

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.



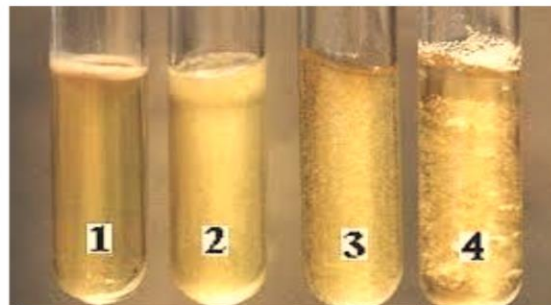
According to the physical state media may be :

Fluid Media

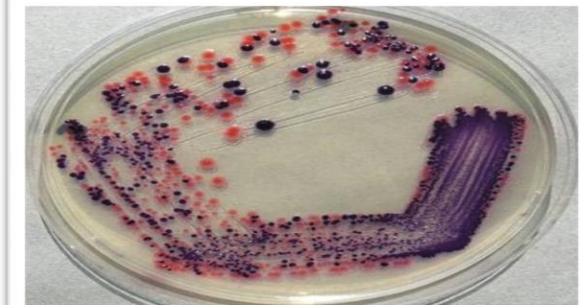
Solid Media

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



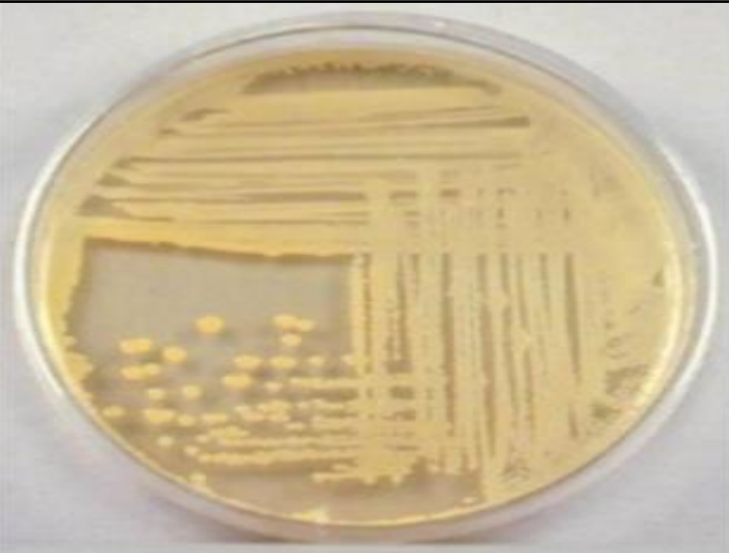
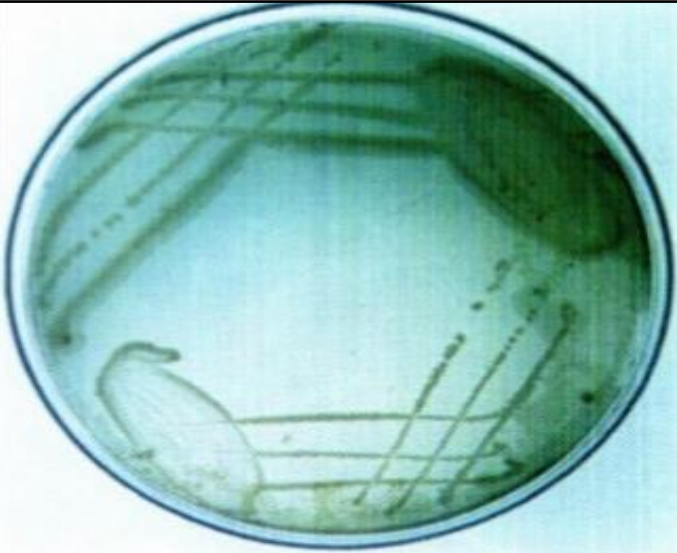

1. Simple Media

- 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

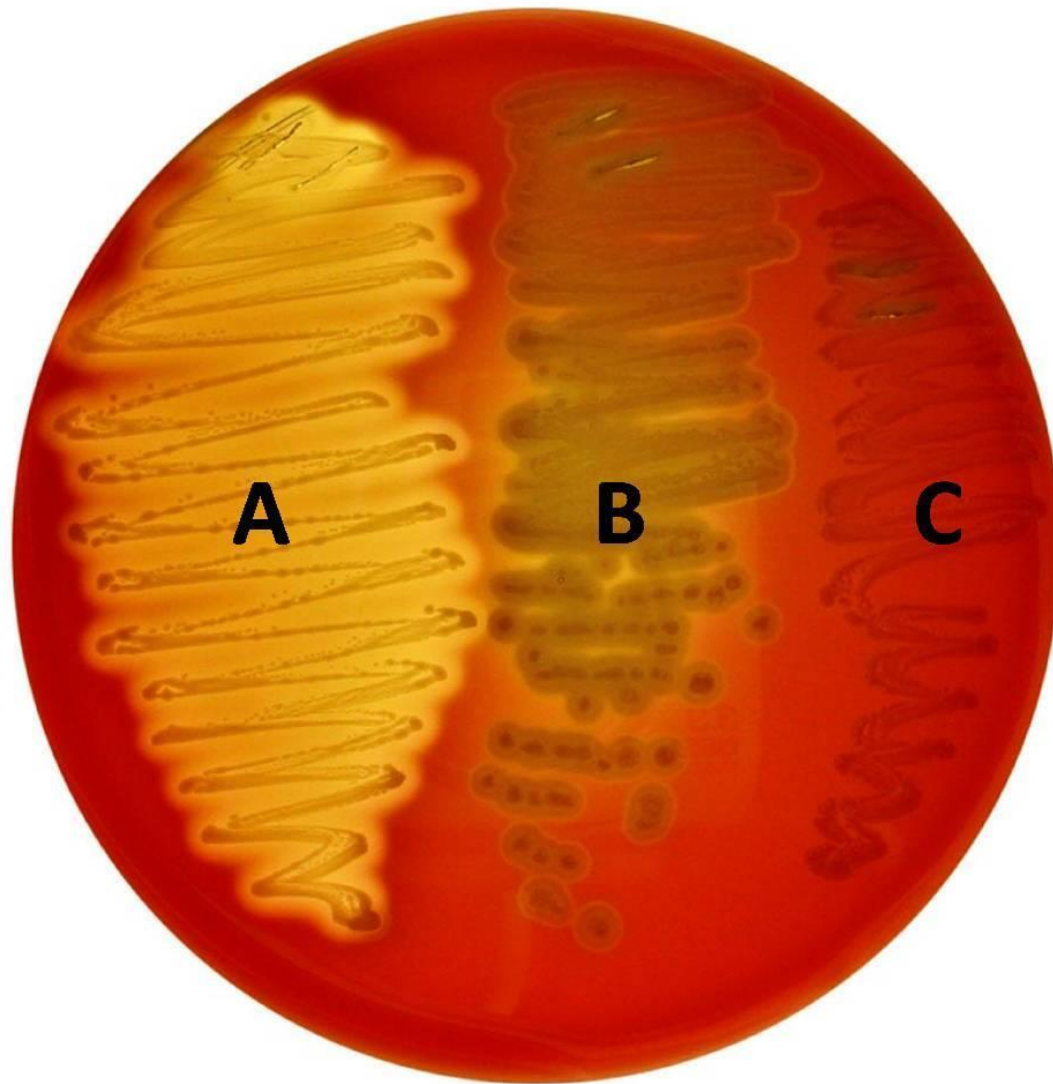
Staphylococcus aureus

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

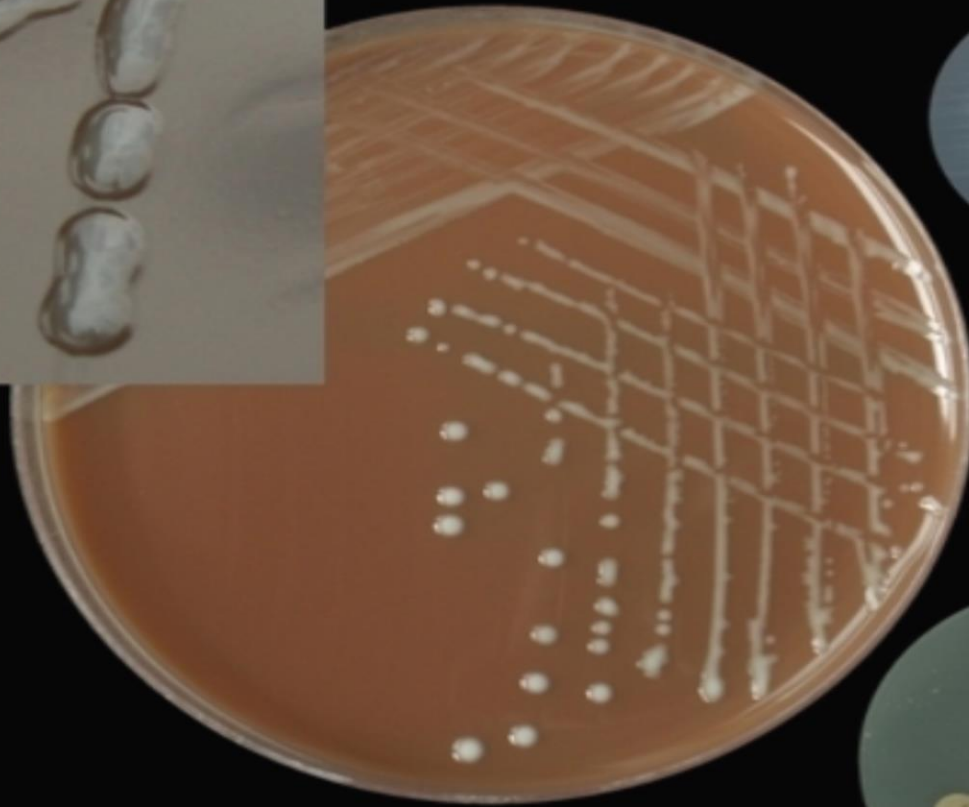
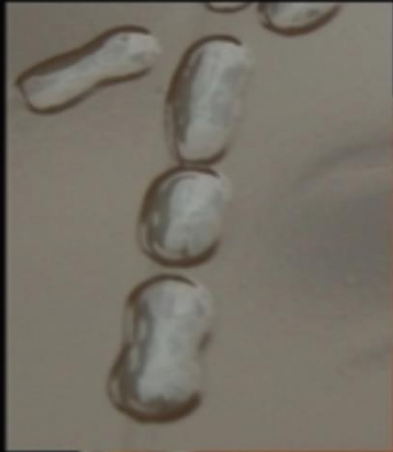
Haematin



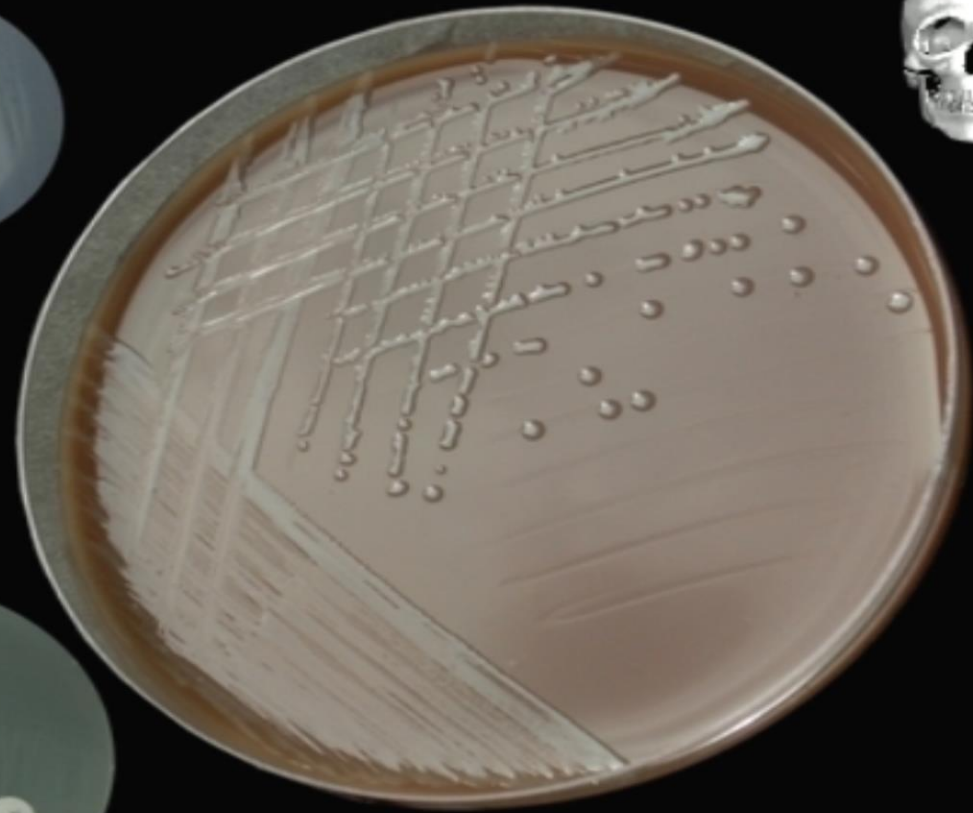
- Chocolate agar is used for growing fastidious respiratory bacteria, such as *Haemophilus influenza*.
- These bacteria need growth factors, like NAD - Nicotinamide Adenine Dinucleotide - (V factor) and hematin (X factor), which are inside erythrocytes; thus, a prerequisite to growth is lysis of the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



©



X + V
factors



X factor

V factor



Haemophilus influenzae
cultivation chocolate agar
24 hours, 37°C, 5% CO₂

H. S. 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.

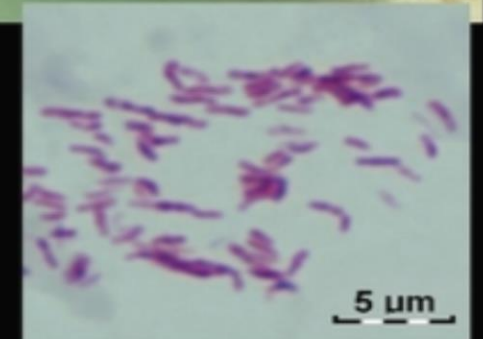
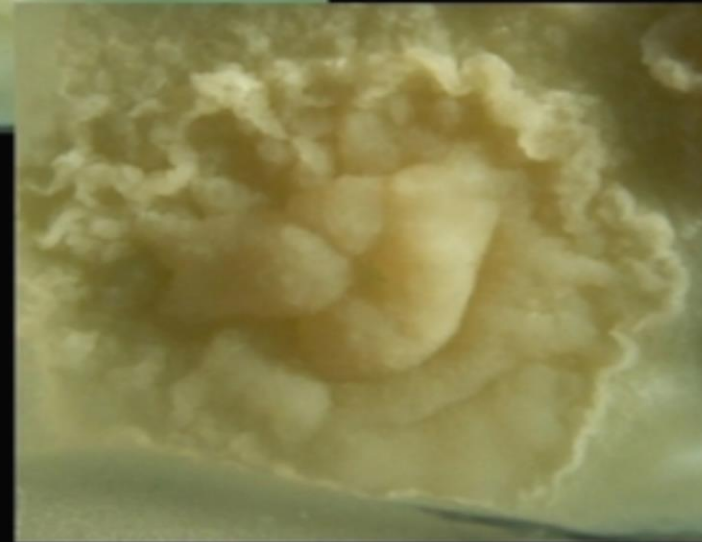
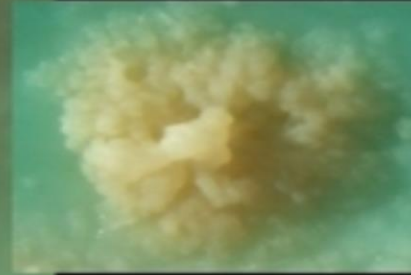
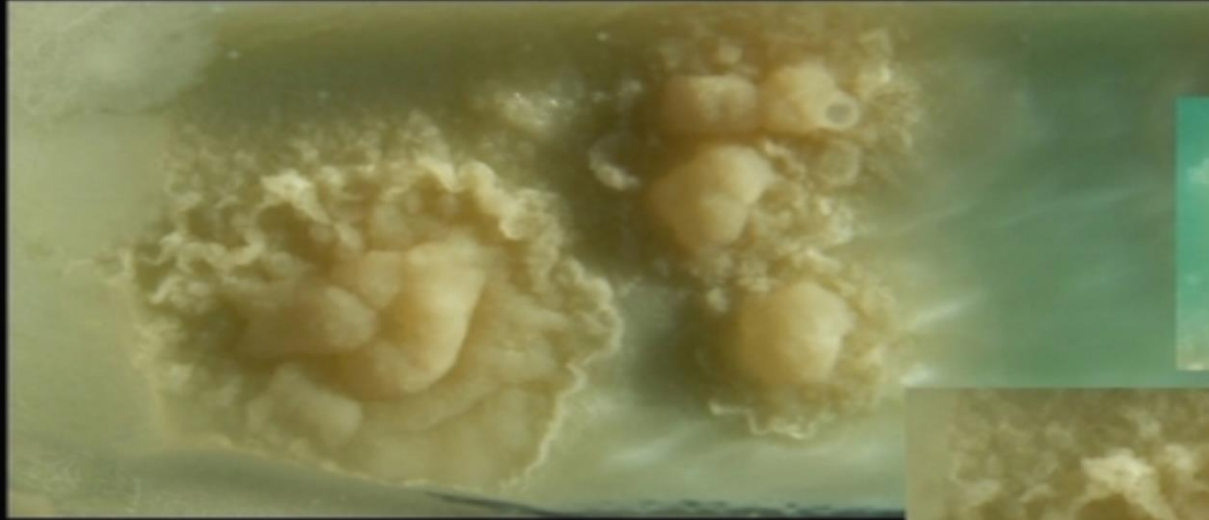


Uninoculated
Tube



*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



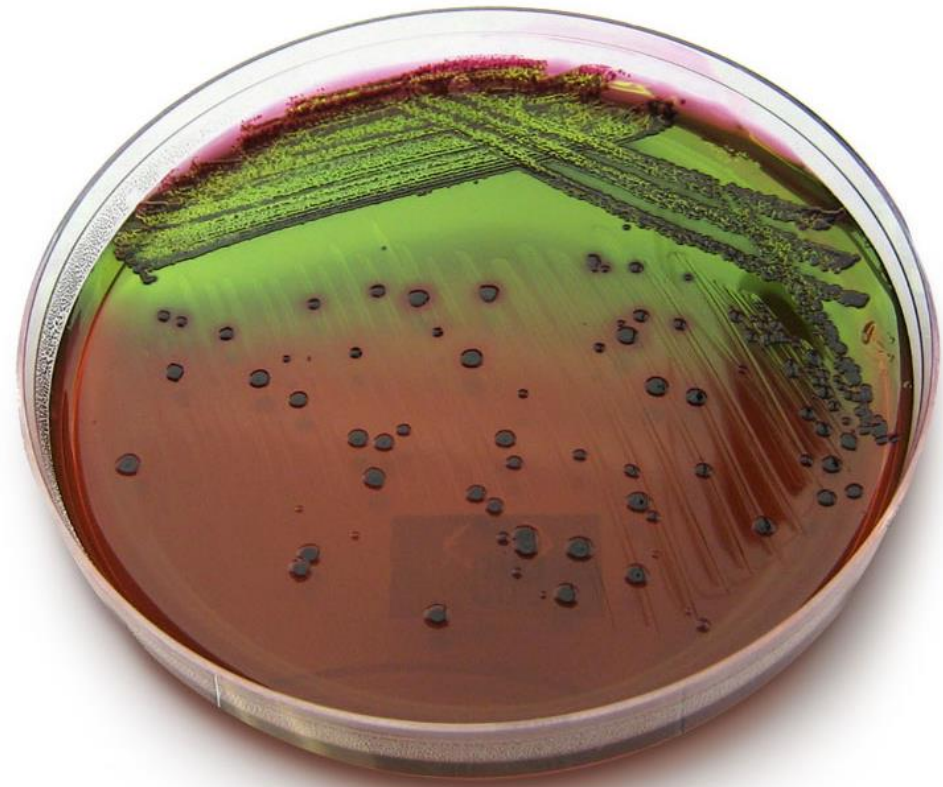
Alpha Hemolysis



Non-hemolytic

5. Selective and Differential Media

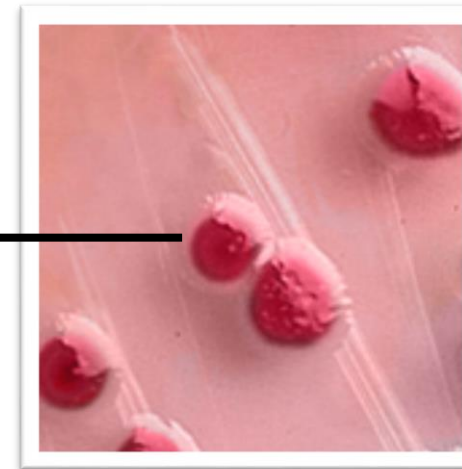
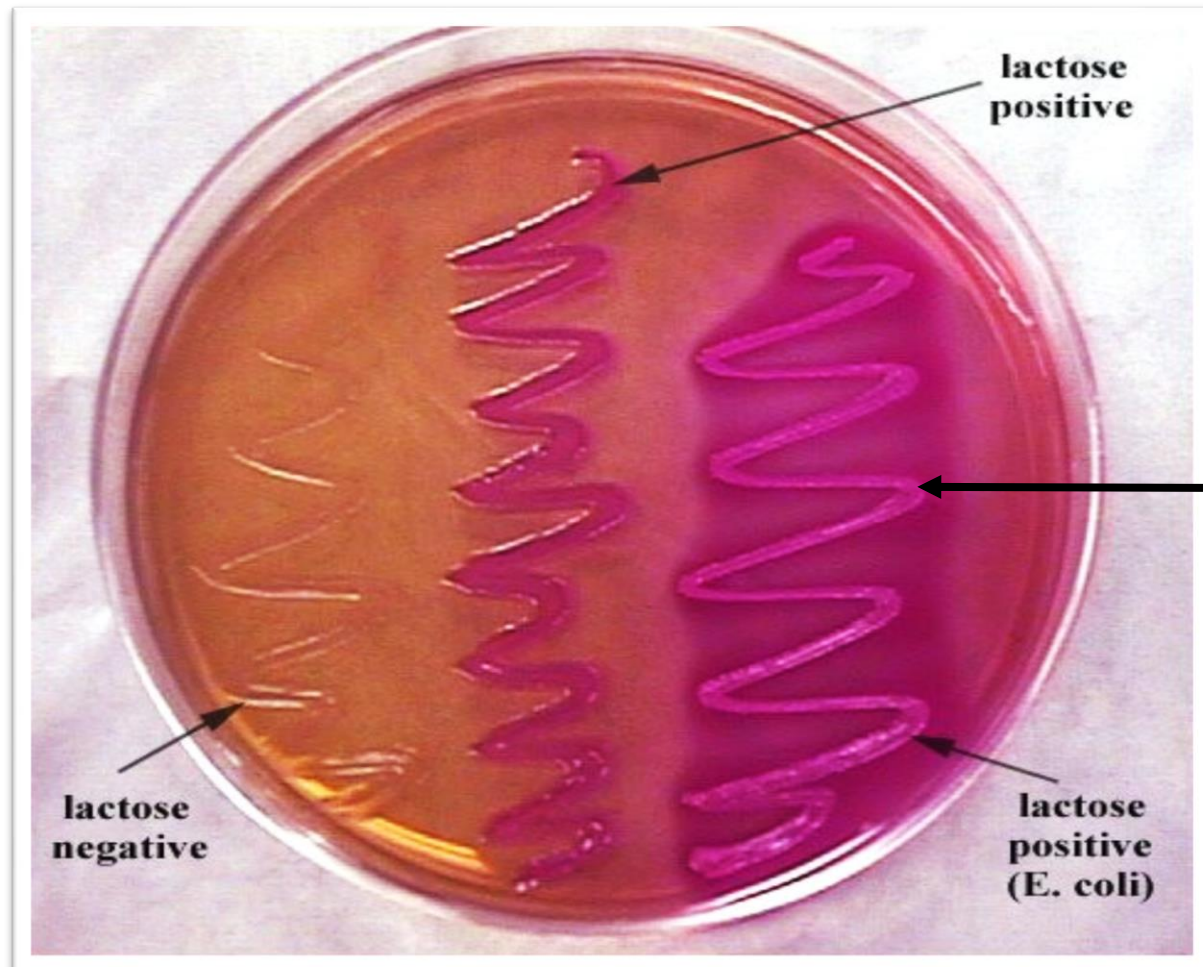
- Some medias considered as selective and differential media, such as:
 - a. MacConkey media.
 - b. Eosin methylene blue.
 - c. Mannitol salt agar



A. MacConkey Agar

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

A. MacConkey Agar

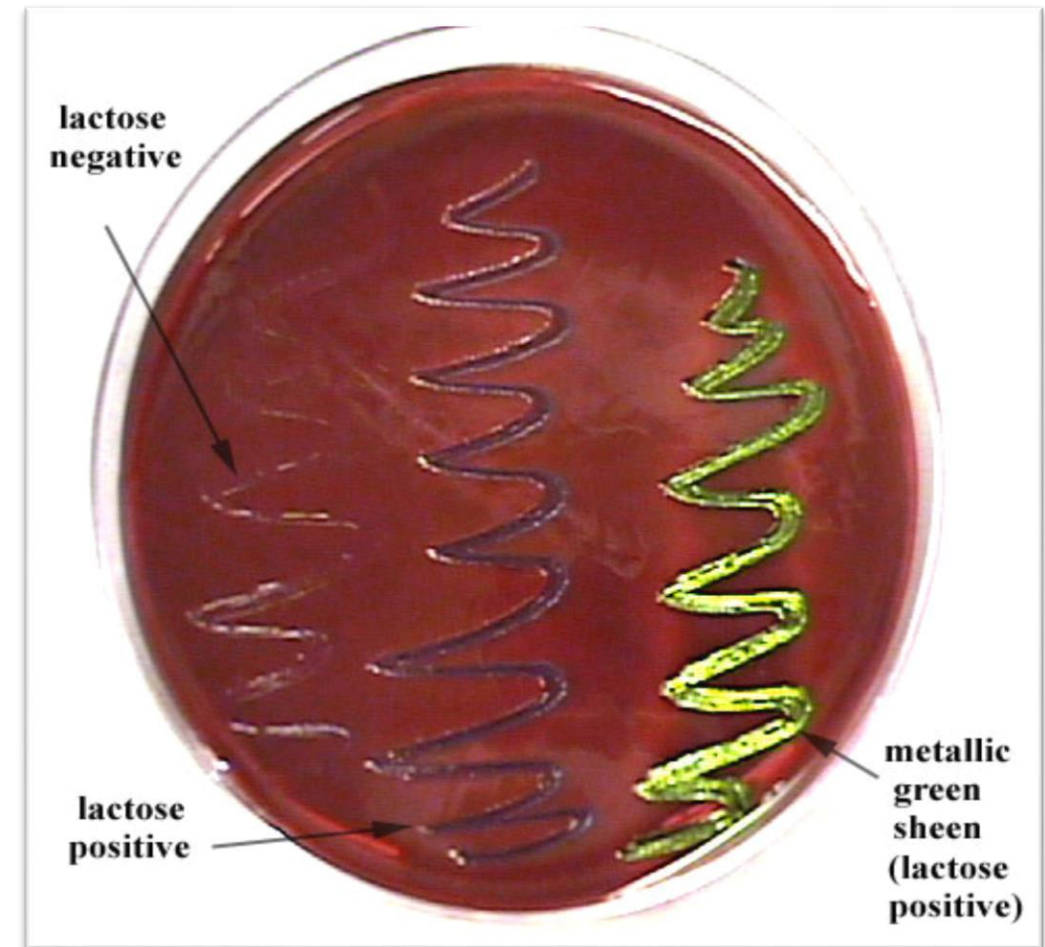


B. Eosin methylene blue

- 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.





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

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