

General Microbiology  
Biochemical reactions  
Lab 5

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## Objective

To become familiar with the biochemical tests used to isolate 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

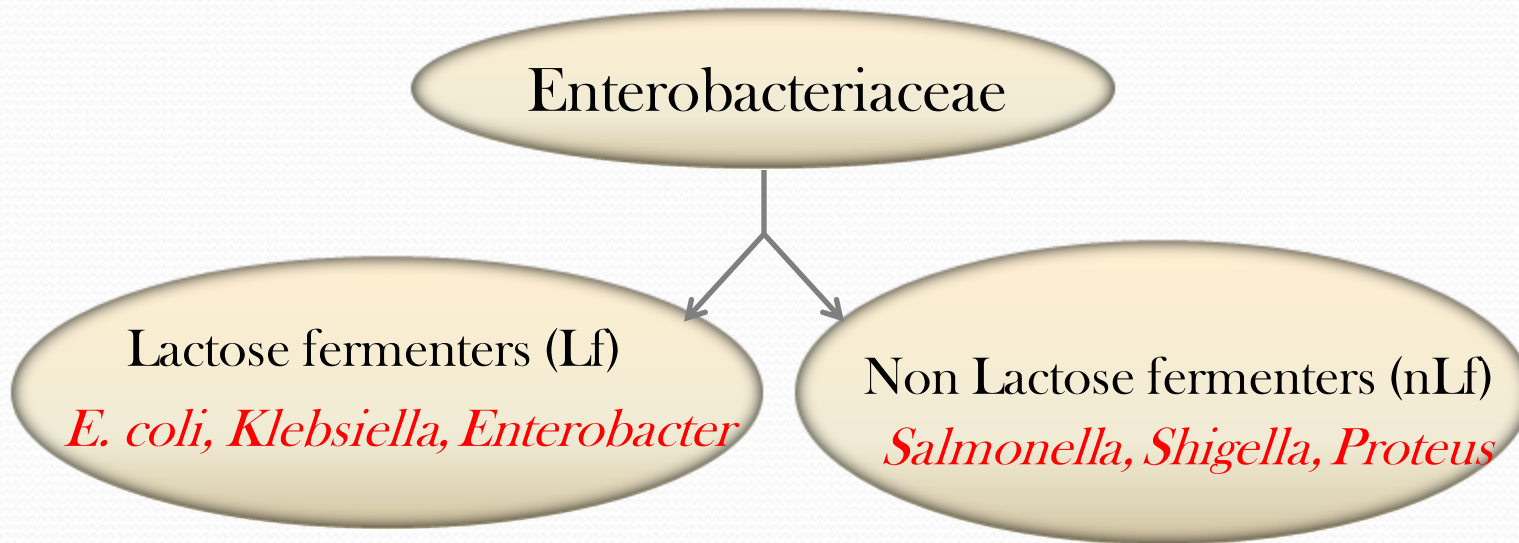
# Identification of *Enterobacteriaceae*

- 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

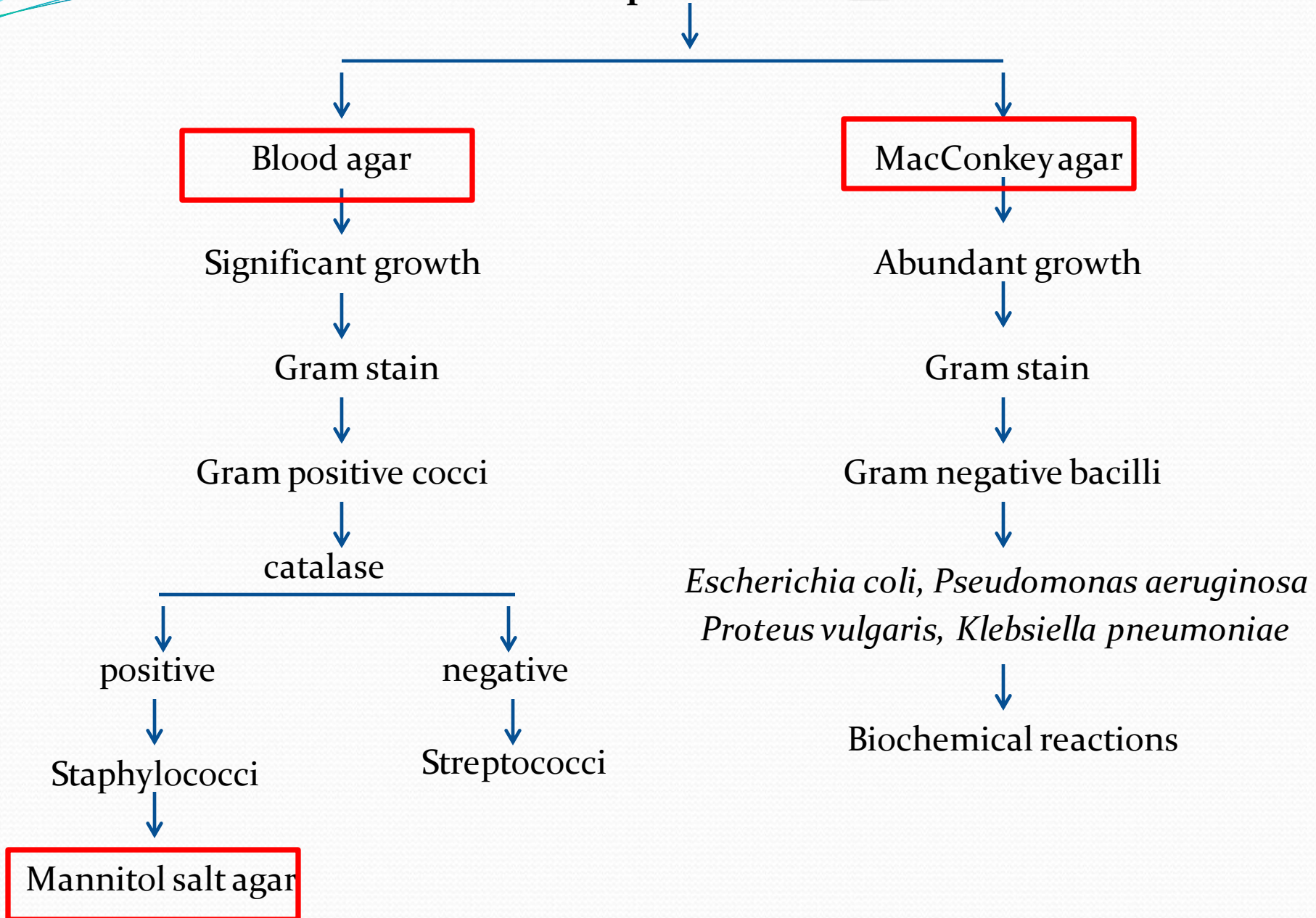


There are several selective and differential media used to isolate and distinguish between Lf & nLf including

- ✓ 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 properties 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: **M**ethyl Red, **V**oges 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

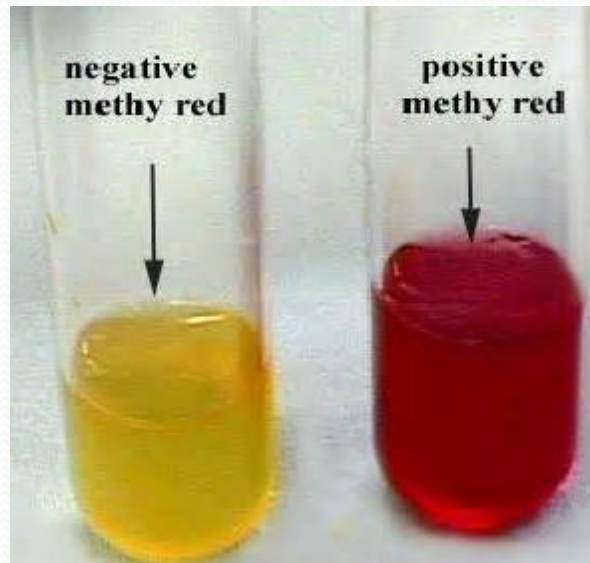
Ex: acetylmethyl carbinol

## Procedure

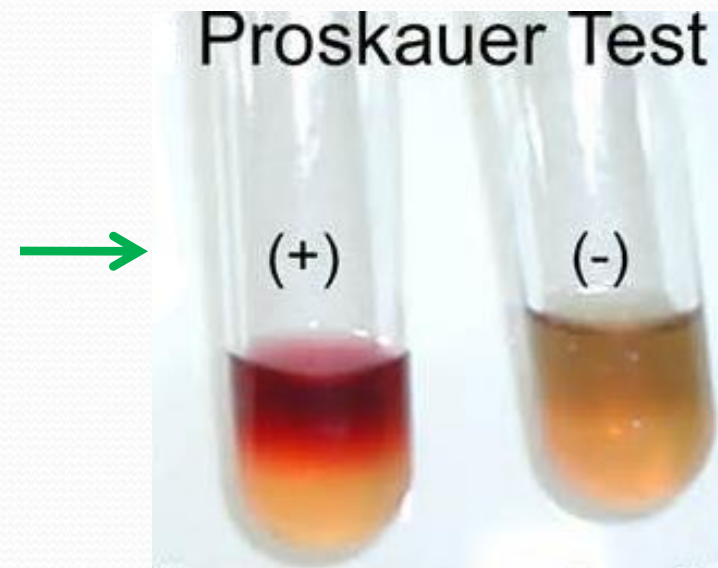
- ✓ Inoculate the tested organism into ONE tube of **MR-VP broth**
- ✓ After incubation: pour 1/3 of the broth into a clean tube
- ✓ Run the tests as following
  1. For methyl red: run in the tube containing the 2/3 by adding 6-8 drops of **methyl red reagent**

# IMViC: Methyl Red, Voges Prosakaur

2. For **Voges Prosakaur**: in the tube containing the 1/3
- add 12 drops of **Barritt's reagent A ( $\alpha$ -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*)



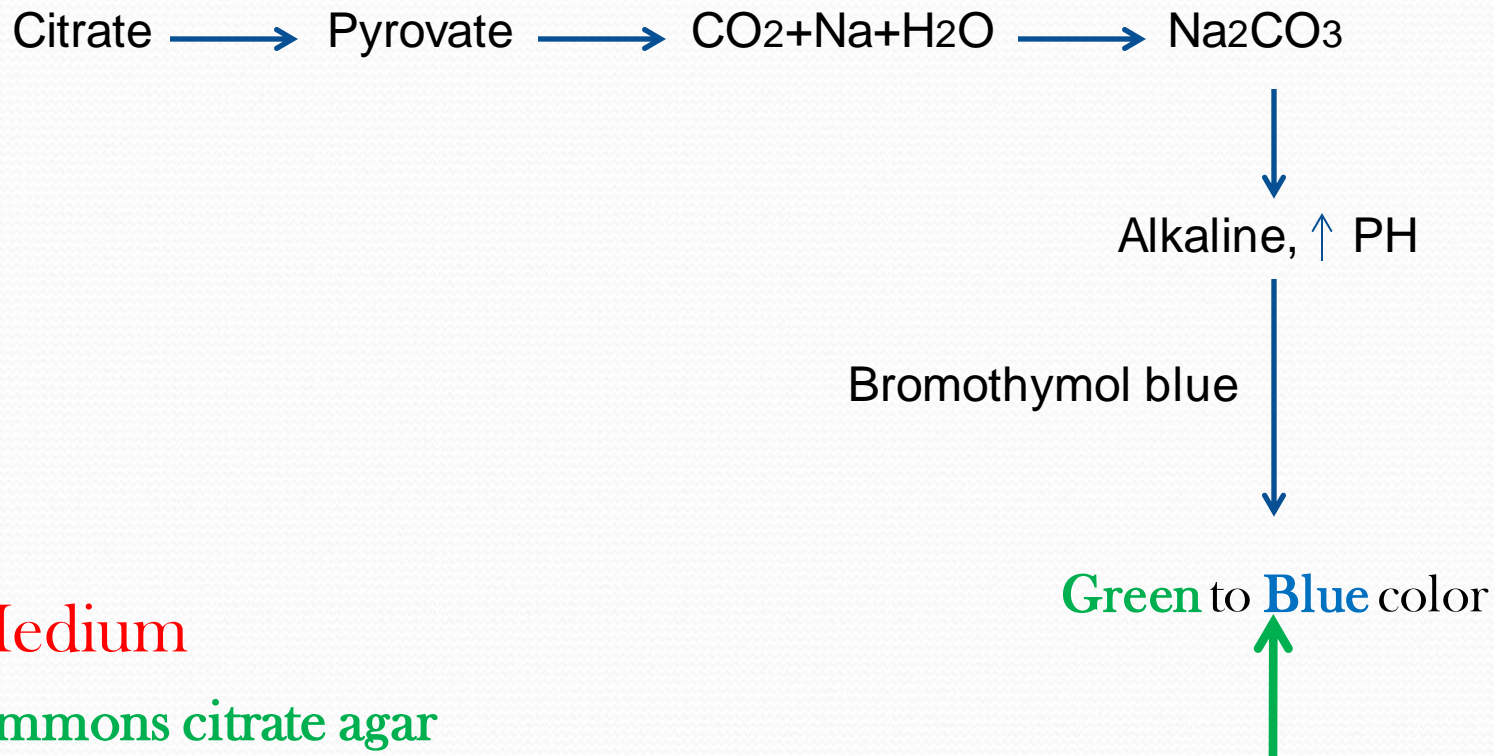
- ✓ Pink: Positive VP (*Klebsiella*)
- ✓ No pink: Negative VP (*E. coli*)

# IMViC: Citrate utilization test

## Purpose

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

## Principle



## Medium

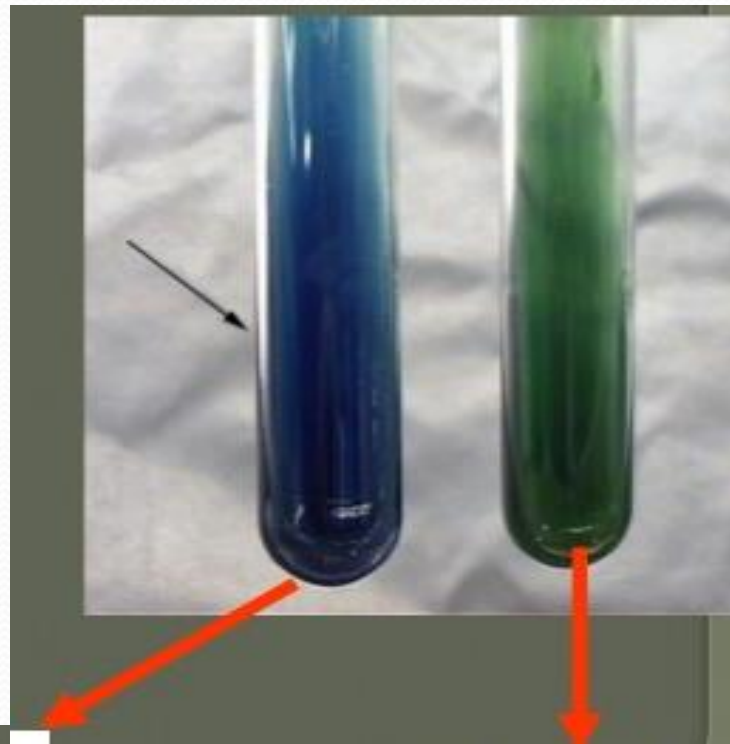
Simmons citrate agar

# 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



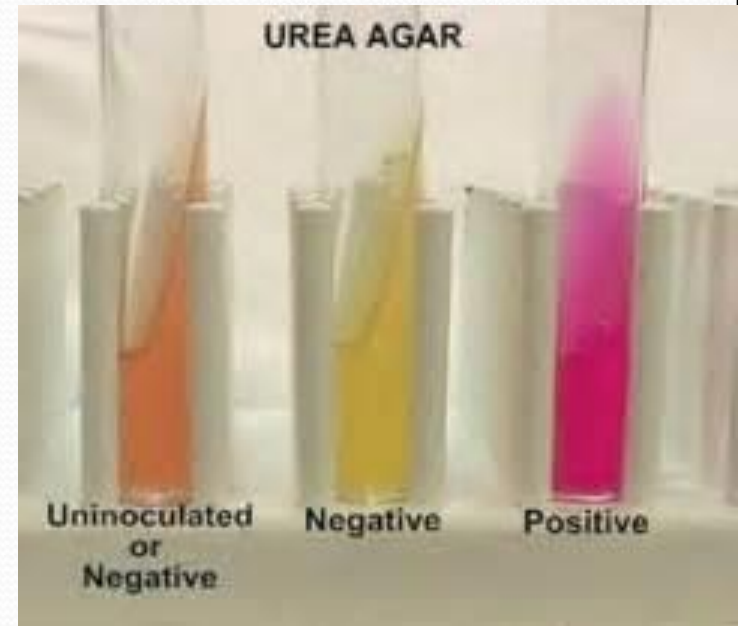
## Medium

Urea agar

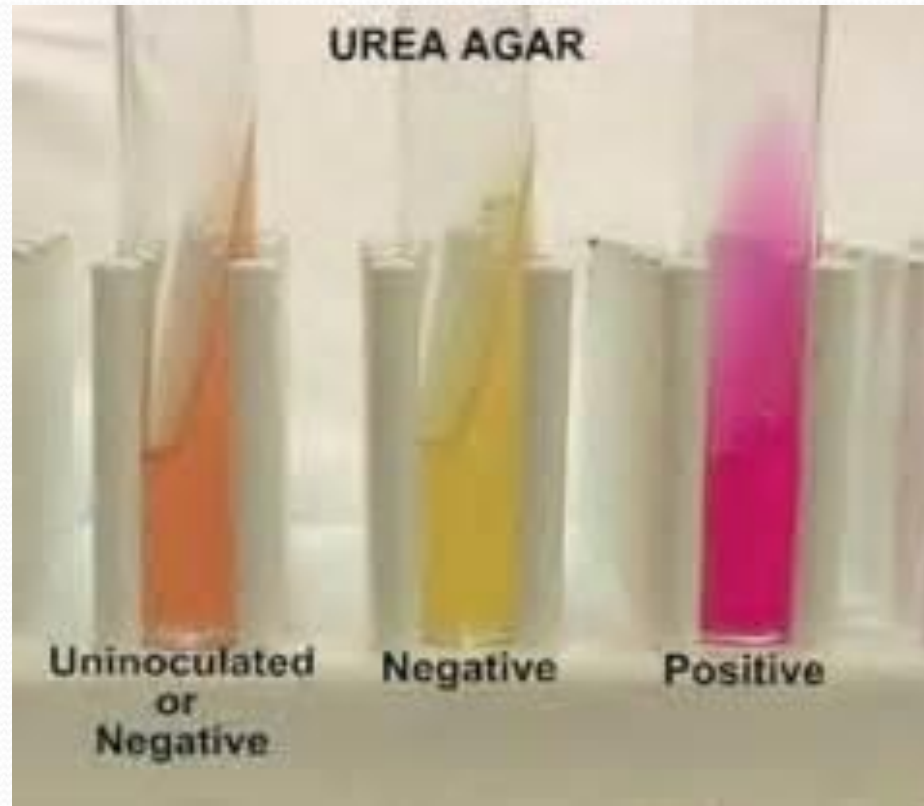
## Results

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**

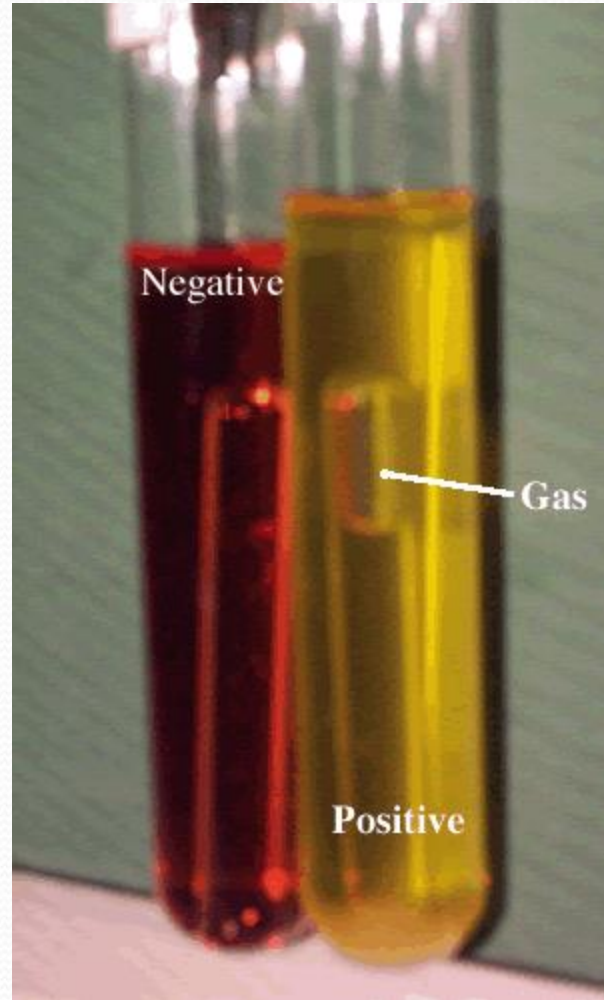
- Glucose - Lactose - Maltose - Mannitol - Sucrose

## **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 <sub>2</sub> S	-ve	-ve	-ve	-ve	-ve	

### Key

**A: acid**

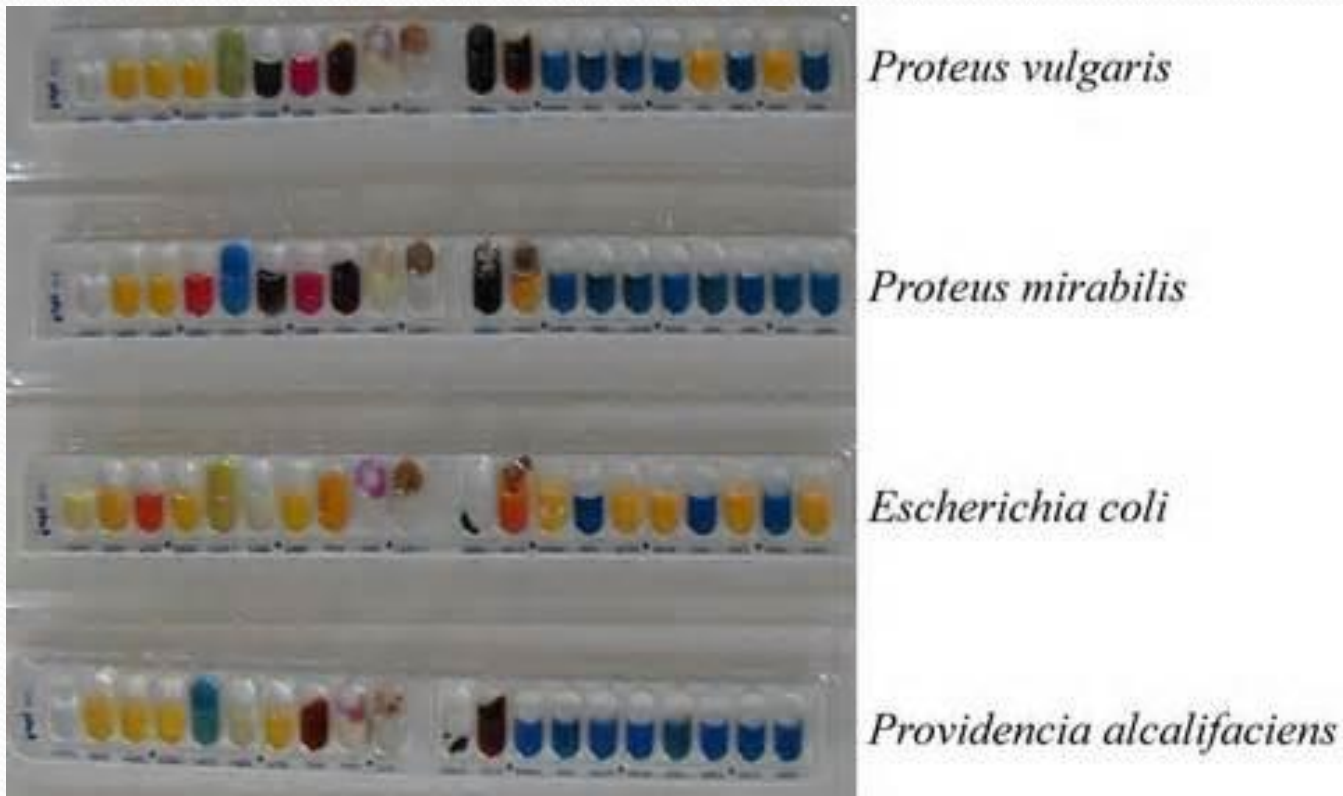
**No G: No gas**

**-ve: negative**

**+ve: positive**

# 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.



# Bacterial Barcodes



*E. Coli*



*Salmonella*