

Lab 3.

Primary Media for Isolation of Microorganisms

MICROBIAL DIAGNOSIS

320 MIC

PRACTICAL



What is Primary Media?

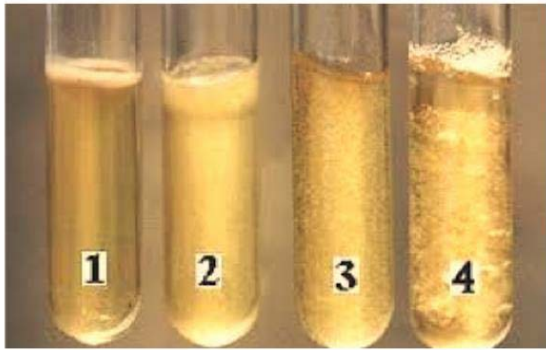
- Many clinical specimens contain a mixed flora of microorganisms.
- Instead, the microbiologist uses several types of **primary media** to culture the specimen initially.
- Culture media provide optimal conditions for growth and multiplication of bacteria.



According to the physical state, media could be:

Broth Media

Turbidity

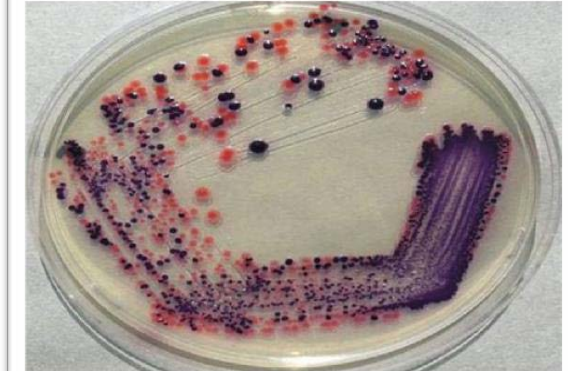


The growth is visualized as



Solid Media

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.

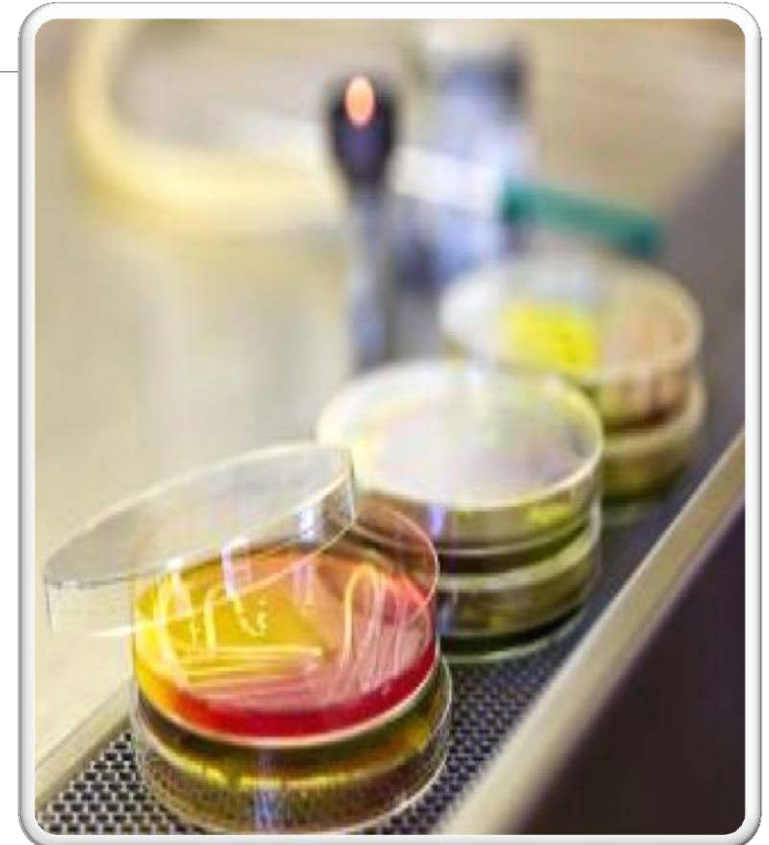
2. Enriched media

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

1. Simple Media:

Some bacteria growth on Simple media (Nutrient agar):

Sample

Staphylococcus aureus

Pseudomonas aeruginosa

Proteus sp.

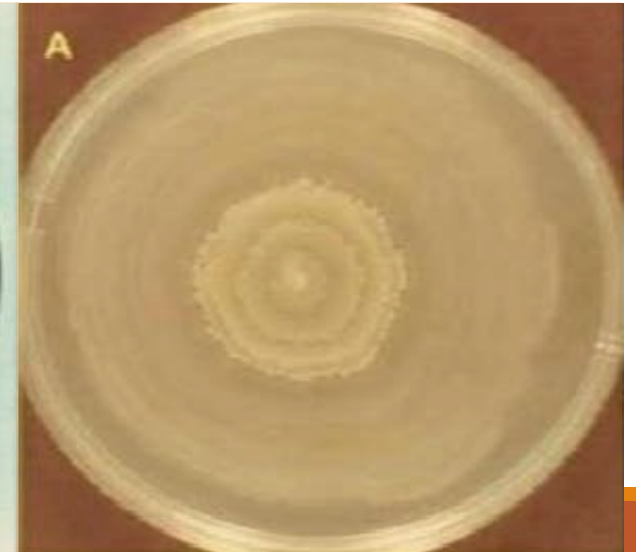
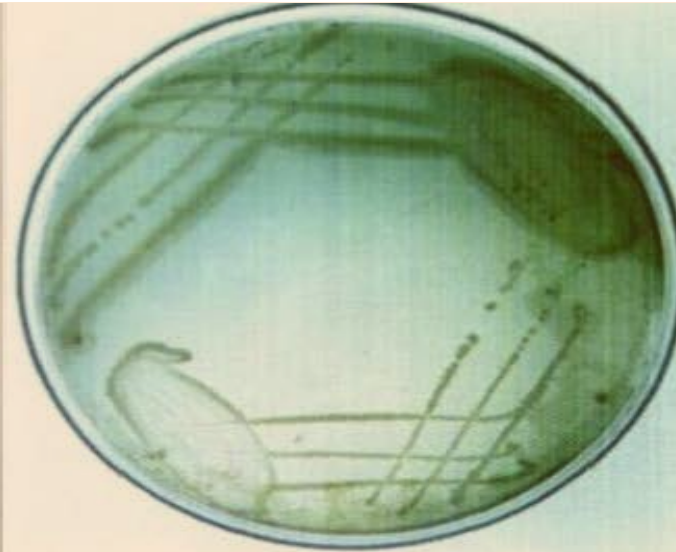
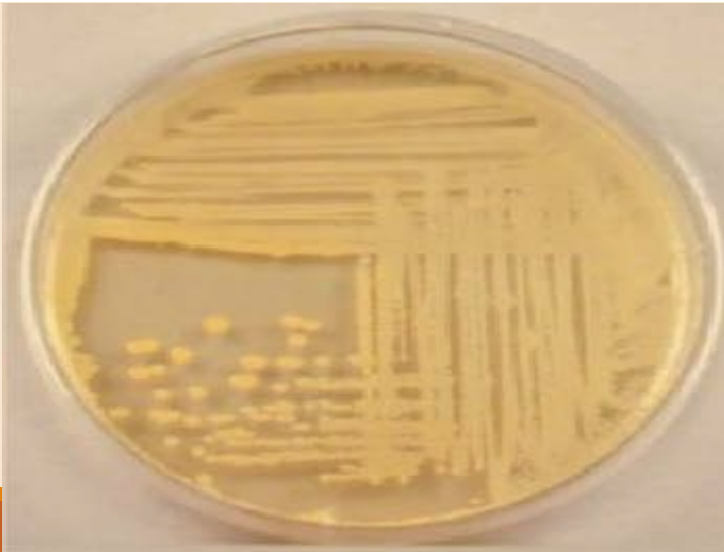
Description
of growth

Golden yellow
endopigment

Greenish exopigment

Swarming growth

The growth



2. Enriched Media:

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



Nutrient agar
+
5-10% Blood

1st: Blood Agar:

- Blood medium is enriched media. Fastidious organisms, such as Streptococci do not grow well in ordinary media.
- Blood contains inhibitors for certain bacteria such as Neisseria and Haemophilus genera.



1st: Blood Agar:

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

1

Beta hemolysis: Bacterial enzymes lyse the blood cells completely producing a clear around 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.

1st: Blood Agar:

Blood Agar as a differential media

Type of
hemolysis

Beta

Alpha

Gamma

Sample

Staphylococcus aureus

E. coli

Staphylococcus
epidermidis

The growth



2nd: Chocolate Agar:

- A non-selective, enriched growth medium.
- A variant of the blood agar media.
- Used for growing fastidious respiratory bacteria, such as Haemophilus influenza.



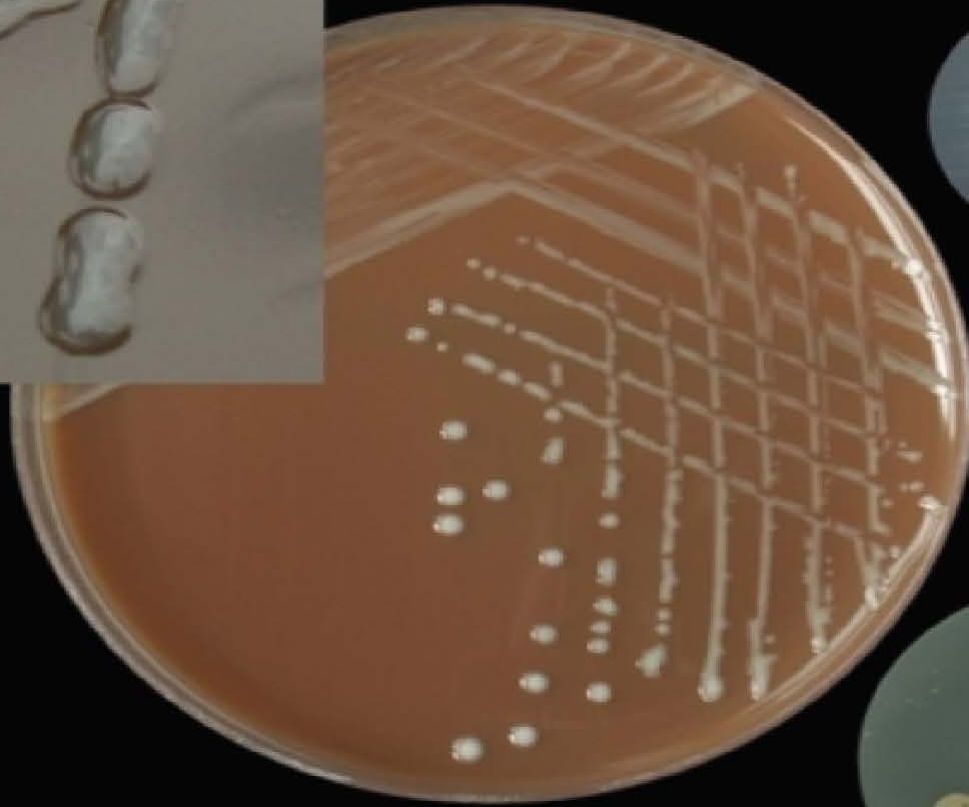
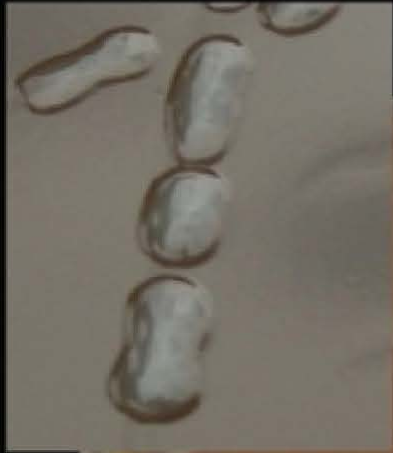
- These bacteria need growth factors, like NAD - Nicotinamide Adenine Dinucleotide - (V factor) and haematin (X factor), which are inside erythrocytes; thus, a prerequisite to growth is lysis of the red blood cells.

Heated Blood agar
very slowly to 56 °C
Haemoglobin
↓ Heat
Haematin

2nd: Chocolate Agar:

©

www.microbiologyinpictures.com



X + V
factors



X factor

V factor



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

Hans N.

3. Selective Media

- Allows the growth of certain types of organisms, while inhibiting the growth of other organisms. Example: LJ medium.
- Become selective by the addition of certain inhibitory agents that don't affect the pathogen.
- Approaches to make a medium selective include:
 - addition of antibiotics, dyes, chemicals,
 - alteration of pH,
 - Or a combination of these.

Example: Lowenstein-Jensen Medium (L J):

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



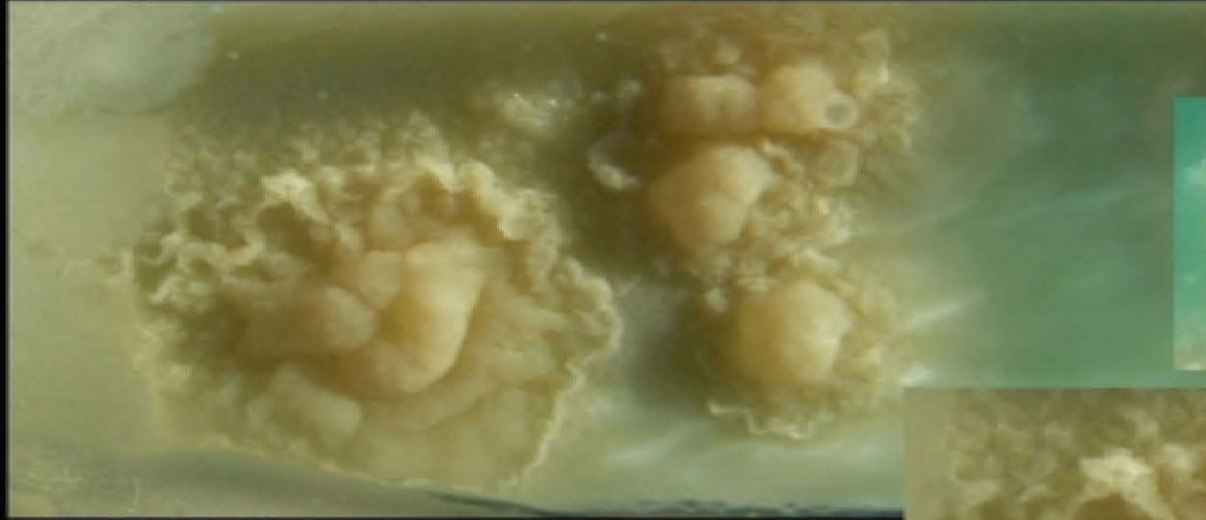
Uninoculated
Tube



*Mycobacterium
fortuitum*
ATCC™ 6841

A selective media: Lowenstein-Jensen Medium (LJ):

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



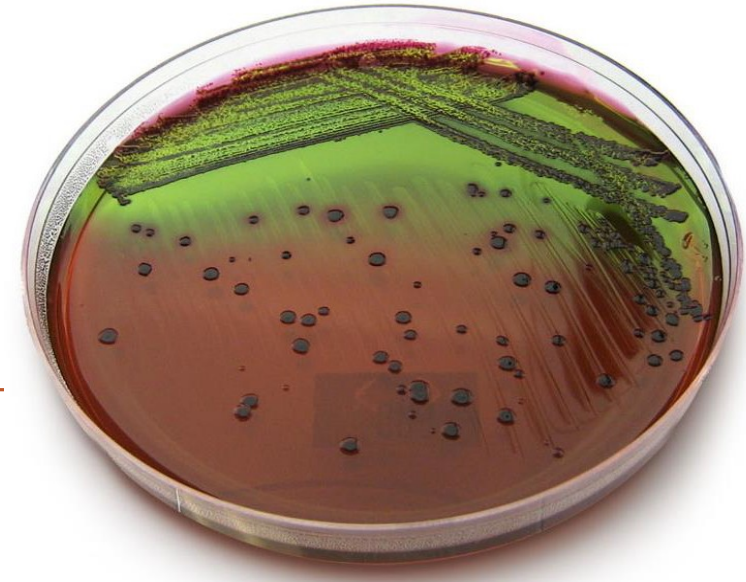
Ziehl-Neelsen stain
(acid-fast rods)

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.**

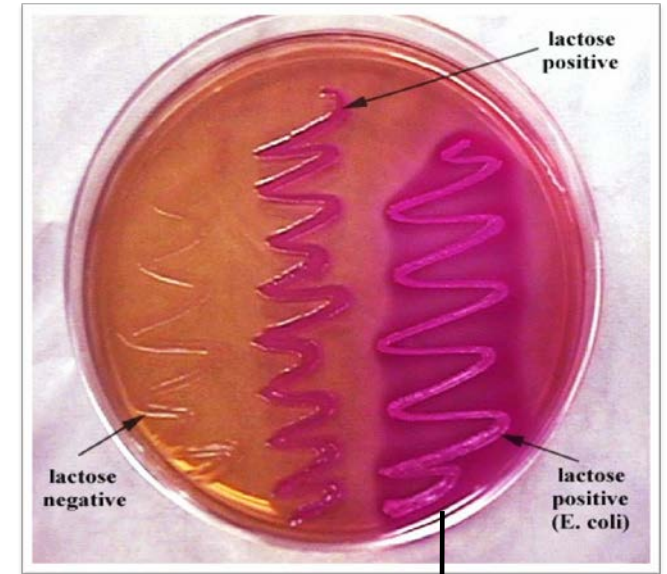
5. Selective and Differential Media:

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



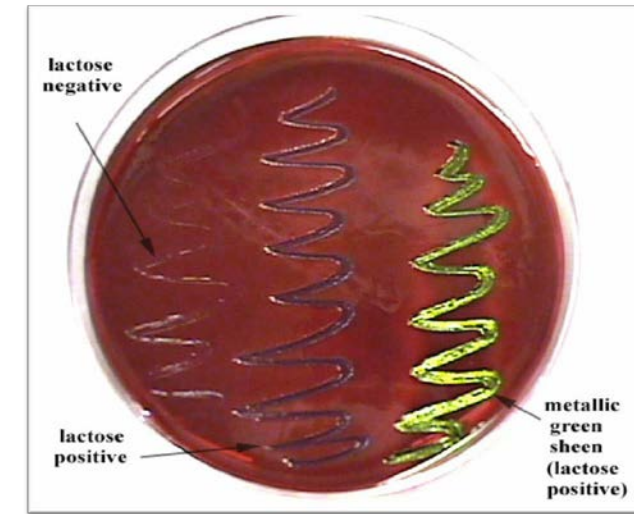
A. MacConkey Agar (EMB):

- 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 and neutral red. When the lactose fermenters, the colonies turn to pink color, while the lactose non-fermenter colonies will be colorless.



B. Eosin Methylene Blue (EMB):

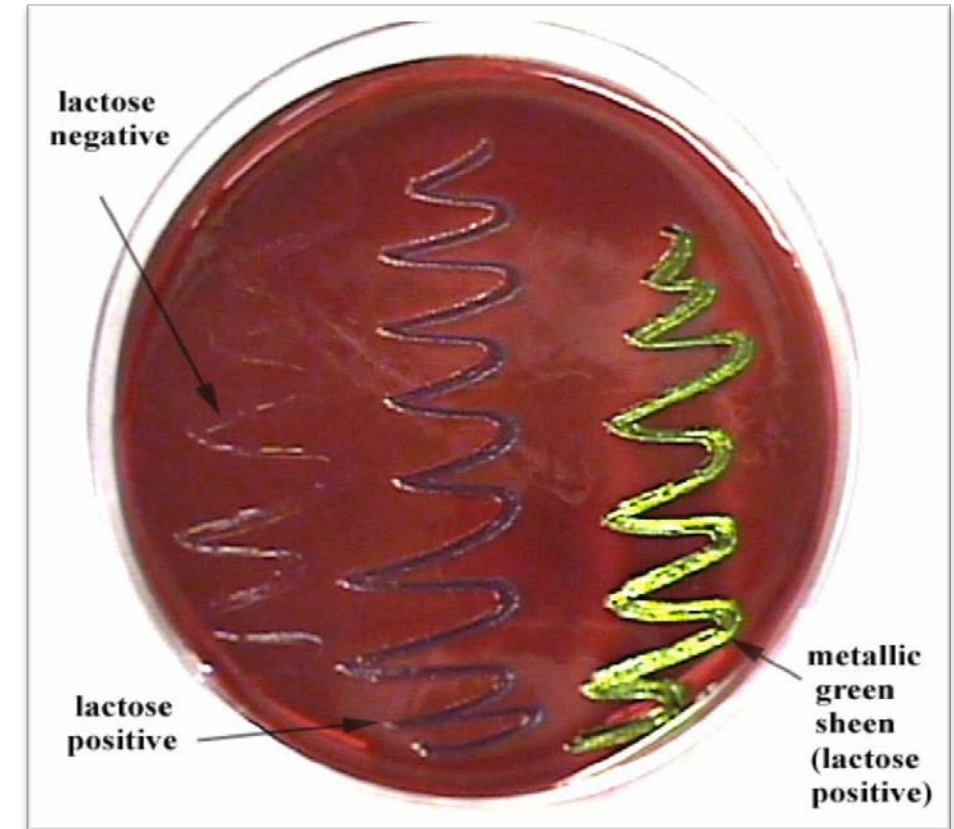
- used to isolate fecal coliforms.
- Eosin Y and methylene blue are pH indicator dyes which combine to form a dark **purple** precipitate at low pH. It inhibit the growth of most Gram positive organisms (**Selective**).
- Sucrose and lactose serve as fermentable carbohydrate sources which encourage the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



B. Eosin Methylene Blue (EMB):

EMB as a differential and selective media

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

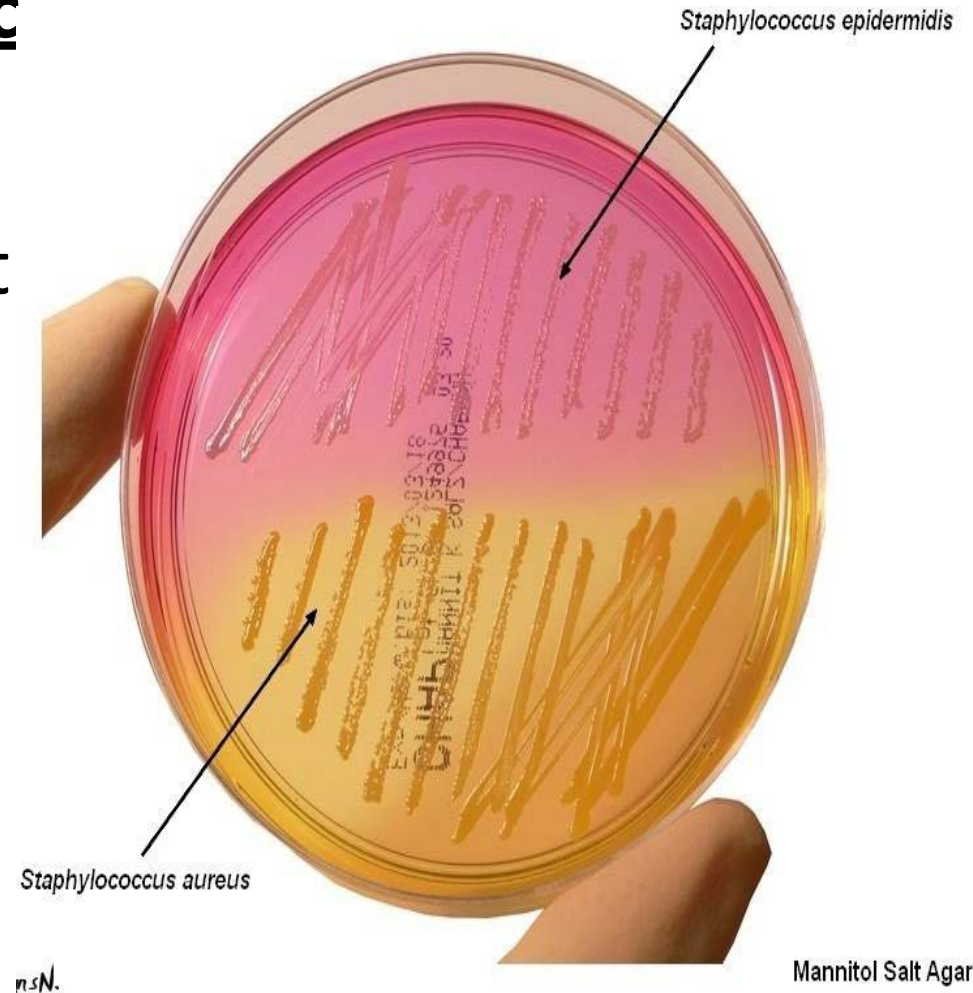


C. Mannitol Salt Agar (MSA):

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

C. Mannitol Salt Agar (MSA):

- 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:

- **e.g.** 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 (VTM).



A scenic landscape photograph showing a bright sun in a blue sky with scattered clouds. The sun's reflection creates a shimmering path on the blue water of the sea. To the right, a dark, forested hill slopes down towards the water.

Thanks for Listening!

For Questions:
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