

Microbiology

Lab 5

Biochemical reactions





General Microbiology
Biochemical reactions
Lab 5
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Objective

To become familiar with the biochemical tests used to isolate gram negative bacteria

* If bacteria growth in culture media dose not mean that I can determine its name . Ex: Enterobacteriaceae (Large Family of bacteria)

تنوع على ← Macconkey agar (selection for gram negative bacilli) ← لكن لم يتم تحديد اسم البكتيريا

* To determine the name of the bacteria → Biochemical reaction
بواسطة

Enterobacteriaceae

Identification of *Enterobacteriaceae*

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

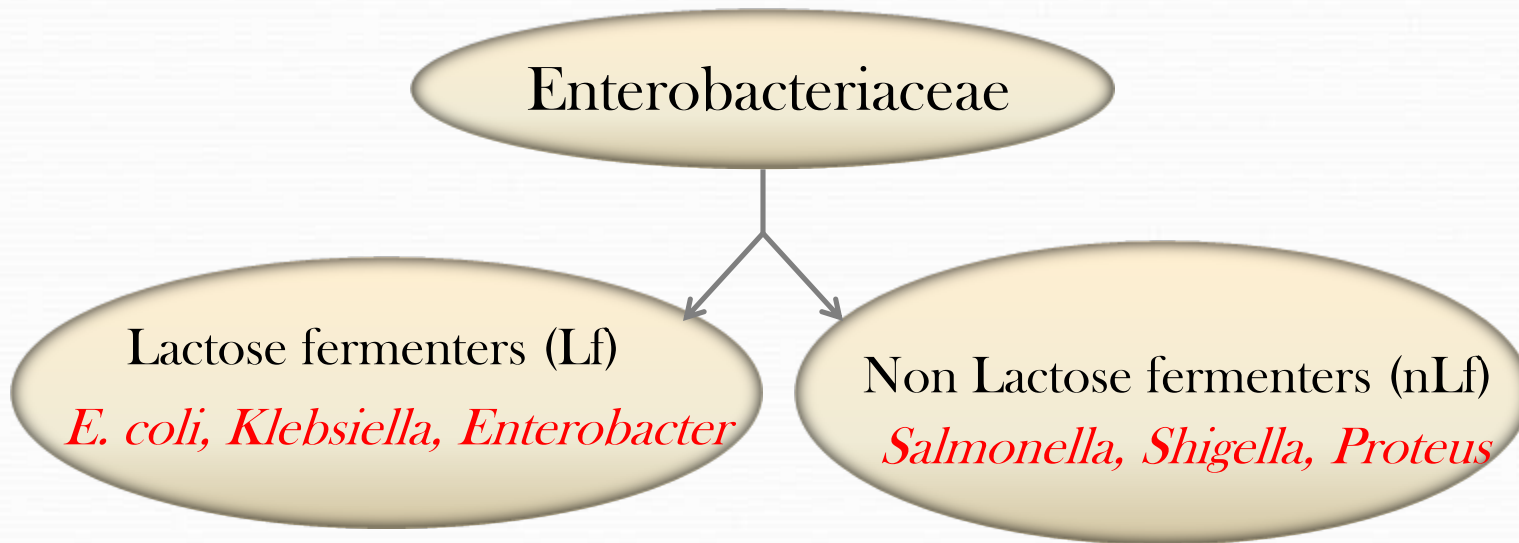
General Characteristics

- 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

Enterobacteriaceae divided into two main groups according to lactose fermentation

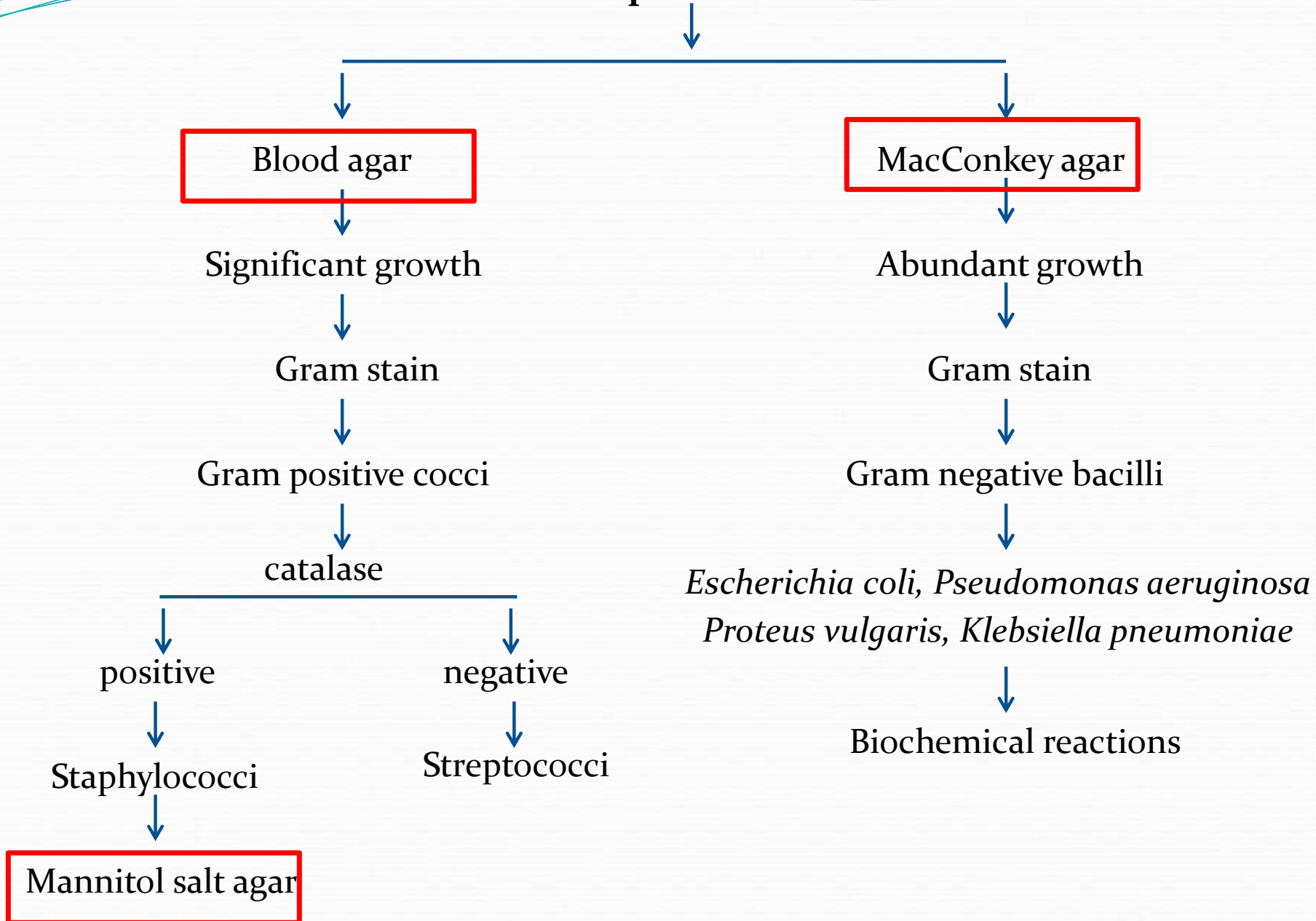


There are several selective and differential media used to isolate and distinguish between Lf & nLf including

- ✓ 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: Methylene blue

V: Voges-Proskauer

C: Citrate utilization tests

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

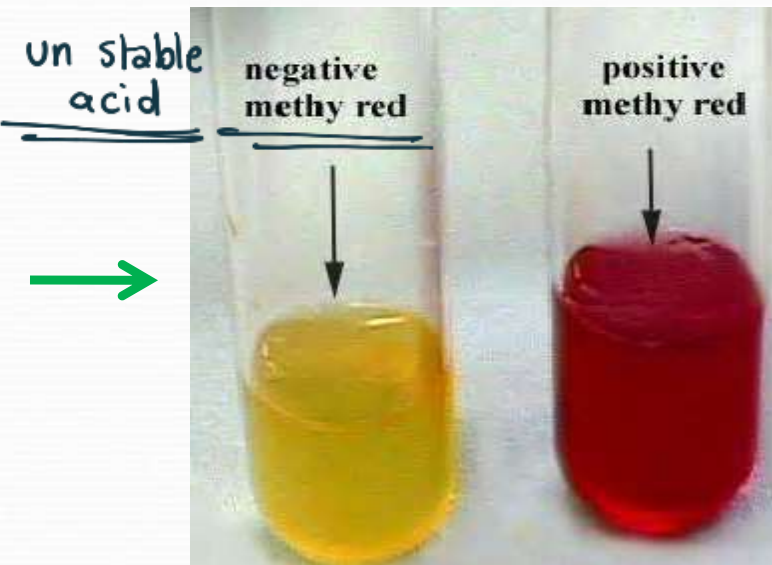
Ex: acetylmethyl carbinol

Procedure

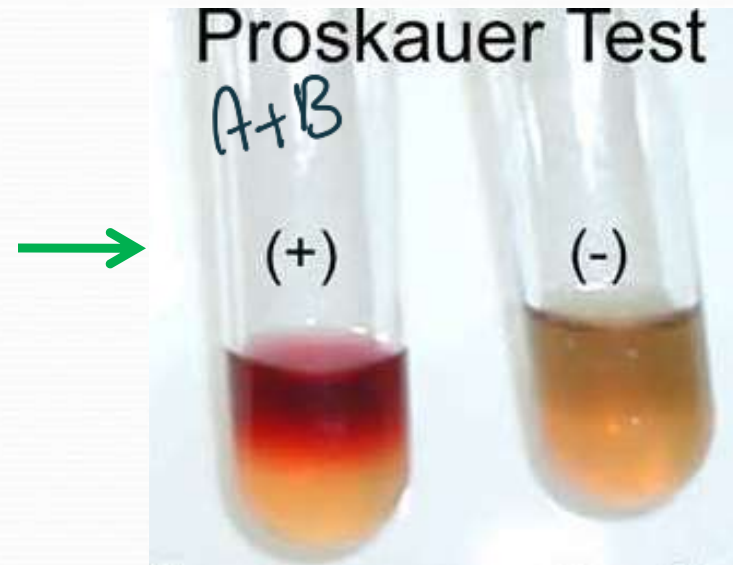
- ✓ Inoculate the tested organism into ONE tube of **MR-VP broth**
- ✓ After incubation: pour 1/3 of the broth into a clean tube
- ✓ Run the tests as following
 1. For methyl red: run in the tube containing the 2/3 by adding 6-8 drops of **methyl red reagent**

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*)



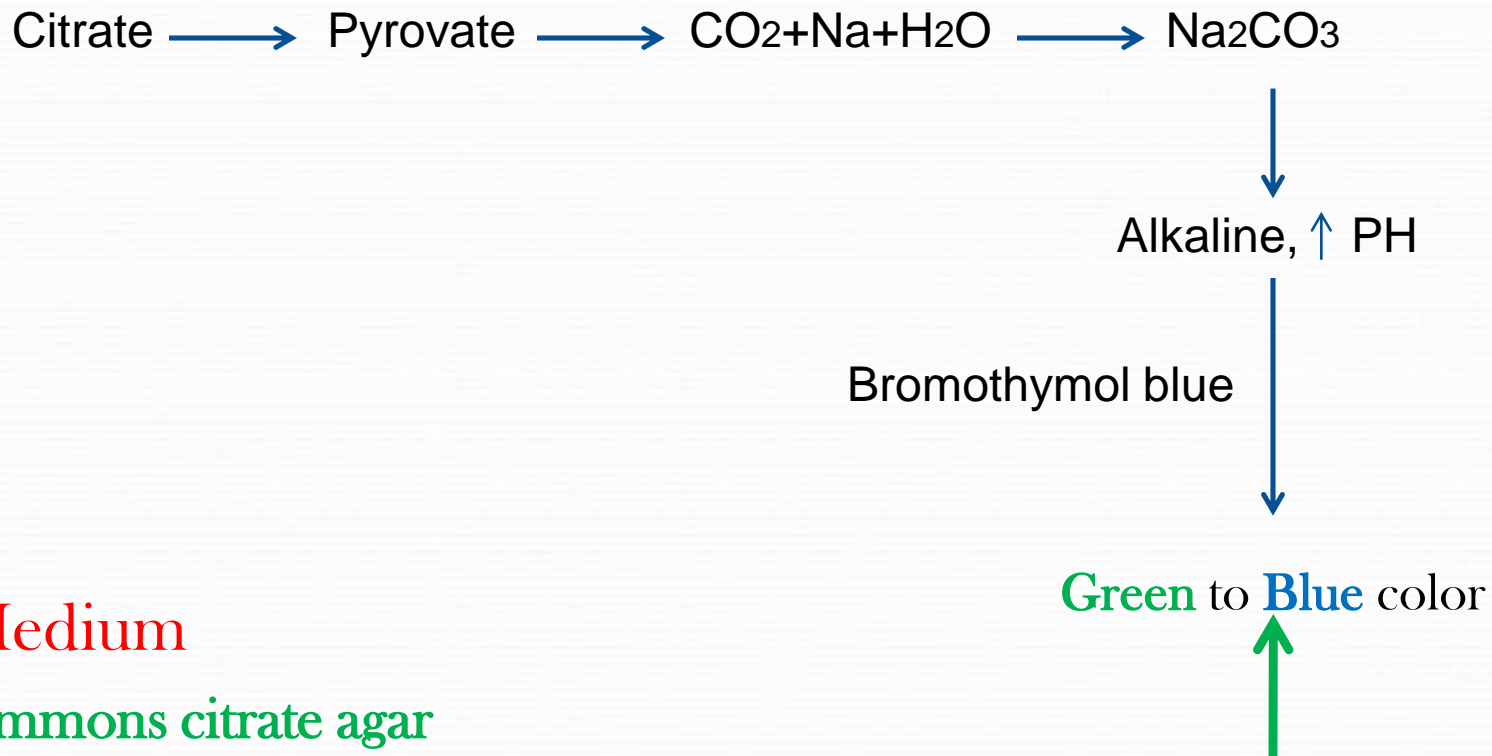
- ✓ Pink: Positive VP (*Klebsiella*)
- ✓ No pink: Negative VP (*E. coli*)

IMViC: Citrate utilization test

Purpose

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

Principle



Medium

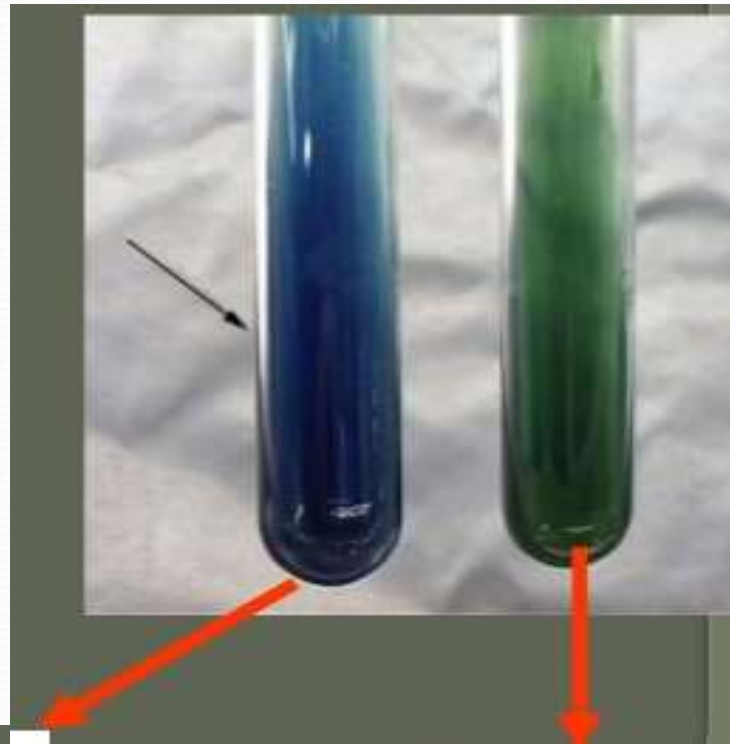
Simmons citrate agar

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



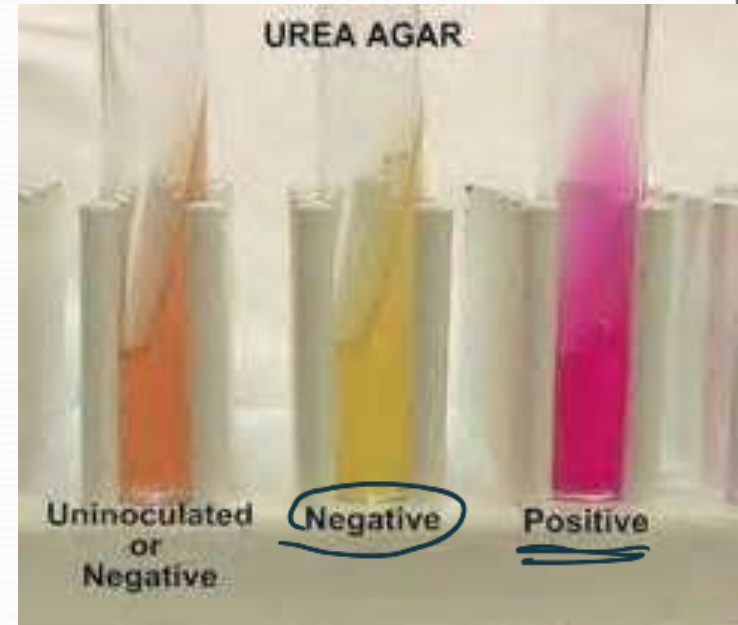
Medium

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

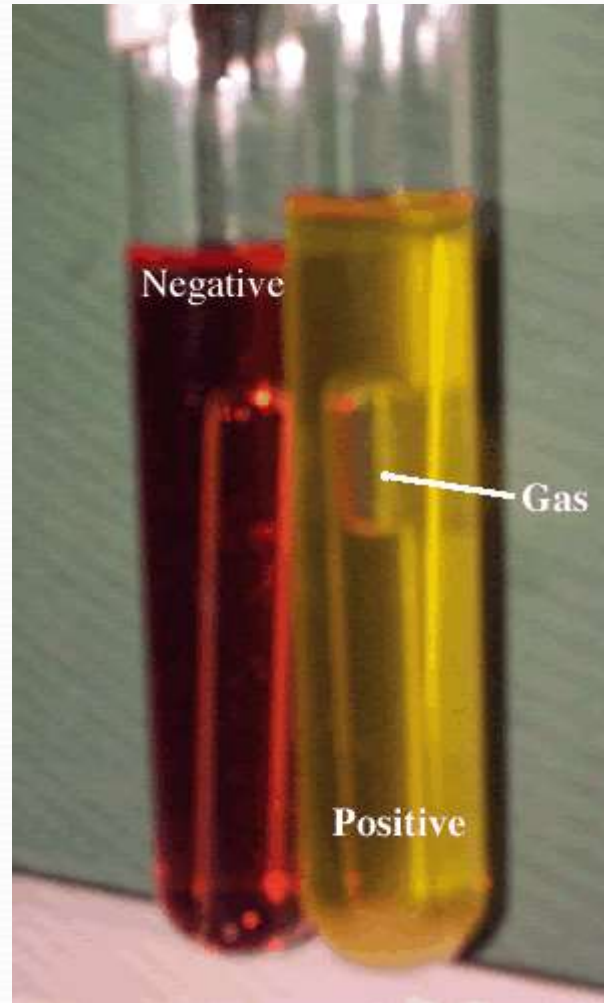
- Glucose - Lactose - Maltose - Mannitol - Sucrose

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



Bacterial Barcodes



E. Coli



Salmonella

X

	<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	
H2S	-ve	-ve	-ve	-ve	-ve	

Key

A: acid

No G: No gas

-ve: negative

+ve: positive

X

Analytical Profile Index System (API) for bacterial identification

another test
used color



Proteus vulgaris

Proteus mirabilis

Escherichia coli

Providencia alcalifaciens

* Biochemical Reaction.

** Enterobacteriaceae

- To identify

selective and differential media

special biochemical reactions.

General characteristics:

- Gram negative bacilli
- Oxidase -ve
- Catalase +ve
- Ferment glucose with or without gas product
- Facultative anaerobes
- Motile, motility like shigella
- Flagella.

* To isolate the Enterobacteriaceae we can use:

- ① Macconkey agar
- ② Salmonella shigella agar (SS agar)

Slid 6-

* IMViC → a set of examinations to identify enterobacteriaceae

① Indole test:

Macconkey ← ② البكتيريا التي لا تستطيع

Metabolize tryptophan

For the Tryptophane media

Media → Tryptophan ①

or peptone broth

↳ Liquid media

1. Indole

2. pyruvic acid

3. NH₃

↳ 3 product:

Kovac's reagent Indole ← إنتاج انديول *
↓ تظهر

bright pink ring → positive Indole

EX: E-coli.

② Methyl Red, Voges Proskauer

to oxidize glucose ← تأكسد الجلوكوز *

with production and stabilization of high acidic endproducts.

* All bacteria have certain types of Acid endproduct after ~~Fermentation~~ Fermentation.

Acid end product ← إنتاج حمض *
stabilisation → acid ← تثبيت الحمض

stable in Growth like

Lactic acid

Formic acid

Other bacteria is unstable and it will convert *
to other product

↓ تحويل
Voges proskauer.

* positive methyl red → when add → Red color

MR-VP

borth

↓ إنتاج حمض
Stable acid end product

3 Citrate utilization test

من كل انواع البكتريا باستثناء
Citrate as a carbon source.

* Medium → Simmons citrate agar.

** Citrate → pyruvate → $CO_2 + Na + H_2O$ → Na_2CO_3 → Alkaline.

↑ pH يرتفع عن قيم في هذه الخطوة

Bromothymol blue

utilization ان البكتريا على Citrate ل
• citrate ل Blue ← Green in Media
Ex → klebsiella, Enterobacter

** Sugar Fermentation test :

The aim of this test → to see this bacteria, what type of sugar can use For Fermentation.

* Media → sugar media

— The type of sugar that can we use :

Lactose, Glucose, Maltose, Mannitol, sucrose.

* The Results → pH indicator → phenol-Red is red at pH > 7
if we have Fermentation → change the Form
Red to yellow.

* الخطوات :-

- 1. كل tube ينوع فيه نوع واحد من السكر
- 2. one colony of macconki بنوعين عليهم
- 3. كأي يوم بثوب أي tube تغير لونه

* ايضاً يعني تغير اللون في tube :-

This bacteria do Fermentation

to the suger and convert
the ~~ph~~ phenol From
Red to yellow.

4. بكتب حسب النتيجة ← Fermenters Lactose
or Non - Fermenters Lactose.