

A **culture medium** is a liquid, solid or semi solid preparation of nutrients used to grow/ proliferation, transport, and store microorganisms. Although all microorganisms require energy sources, carbon, nitrogen, phosphorus, sulphur and various minerals.

Remember: Knowledge about microorganisms natural habitat Often is useful in selecting an appropriate culture medium because of its natural surroundings reflect its nutritional requirements.

Selective Media:

A selective medium is one that prevents the growth of unwanted microorganisms while permits and promotes the growth of wanted microorganisms .

The selective action of the medium : is due to the addition of certain chemicals to the medium.

For example, addition of crystal violet dye in the culture medium selectively inhibits the growth of **Gram-positive bacteria** and permits and promotes the growth of Gram-negative bacteria.

Such as : MacConkey-Agar

Another example: addition of salt content (7.5% NaCl) of culture medium ; inhibits the growth of **Gram-negative bacteria**, while allows only salt-tolerant bacteria (Gram-positive bacteria).

Such as : Mannitol-Salt-Agar

While addition the methylene blue: which is toxic to Gram-positive bacteria, selecting for Gram-negative bacteria. Such as : Eosin Methylene Blue (EMB) Agar.

The bile salts and brilliant green were added to **Salmonella-Shigella Agar** inhibit the growth of most Gram-positive bacteria & most coliform bacteria, but *Salmonella spp.* and *Shigella spp.* are not affected by these inhibitors.

****Ordinary solid media contain 1.5-2.0% Agar****

Differential (Indicator) Media:

A differential (indicator) medium is one which causes a visible change between different groups of bacteria growing in the medium and even permits previous identification of microorganisms .

 **MacConky culture medium** : The medium contains lactose and a pH indicator called neutral red.

- When lactose is fermented by bacteria , it produces acid, which lowers the pH of the agar. The neutral red indicator turns pink under acidic conditions.
- Non-lactose-fermenting bacteria appear as off-white or colorless colonies.
- Pink colonies : *Escherichia coli*, *Enterobacter* & *Klebsiella*
- Lactose fermenter slowly : *Serratia* & *Citrobacter*
- Pale Colonies : *Pseudomonas aeruginosa* , *Proteus species*, *Salmonella species* & *Shigella species*.

✚ **Mannitol Salt medium** : it contains mannitol and a phenol red pH indicator.

- Ferment mannitol by Bacteria produce acid $\longrightarrow$ lowers the agar's pH $\longrightarrow$ yellow colonies : *Staphylococcus aureus*.
- NO ferment mannitol : Pink colonies or no change of color such as: *Staphylococcus epidermidis*.

Eosin Methylene Blue (EMB) agar : Lactose & pH Indicator dyes : eosin and methylene blue.

Lactose-fermenting bacteria: result in pink or brown-pink coloured colonies, except *Escherichia coli* produce :metallic green sheen.

Salmonella-Shigella Agar:

✚ The medium also contains Neutral Red ,Ferric (iron compound) & Lactose .

- Colony Appearance: Shigella colonies are colorless .
Lactose Fermentation: Shigella cannot ferment lactose $\implies$ the neutral red indicator is not activated.
- *Salmonella species* are also lactose non- fermenters but produce H₂S that converts the colonies to black colour or black centres a result to formation :Ferrous sulfide (FeS)

Enriched media :

culture media that contain added nutrients, such as blood, serum, or egg yolk, to support the growth of fastidious microorganisms.

Examples:

- Blood Agar: Contains 5–10% sheep blood, rich nutrients to survive and grow, used for isolation of *Streptococcus spp.*

- **Types of Hemolysis:**

Beta-Hemolysis (β): Complete lysis of red blood cells..

Alpha-Hemolysis (α): Partial lysis of red blood cells.

Gamma-Hemolysis (γ): No lysis of red blood cells .

Blood-Agar medium is also used to differentiate between hemolytic and non-hemolytic bacteria.

- Chocolate Agar: red blood cells are lysed by heating, releasing intracellular nutrients.

Chocolate agar for isolation of *Neisseria* and *Haemophilus*.

❖ **Remember: to obtain pure colonies by streaking**

