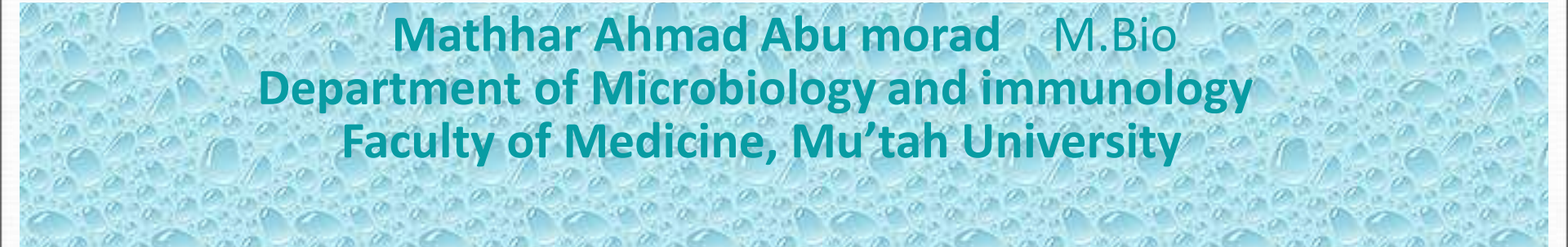




General Microbiology
Biochemical reactions
Lab 5



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Objective

To become familiar with the biochemical tests used to identify isolated gram-negative bacteria.

Enterobacteriaceae

General Characteristics

- Gram-negative bacilli
- Oxidase -ve
- Catalase +ve
- Ferment glucose with or without gas production
- facultative anaerobes
- If motile, motility by flagella

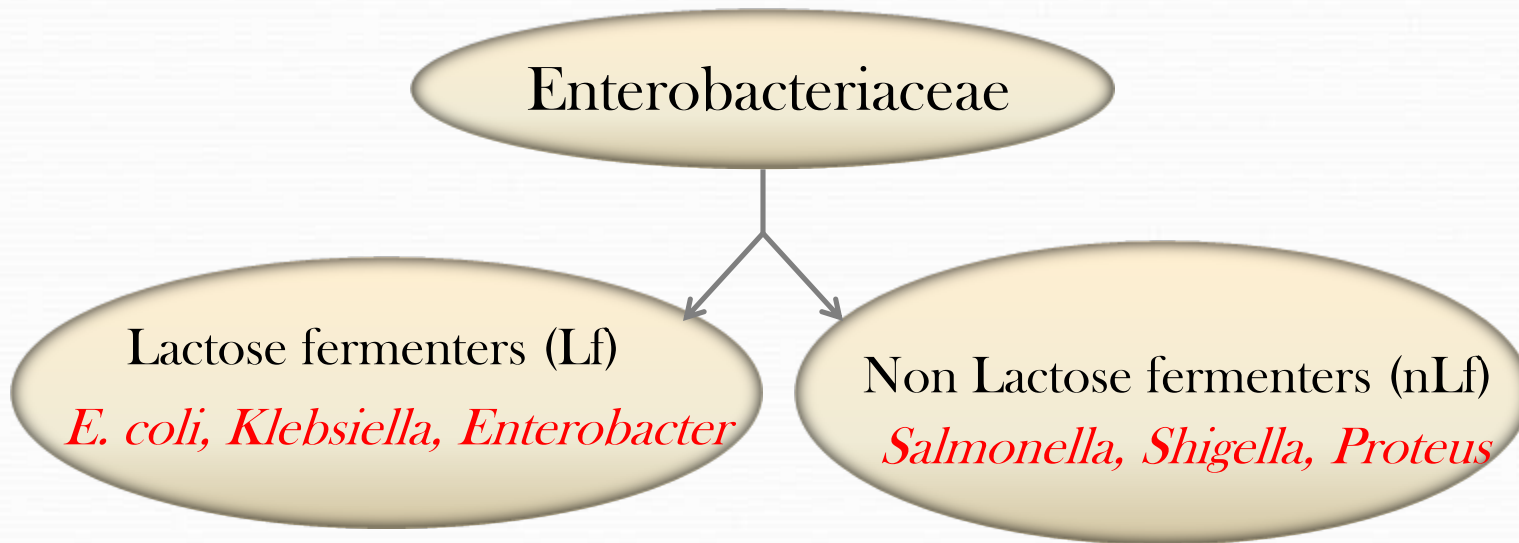
Identification of *Enterobacteriaceae*

- 1- Using selective and differential media
- 2- Using special biochemical reactions

Identification of *Enterobacteriaceae*

1- Using selective and differential media

Enterobacteriaceae divided into two main groups according to lactose fermentation

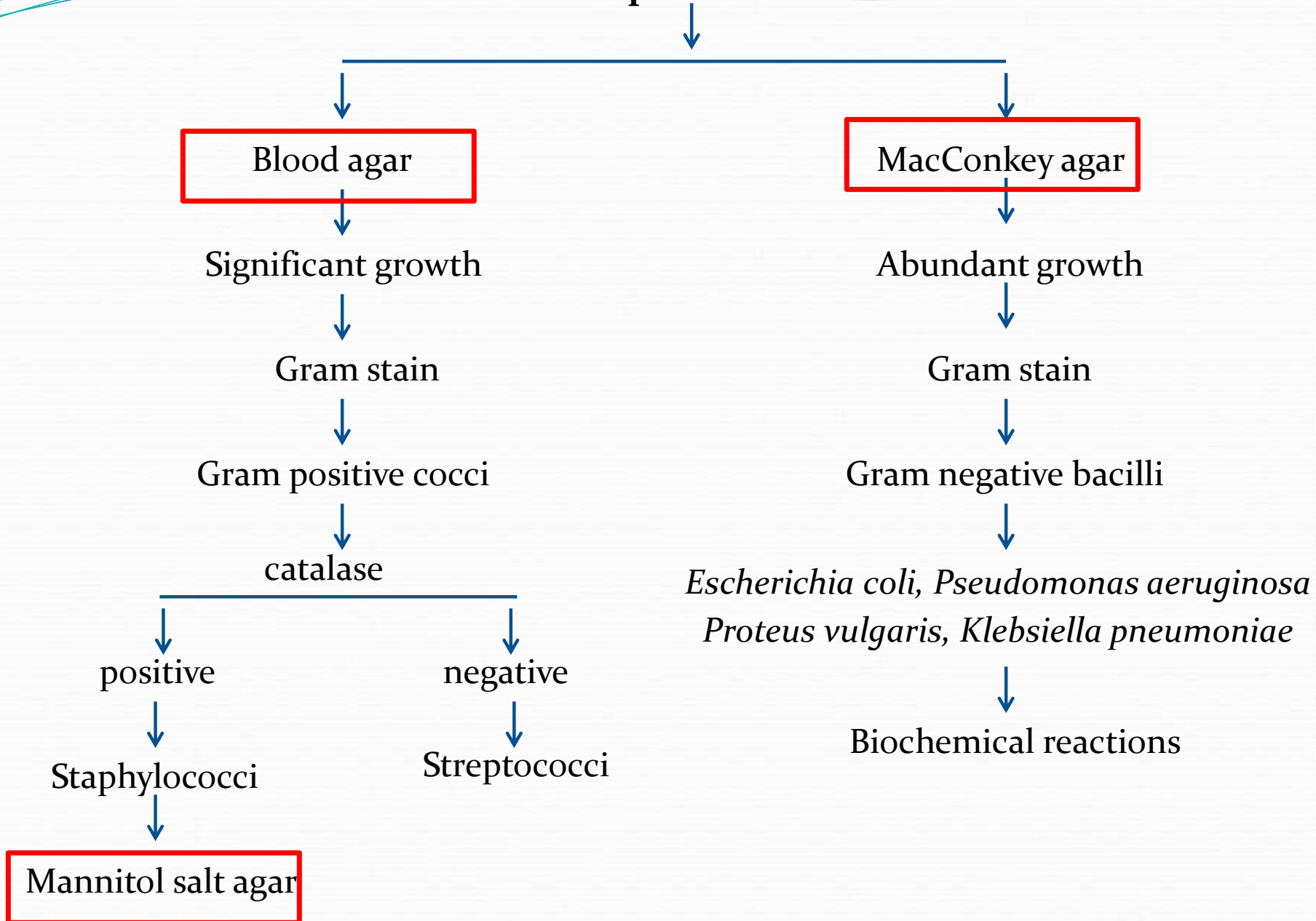


There are several selective and differential media used to isolate and distinguish between Lf & nLf including

- ✓ MacConkey agar
- ✓ *Salmonella Shigella* agar (SS agar)

Urine analysis

Midstream urine sample
Sample inoculation



Identification of *Enterobacteriaceae*

2- Using special biochemical reactions

The differentiation of the principle groups of *Enterobacteriaceae* can be accomplished on the basis of their biochemical prosperities and enzymatic reactions in the presence of the specific substrate

One important group of biochemical reactions is:

IMViC

I: Indole

M: Methyl red

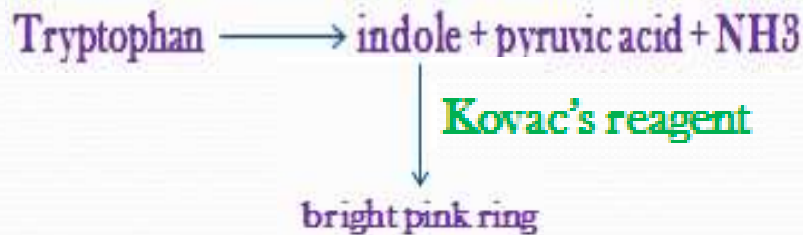
V: Vogus Proskauer

C: Citrate utilization tests

IMViC: Indole test

Principle

- ✓ Some microorganisms can metabolize tryptophan by **tryptophanase**
- ✓ The enzymatic degradation leads to the formation pyruvic acid, indole, and ammonia
- ✓ The presence of indole is detected by addition of Kovac's reagent

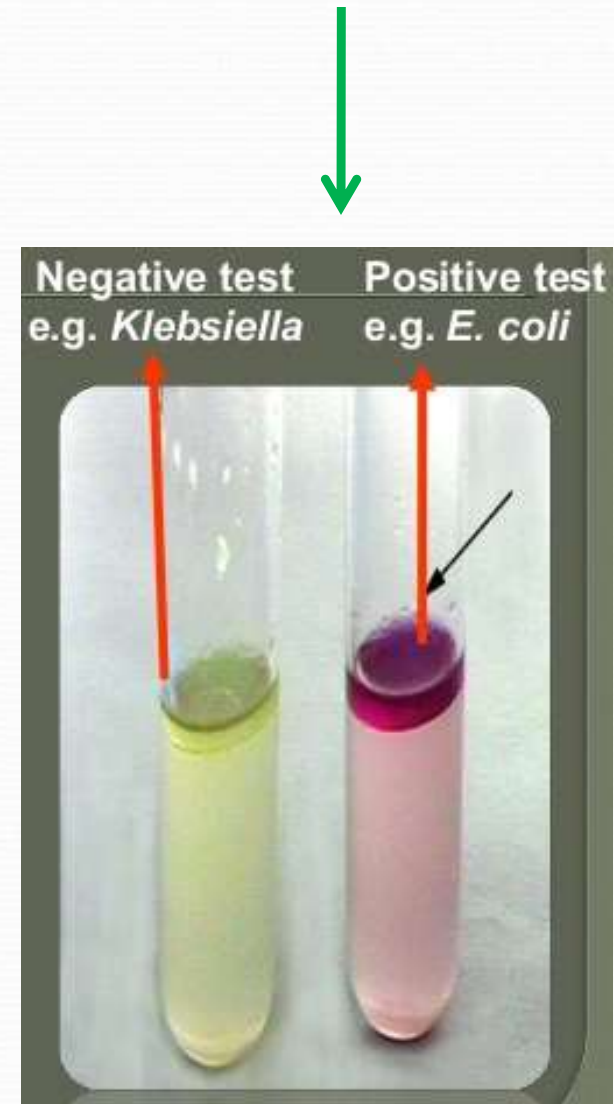


Media

tryptophan or peptone broth

Results

A bright pink color on the top layer indicated the presence of indole



IMViC: Methyl Red, Voges Prosakaur

Principle

- ✓ **Methyl Red test:** to determine the ability of bacteria to oxidize glucose with the production and stabilization of high acidic end products.

Ex: Lactic acid, formic acid

- ✓ **Voges Prosakaur:** to determine the ability of bacteria to produce non-acidic or neutral end products

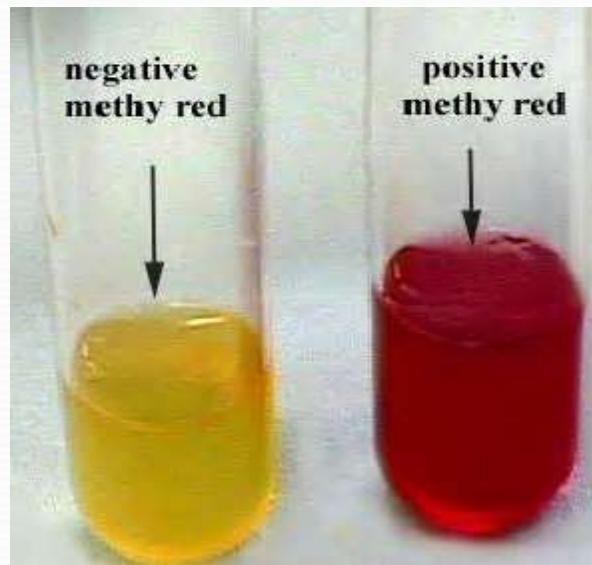
Ex: acetylmethyl carbinol

Procedure

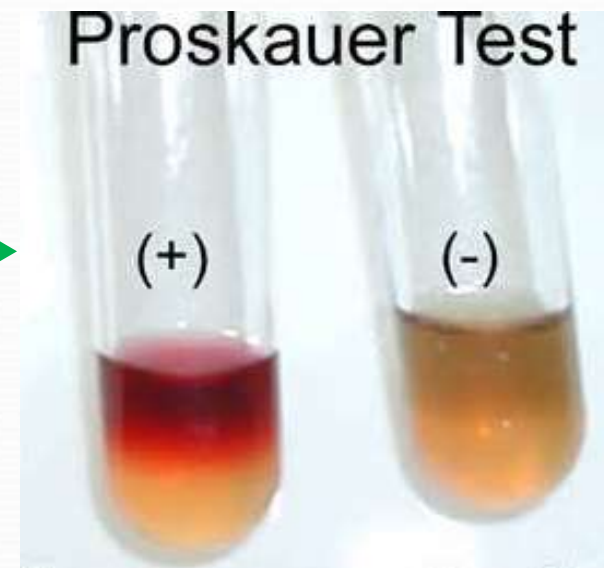
- ✓ Inoculate the tested organism into ONE tube of **MR-VP broth**
- ✓ After incubation: pour 1/3 of the broth into a clean tube
- ✓ Run the tests as following
 1. For methyl red: run in the tube containing the 2/3 by adding 6-8 drops of **methyl red reagent**

IMViC: Methyl Red, Voges Prosakaur

2. For **Voges Prosakaur**: in the tube containing the 1/3
- add 12 drops of **Barritt's reagent A (α -naphthol)**, Mix
 - add 4 drops of **Barritt's B reagent (40% KOH)**, Mix
 - Let undisturbed for at least 1 hour
- **Methy red** is red in pH under 4.4, yellow in pH over 6.2



- ✓ Red: Positive MR (*E. coli*)
- ✓ Yellow or orange: Negative MR (*Klebsiella*)



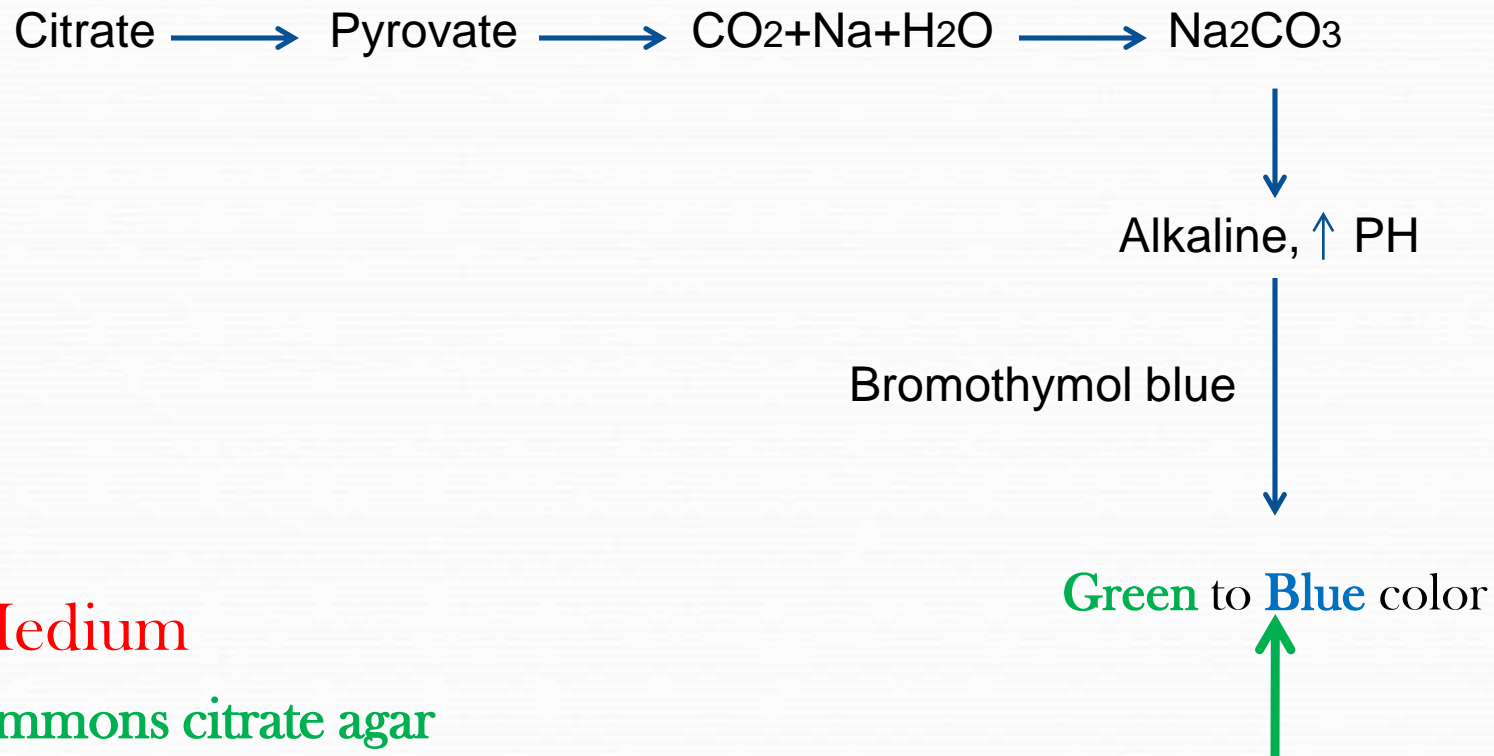
- ✓ Pink: Positive VP (*Klebsiella*)
- ✓ No pink: Negative VP (*E. coli*)

IMViC: Citrate utilization test

Purpose

To determine the organisms that are able to ferment citrate as a sole carbon source

Principle



Medium

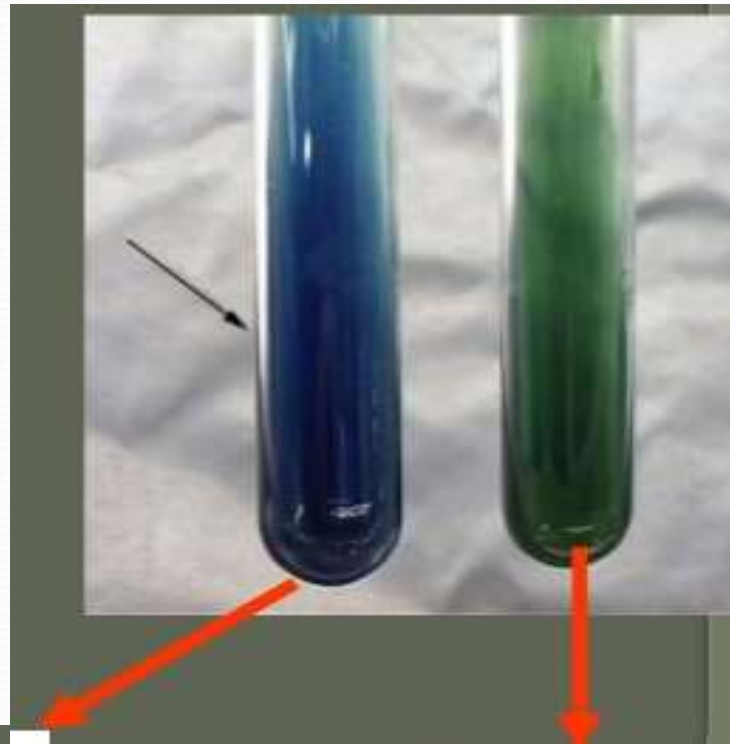
Simmons citrate agar

IMViC: Citrate utilization test

Results

Positive results: blue color (*Klebsiella*)

Negative results: green color (*E. coli*)



Positive
Klebsiella, *Enterobacter*

Negative
E. coli

Urease test

Purpose

To isolate organisms that are urease positive

Principle



Medium

Urea agar

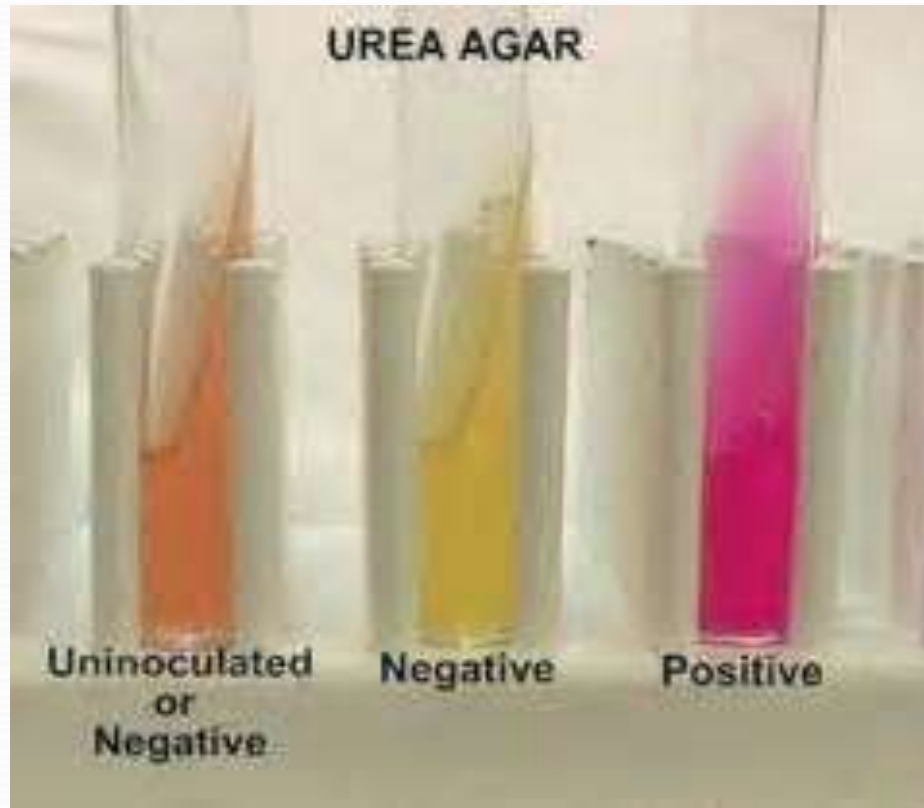
Results

Negative = yellow color e.g., *E. coli*

Positive = pink color e.g., *klebsiella aregenes*



Urease test



Sugar fermentation test

Purpose

Carbohydrate fermentation tests detect the ability of microorganisms to ferment a specific carbohydrate.

Media

Sugar media

Sugars used

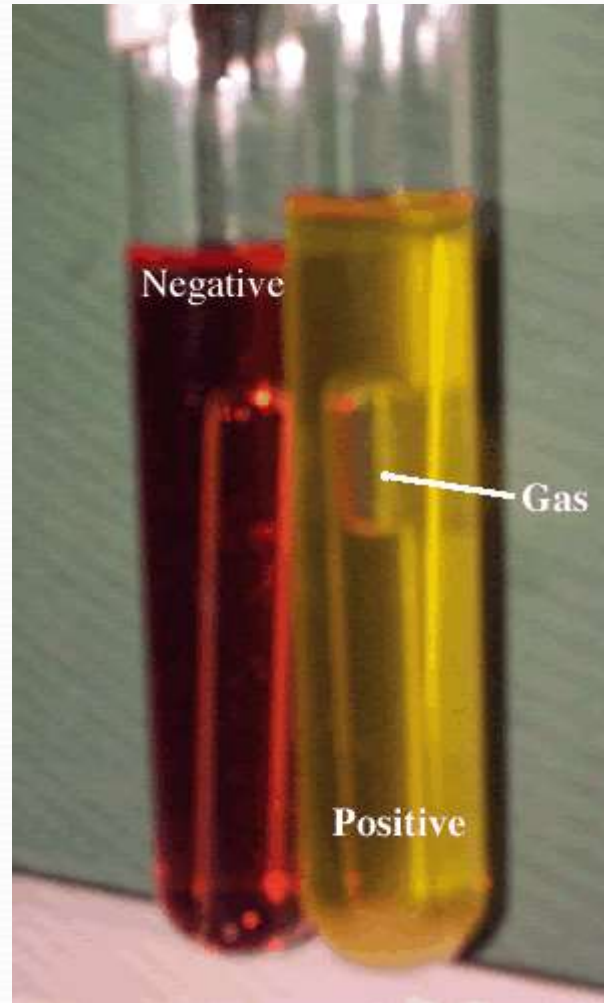
- Glucose - Lactose - Maltose - Mannitol - Sucrose

Results

pH indicator: Phenol-Red is red at pH > 7

If fermentation occurs, the acidic by-products will change the color from red to yellow.

Sugar fermentation test



	<i>S. Dysenteriae</i>	<i>S. flexneri</i>	<i>S. sonni</i>	<i>Klebsiela</i>	<i>E. coli</i>	<i>V. cholera</i>
Glucose	A, No G	A, No G	A, No G	A, G	A, G	A, No G
Lactose	-ve	-ve	A, No G	A, G	A, G	A, No G
Maltose	-ve	-ve	-ve	A, G	A, G	A, No G
Mannitol	-ve	A, No G	A, No G	A, G	A, G	A, No G
Sucrose	-ve	-ve	-ve	A, G	A, G	A, No G
indole	-ve	-ve	-ve	-ve	+ve	+ve
MR	+ve	+ve	+ve	-ve	+ve	
VP	-ve	-ve	-ve	+ve	-ve	
Citrate	-ve	-ve	-ve	+ve	-ve	
Urease	-ve	-ve	-ve	+ve	-ve	
H ₂ S	-ve	-ve	-ve	-ve	-ve	

Key

A: acid

No G: No gas

-ve: negative

+ve: positive

Analytical Profile Index System (API)



Analytical Profile Index System (API)

Negative Tests



Positive Tests



Analytical Profile Index System (API) for bacterial identification

• **API (Analytical Profile Index) 20E** is a biochemical panel for identification and differentiation of members of the family Enterobacteriaceae.

