

# 320 MBIO

## Microbial Diagnosis

### Lab 3

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# Primary Media for Isolation of Microorganisms



- As we know, many clinical specimens contain a mixed flora of microorganisms.
- Thus when the specimen was cultured it will take a great deal of subsequent time to subculture and sort through the isolated bacterial species.



- Instead, the microbiologist uses several types of primary media to culture the specimen initially.
- Culture media provide optimal conditions for growth and multiplication of bacteria.





According to the physical state media may be :

Fluid Media

Solid Media

Growth is visualized as

Turbidity

Colonies



- In general, the primary media has three basic purposes, accomplished simultaneously :

1

To culture all bacterial species present and see which if any predominate.

2

To differentiate species by certain characteristic responses to ingredients of the culture medium

3

To selectively encourage growth of those species of interest while suppressing the normal flora.

# Types of culture media

1. Simple (general) media

2. Enriched media

3. Selective media

4. Differential media

5. Selective and differential media

6. Transport media



# 1. Simple Media


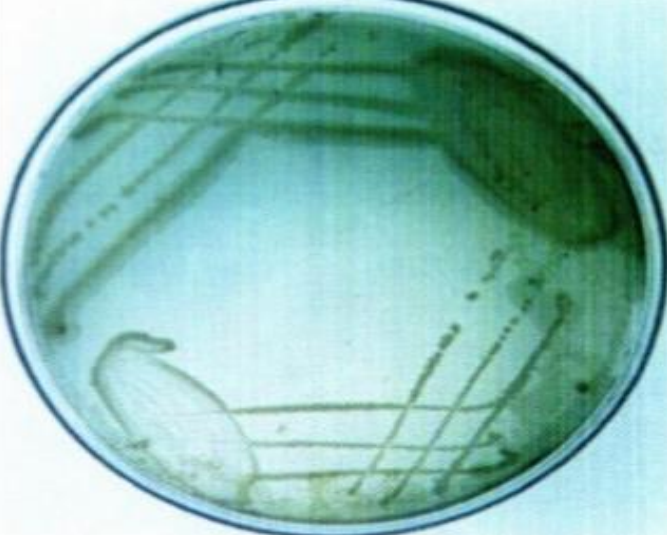

- It used to support the total flora of a clinical specimen, such as peptone water, nutrient agar.
- It can support most non-fastidious bacteria.
- Simple media can be a broth or solid by adding agar-agar to the nutrient broth.



Nutrient Broth  
+  
2% agar-agar



# Some bacteria growth on Simple media (Nutrient agar)

Sample	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus sp.</i>
Description of growth	Golden yellow endopigment	Greenish exopigment	Swarming growth
The growth			

## 2. Enriched Media

- Addition of extra nutrient in the form blood, serum, egg yolk etc. to a simple medium makes them enriched media.
- For example, chocolate agar, blood agar.
- The blood source usually from animal (sheep or rabbits, sometimes horses), but human blood may also be used.



Nutrient agar  
+  
5-10% Blood

## ■ First : Blood Agar

- Blood agar is an enriched, bacterial growth medium. Fastidious organisms, such as streptococci, do not grow well on ordinary growth media.
- Blood contains inhibitors for certain bacteria such as *Neisseria* and *Haemophilus* genera.



- Certain bacteria produce enzymes (hemolysins) that act on the red cells to produce either:

1

**Beta hemolysis:** Enzymes lyse the blood cells completely, producing a clear area around the colony.

2

**Alpha hemolysis:** Incomplete hemolysis produces a greenish discoloration around the colony

3

**Gamma hemolysis:** No effect on the red cells.

## Blood Agar as a differential media

Type of  
hemolysis

Beta

Alpha

Gamma

Sample

*Staphylococcus aureus*

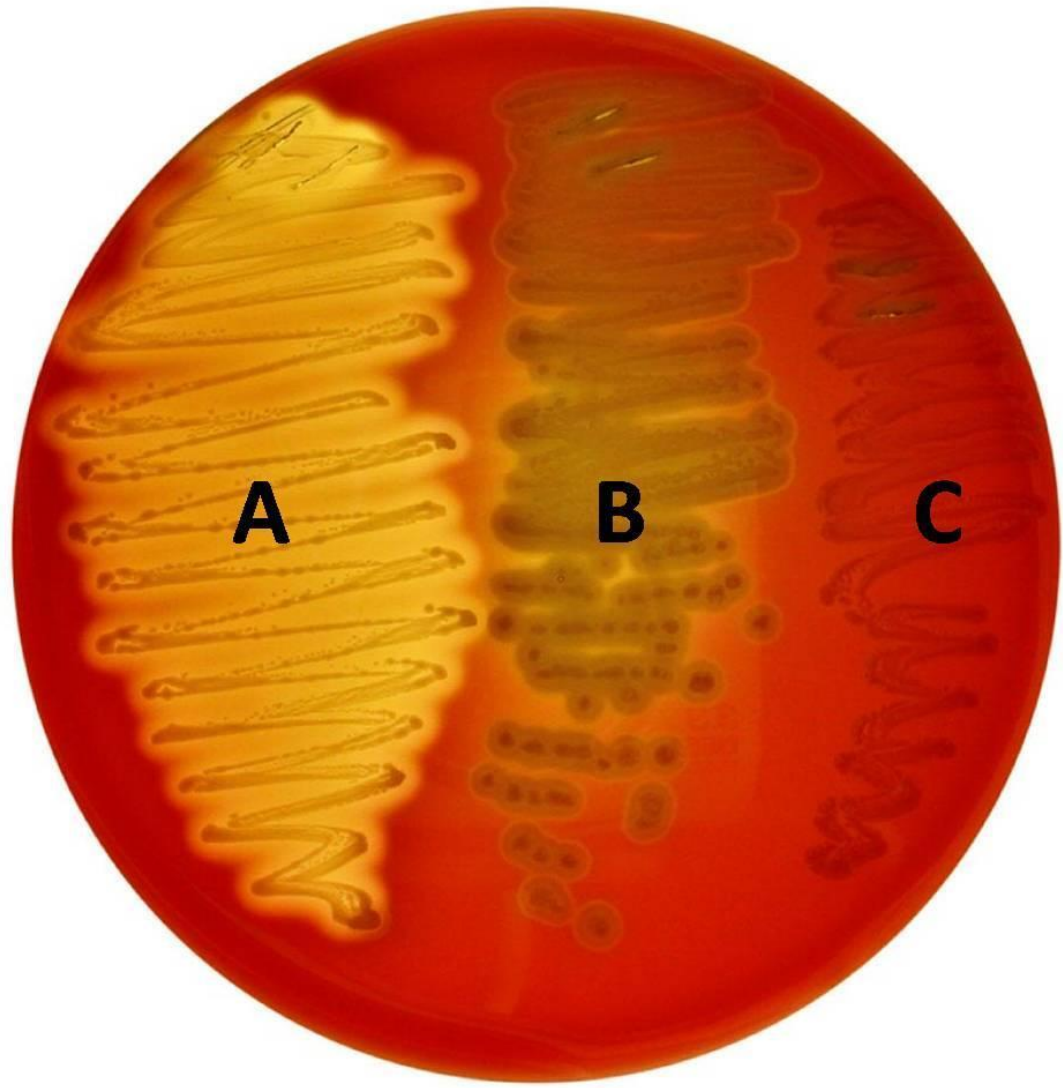
*E. coli*

*Staphylococcus  
epidermidis*

The growth







## ■ Second : Chocolate Agar

- Chocolate agar is a non-selective, enriched growth medium.
- It is a variant of the blood agar plate.
- It contains red blood cells, which have been lysed by heating very slowly to 56 °C.

Heated Blood agar

Haemoglobin



Heat

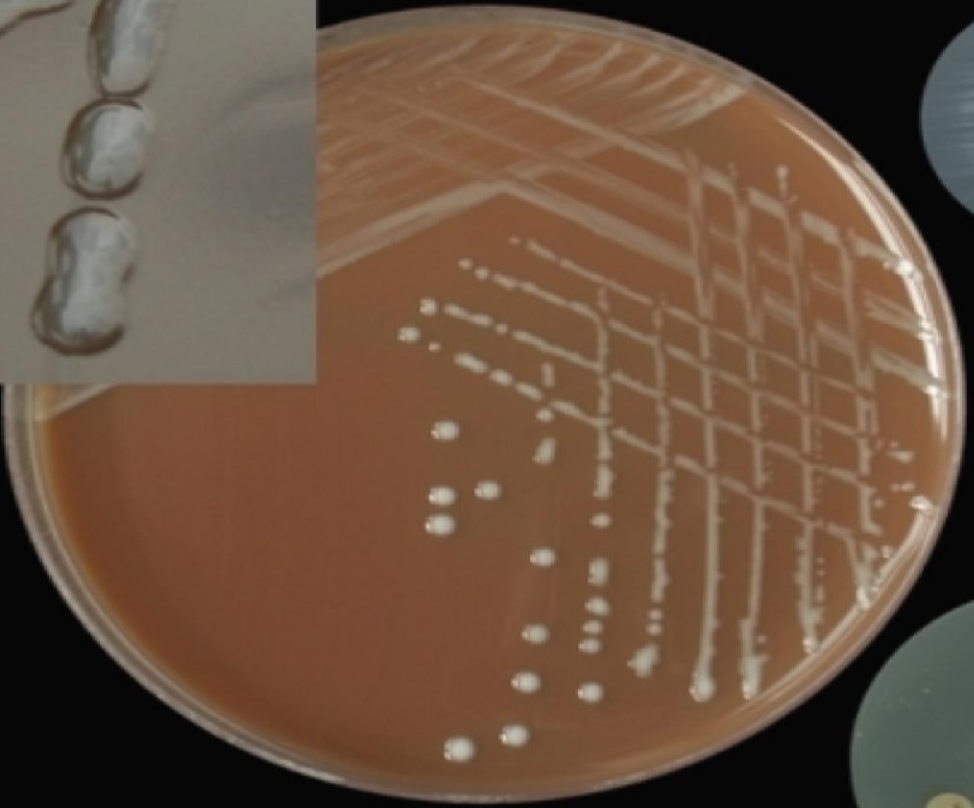
Haematin



- Chocolate agar is used for growing fastidious respiratory bacteria, such as *Haemophilus influenza*.
- These bacteria need growth factors, like NAD - Nicotinamide Adenine Dinucleotide - (V factor) and hematin (X factor), which are inside erythrocytes; thus, a prerequisite to growth is lysis of the red blood cells.



©



X + V factors

X factor

V factor



*Haemophilus influenzae*  
cultivation chocolate agar  
24 hours, 37°C, 5% CO<sub>2</sub>

Hans N.

### 3. Selective Media

- Culture medium that allows the growth of certain types of organisms, while **inhibiting** the growth of other organisms.
- Any agar media can be made selective by addition of certain **inhibitory agents** that don't affect the pathogen.
- Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these medium, for example **LJ**.



## ■ Lowenstein Jensen Agar

- The Lowenstein-Jensen medium, popularly known as LJ medium.
- Is a growth medium specially used for culture of *Mycobacterium tuberculosis*.
- When grown on LJ medium *M. tuberculosis* appears as brown, granule like colonies.

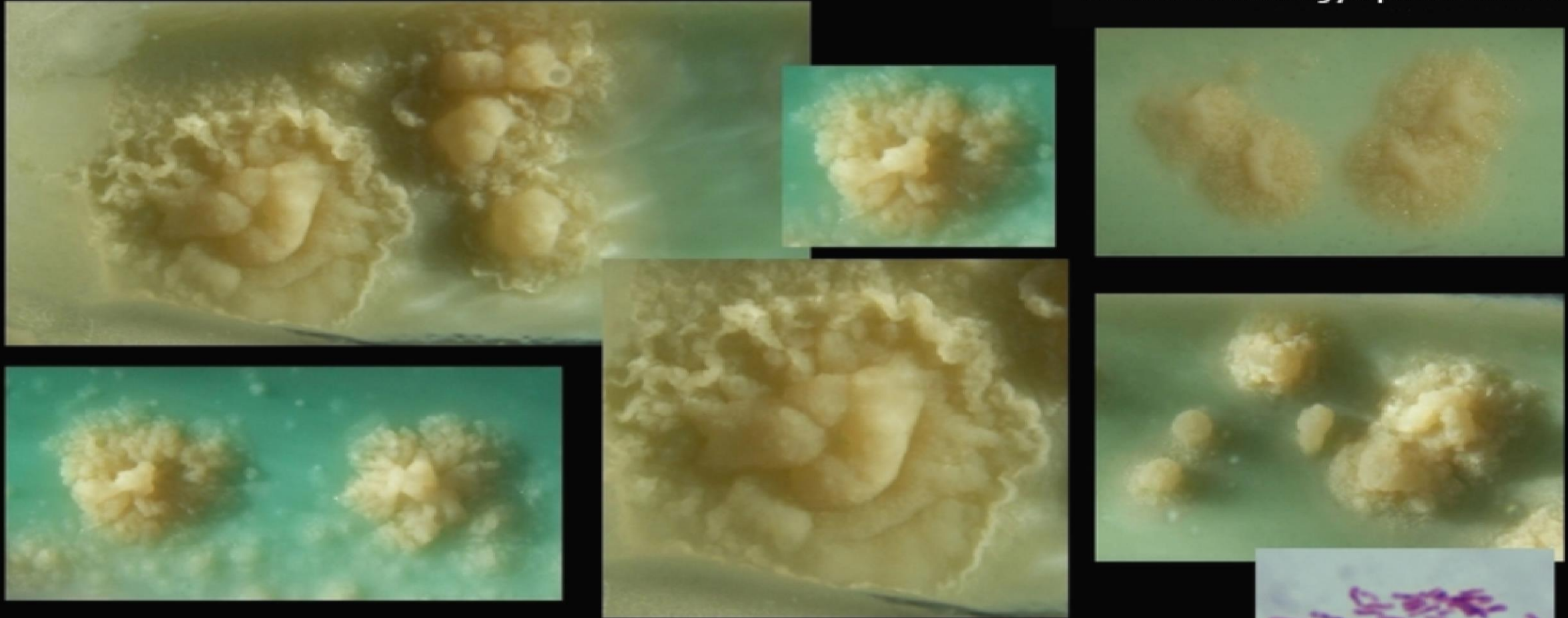


Uninoculated  
Tube



*Mycobacterium  
fortuitum*  
ATCC™ 6841

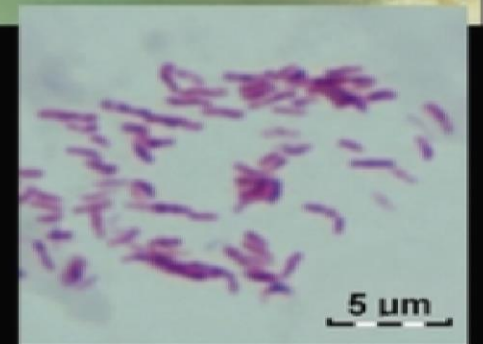
www.microbiologyinpictures.com



*Mycobacterium tuberculosis*  
Löwenstein-Jensen medium  
cultivation 6 weeks, 37°C



Ziehl-Neelsen stain  
(acid-fast rods)



## 4. Differential Media

- Differential media is used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria.
- Examples; **Blood agar and Chocolate agar.**



**Beta Hemolysis**



**Alpha Hemolysis**

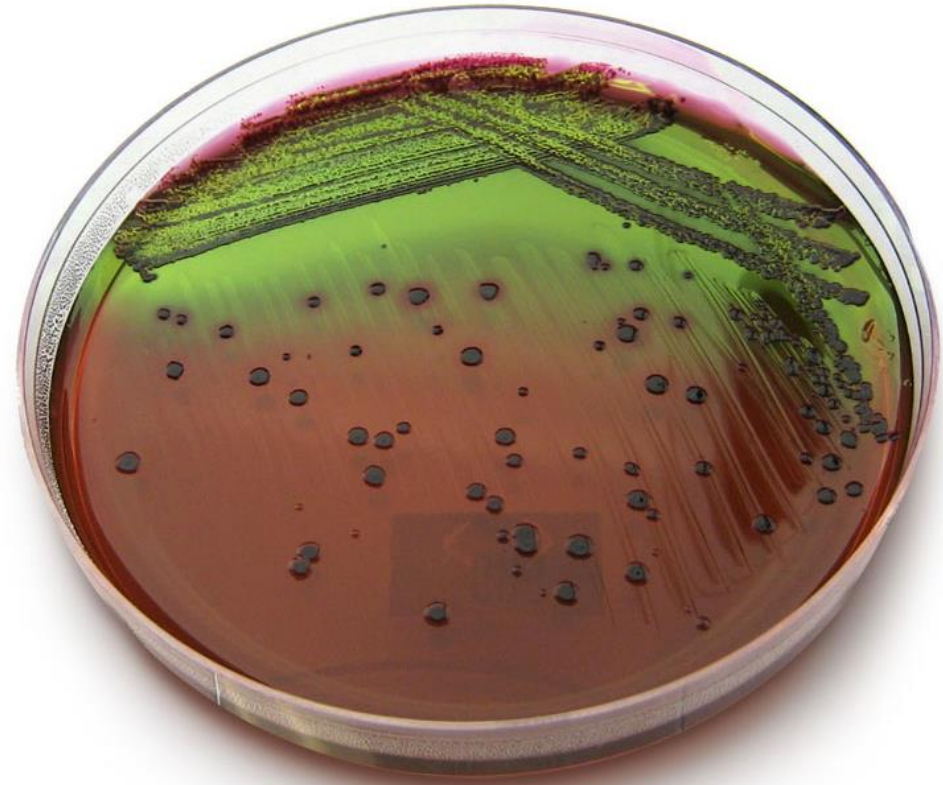


**Non-hemolytic**

## 5. Selective and Differential Media

- Some medias considered as selective and differential media, such as:

- a. MacConkey media.
- b. Eosin methylene blue.
- c. Mannitol salt agar



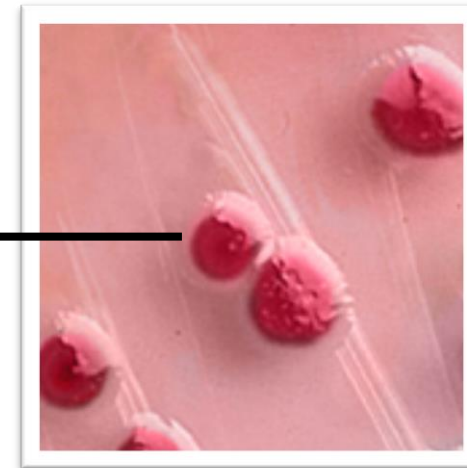
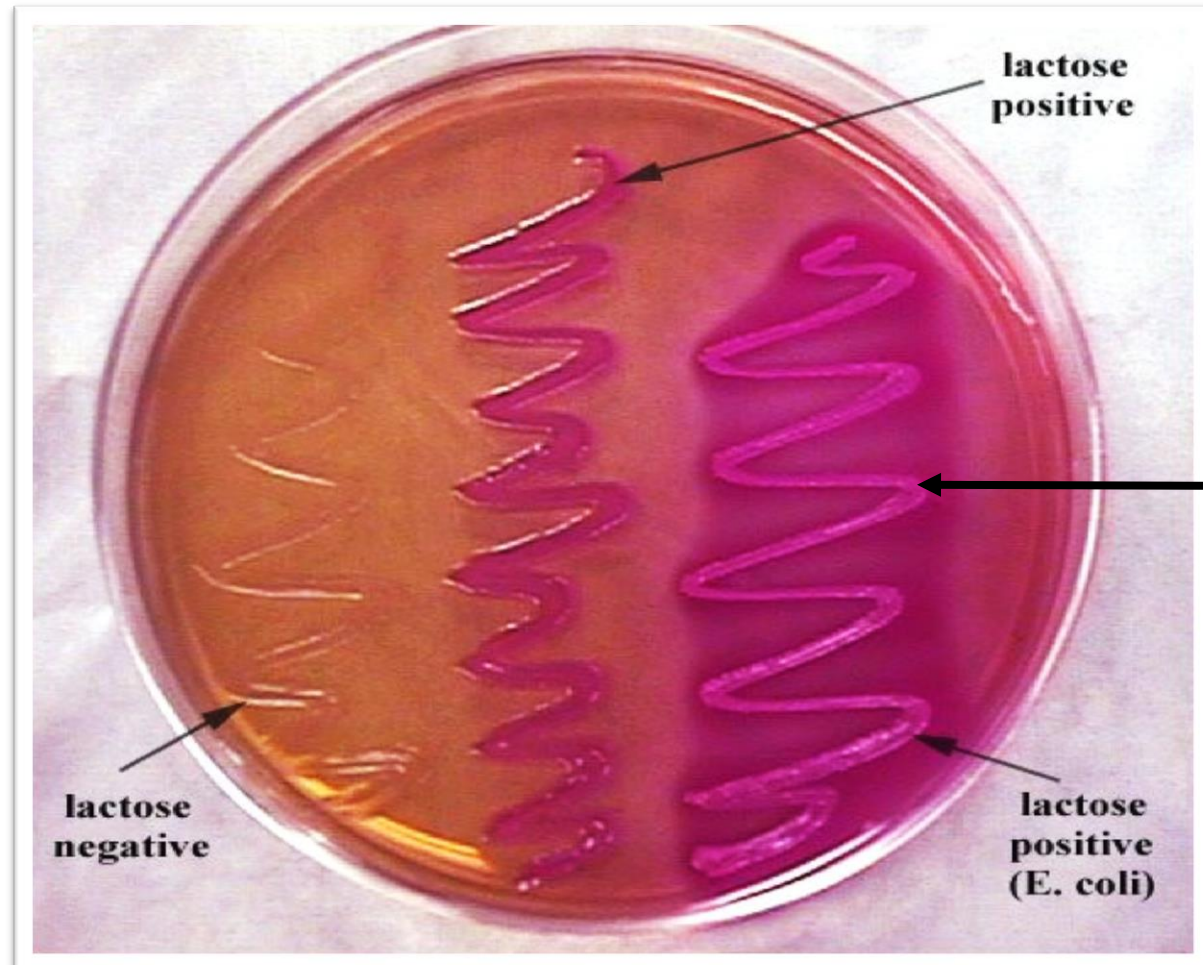


## A. MacConkey Agar

- MacConkey's is **selective media** because it contains a crystal violet which inhibits gram positive organisms.
- MacConkey's is **differential media** because it contains a lactose and a neutral red. When the lactose fermenters, the colonies turn to pink color, while the lactose non-fermenter colonies will be colorless.



# A. MacConkey Agar

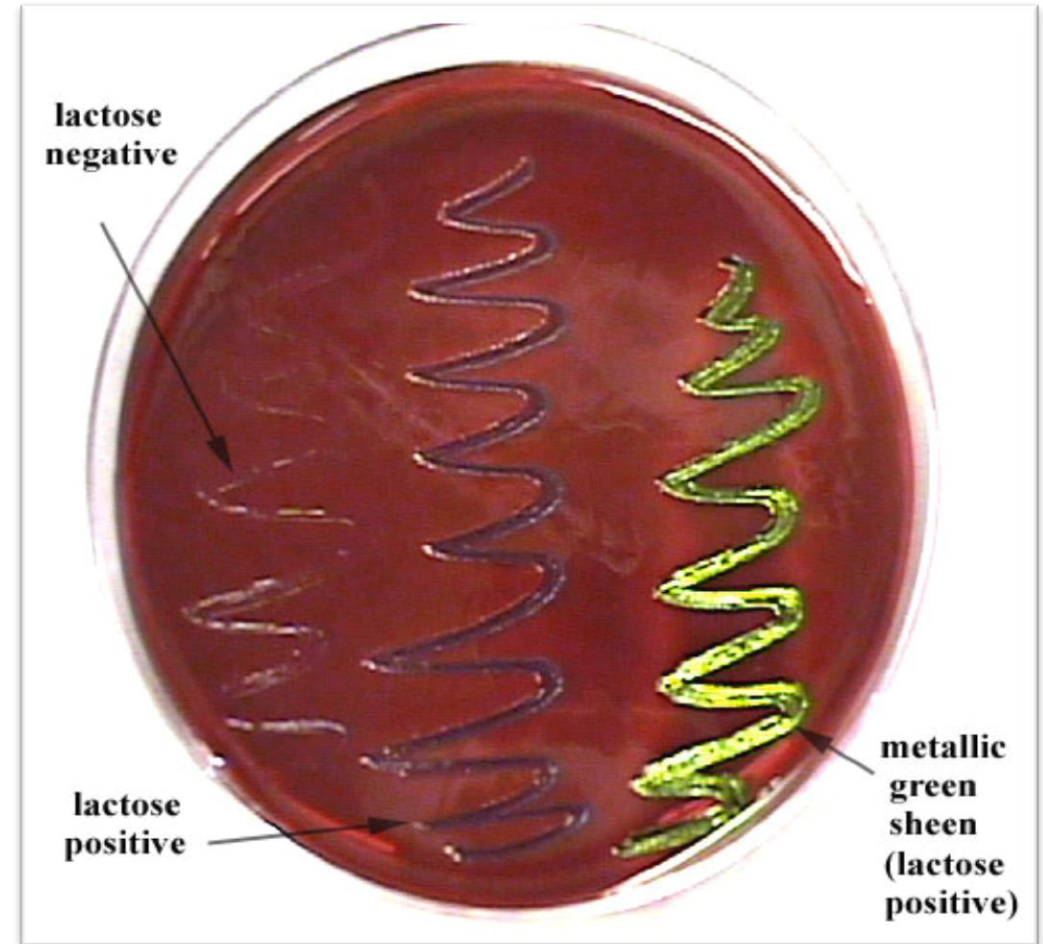


## B. Eosin methylene blue

- EMB is used to isolate fecal coliforms.
- Eosin Y and methylene blue are pH indicator dyes which combine to form a dark purple precipitate at low pH. It inhibit the growth of most Gram positive organisms (**Selective**).
- Sucrose and lactose serve as fermentable carbohydrate sources which encourage the growth of fecal coliforms and provide a means of differentiating them (**Differential**).

# EMB as a differential and selective media

Morphology	Growth	Growth & color change	Green metallic growth
Sample	Lactose Negative	Gram -ve Lactose +	<i>E. coli</i> <i>K. pneumonia</i>



## C. Mannitol salt agar

- **Selective** because it has a high NaCl (7.5%) concentration, and few types of bacteria can grow on this hypertonic medium.
- Members of genus *Staphylococcus* are halophilic, and grow well on this media.
- **Differential** because it contains a pH-sensitive dye to identify organisms that ferment mannitol, producing the change of medium color from red (or pink) to yellow.



- MSA works well for identifying **pathogenic** staphylococci, such as *Staphylococcus aureus*, which will ferment mannitol.
- Most **non-pathogenic** staphylococci (*Staphylococcus epidermidis*) will not ferment mannitol.







*Staphylococcus epidermidis*

*Staphylococcus aureus*

Hans N.

Mannitol Salt Agar

## 6. Transport Media

- Stuart's medium contain reducing agents to prevent oxidation, and charcoal to neutralize certain bacterial inhibitors to *Gonococci*.
- Allows organisms to survive, so it's non-nutritive.
- For bacteria → i.e., Cary Blair.
- For viruses → virus transport media.



Any Questions

