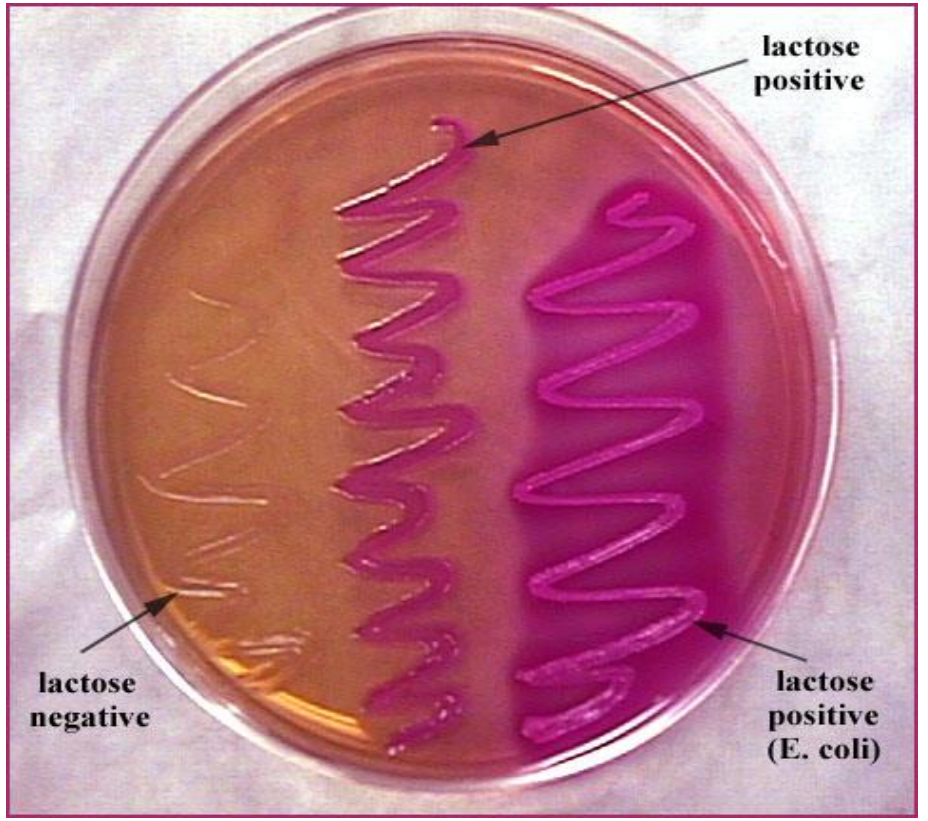
MacConkey agar

- **Lactose fermenter (KEEC):**

1. *Klebsiella*
2. *Enterobacter*
3. *Escherichia*
4. *Citrobacter*

- **Lactose non-fermenter (ShYPS):**

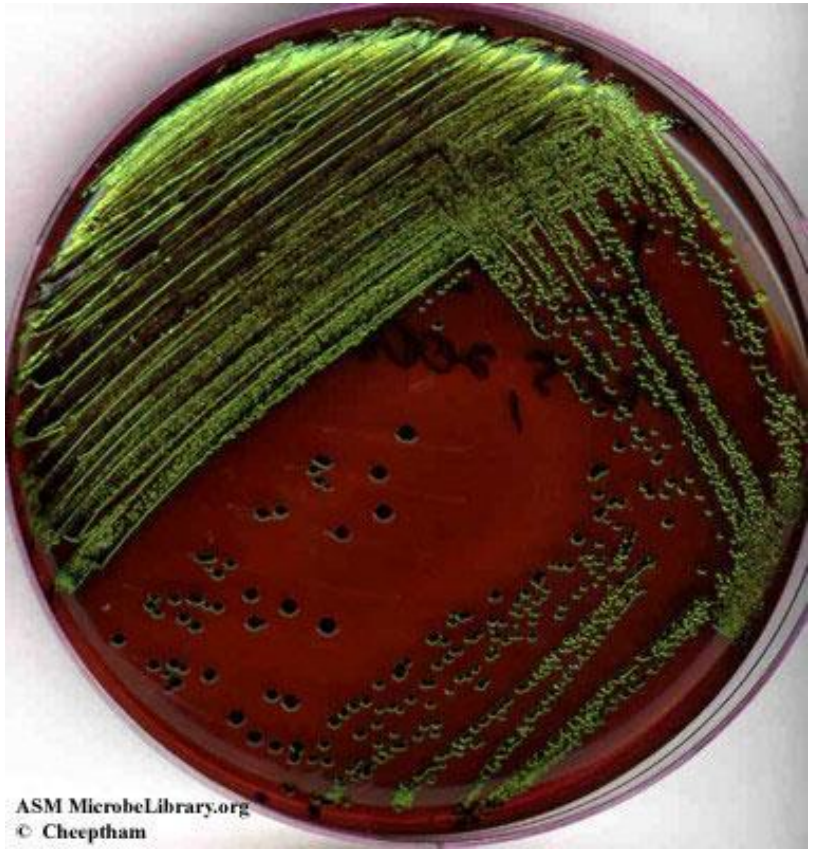
1. *Shigella*
2. *Yersinia*
3. *Proteus*
4. *Salmonella*

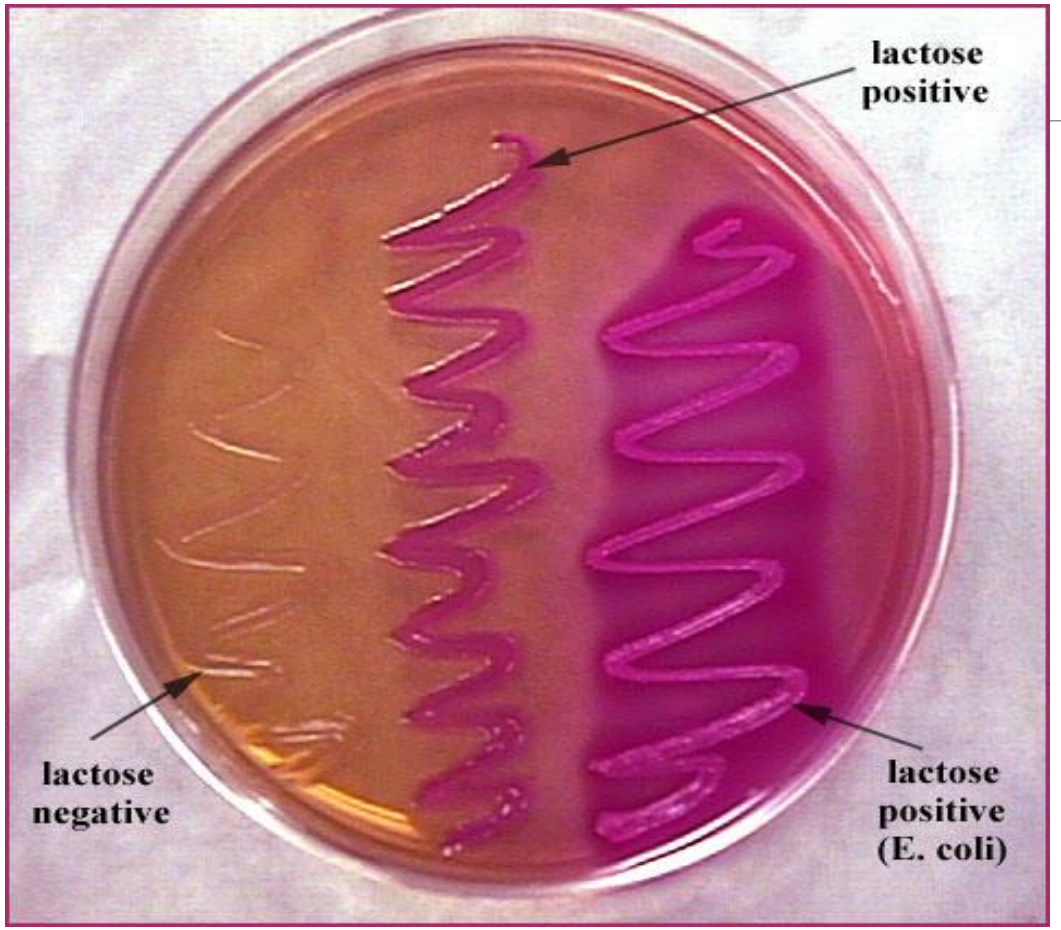


Eosin Methylene blue : EMB

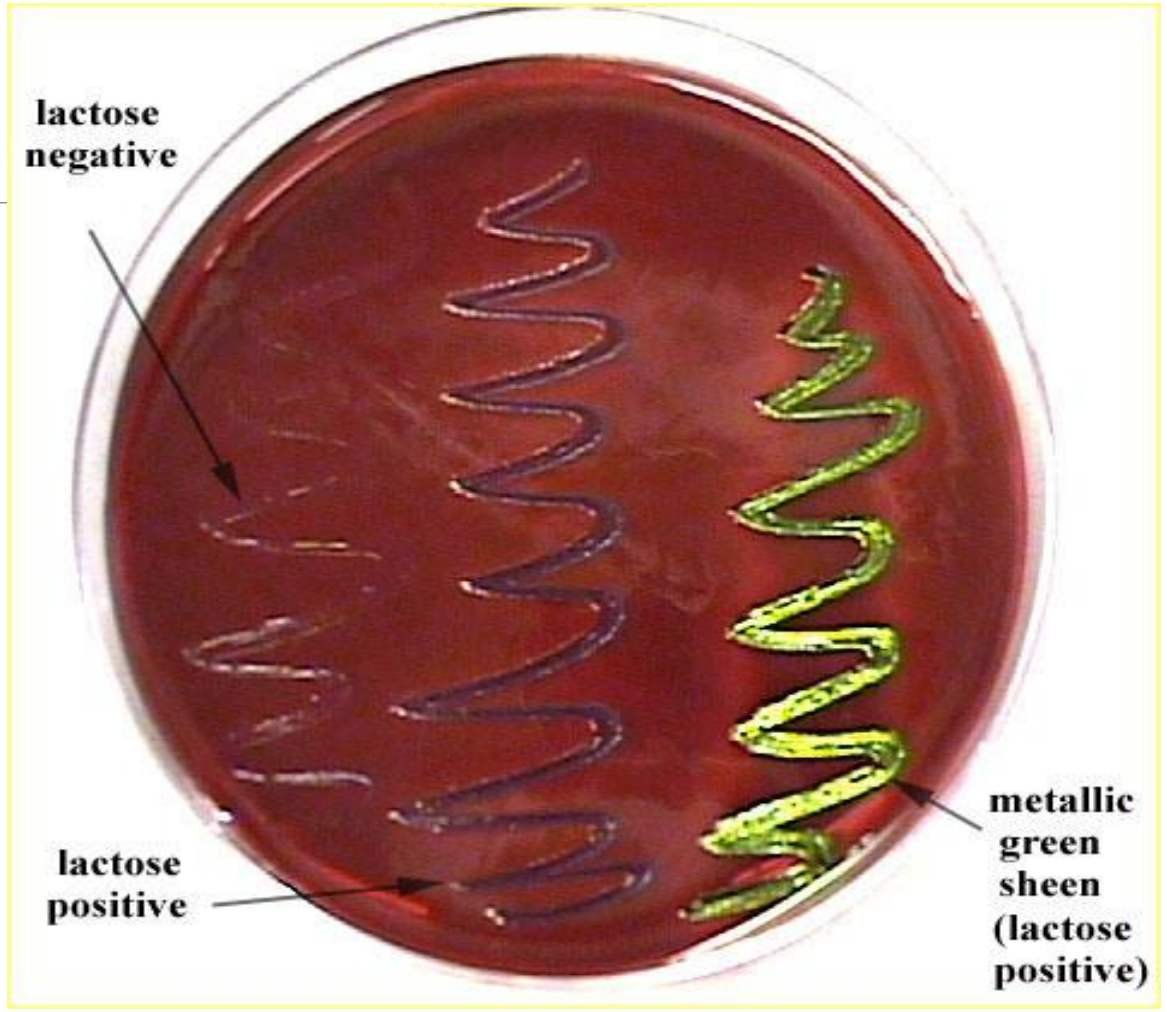
- Selective for enterobacteriaceae (contain **methylene blue** which inhibits Gram +ve pacteria)

- Differential for *E.coli* (**green** metallic sheen) due to the metachromatic properties of the dye, *E. coli* movement using flagella, and strong acid end-products of fermentation.





Lactose fermenter and non-lactose fermenter

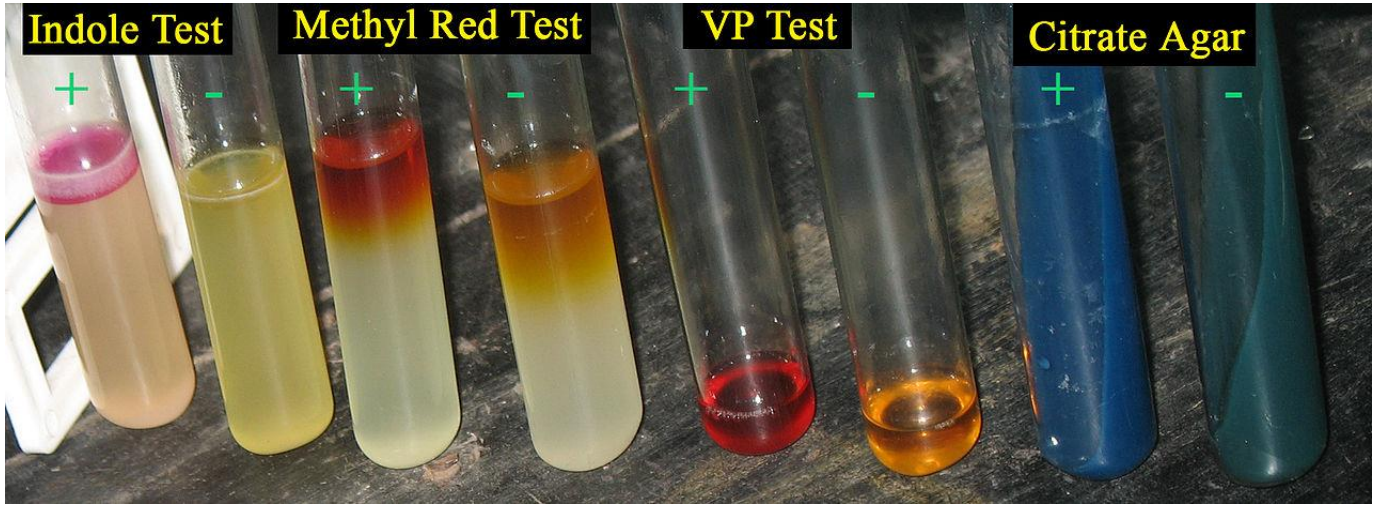


Enteric bacteria on EMB-agar

IMViC test

Consist from 4 tests

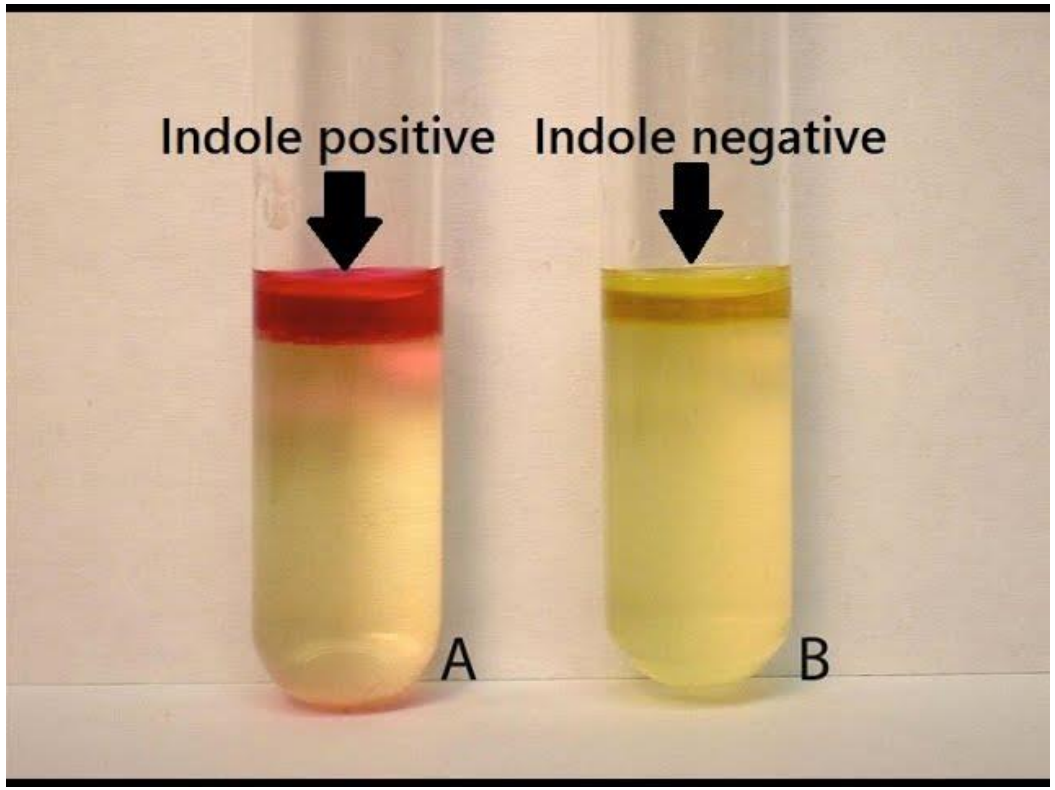
1. Indol.
2. Methyl red.
3. Vogas proskawer.
4. Citrate utilization.



These tests are useful in distinguishing members of Enterobacteriaceae.

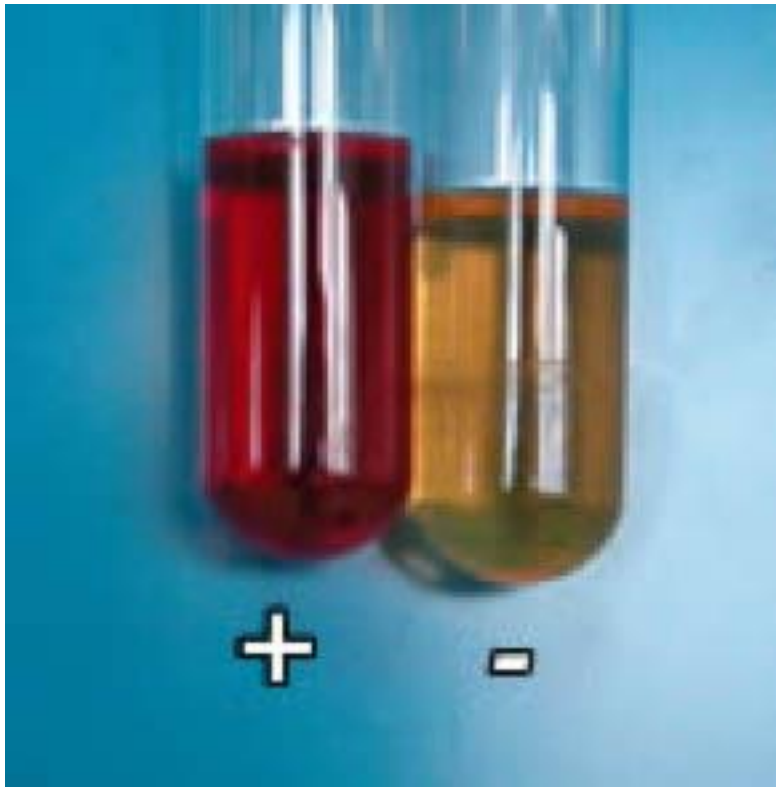
Indole test

- In this test, the organism is grown in peptone water broth. It contains tryptophan, which under the action of enzyme tryptophanase is converted to an indole molecule
- Kovac's reagent is used to determine ability of an organism to separate indole from amino acid tryptophan and it is added after incubation.
- A positive result is indicated by a pink/red layer forming on top of the liquid.
- *E.coli* is indole positive while *P. mirabilis* is indole negative



Methyl red

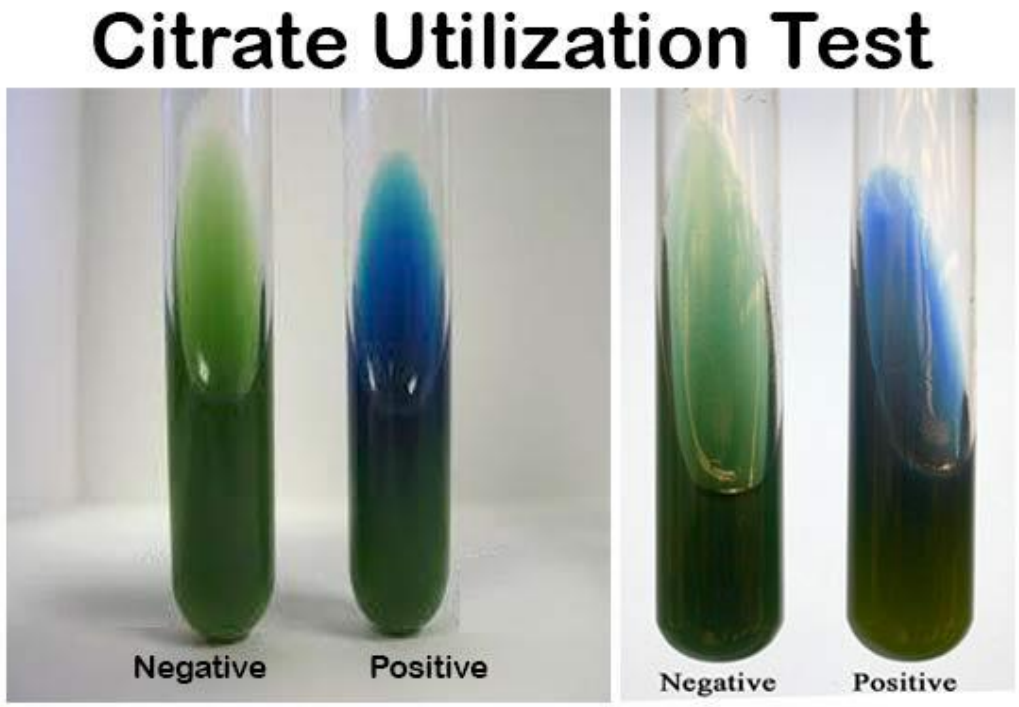
- **Methyl red** is a pH indicator; it is red in pH under 4.4, **yellow** in pH over 6.2, and **orange** in between.
- This makes it an indicator dye that turns red in acidic solutions.
- It is used to identify bacteria producing stable acids by mechanisms of mixed acid fermentation of glucose.
- *E.coli* is MR positive while *Enterobacter aerogenes* is MR negative



Voges–Proskauer test

- **Voges–Proskauer** or VP is a test used to detect acetoin in a bacterial broth culture.
- The test depends on the digestion of glucose to acetylmethylcarbinol (Glucose partial fermentation).
- . Indicator: (KOH+ ∝- naphthol) responsible for color change.
- A **cherry red color** indicates a positive result, while a **yellow-brown** color indicates a negative result.
- *Enterobacter* and *Klebsiella* are VP +ve while *E.coli* is –ve.

Citrate utilization test



- The citrate test detects the ability of an organism to use citrate as the sole source of carbon and energy.
- Bacteria are inoculated on a medium containing sodium citrate and a pH indicator such as (**bromothymol blue**).
- When Simmons Citrate agar is inoculated with *Escherichia coli* , the medium remains green (-ve result).
- With *Salmonella typhimurium* , the medium turns royal blue (positive result).

Urease test

The urease test is used to differentiate urease positive organisms (eg *Proteus*) from other urease negative organisms (*E.coli*).

urease enzyme

Urea ⟶ NH3

Medium: urea agar. Substrate: urea.

Indicator: phenol red.

+ve results: pink color.

-ve results: no change.

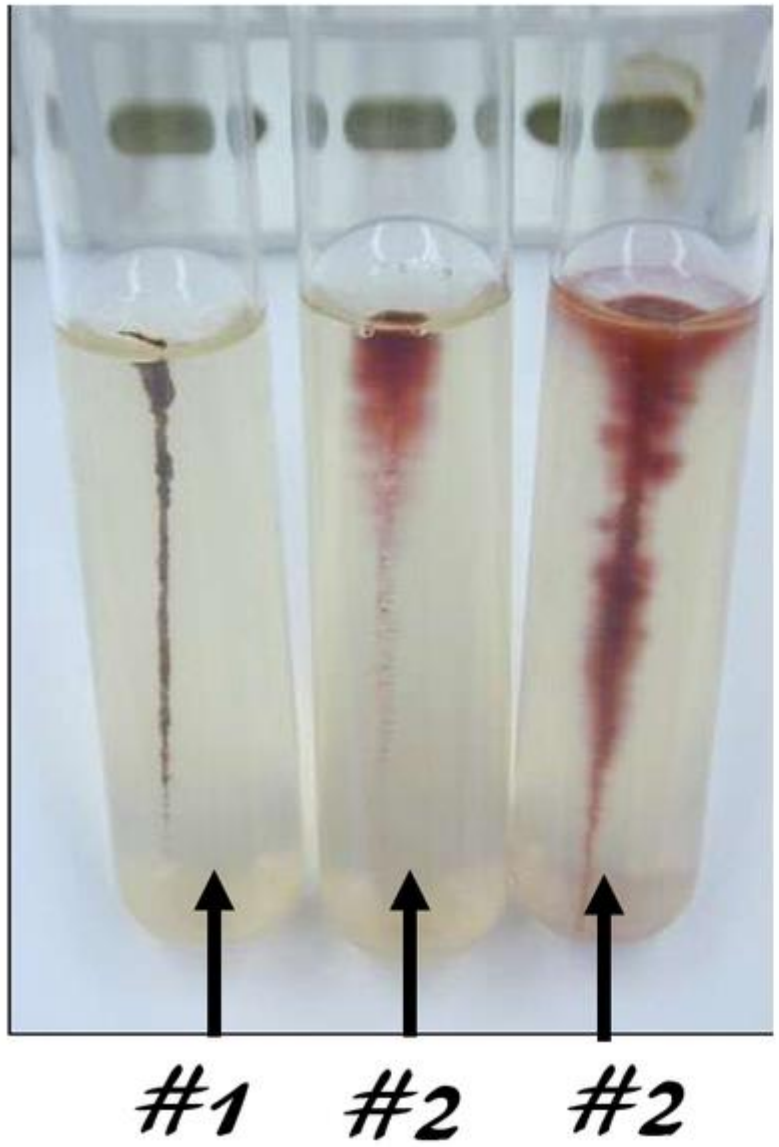
Motility test

Motility test medium is a semi solid medium used to determine the motility of microorganisms. Although there is a single function test medium. **Motility tests** are often used in the differentiation of the Enterobacteriaceae

To test for motility, use a sterile needle to pick a well-isolated colony and stab the medium to within 1 cm of the bottom of the tube. Be sure to keep the needle in the same line as it entered as it is removed from the medium. Incubate at 35°C for 18 hours or until growth is evident

Motile bacteria eg*: E. coli*

Non motile bacteria eg: *Klebsiella pneumoniae*



Motility Stab Test

- Motility is Defined as Any Growth Visible Away From The Stab Point

1. Non-Motile
2. Motile
3. Motile

THANKS FOR YOUR CONSIDERATION