

Culture Media

By:

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Culture media: is a gel or liquid that contains the nutrient and is used for culturing bacteria, yeasts, molds, and algae. Media contain necessary nutrients supporting the growth (replication) of microorganisms.

It is an environmental mixture of essential required nutrients for surviving and growing of microorganisms, that contains: C, N, P, S and elements such as Fe, Cu, Ca, Zn, Mg, Mo, Cl- with vitamins and growth factors.

A suitable growth medium must contain all the nutrients required by the organism to be cultivated, and such factors as pH, temperature, and aeration must be carefully controlled.

Culture media are mediums that provide essential nutrients and minerals to support the growth of microorganisms in the laboratory.

Culturing microorganisms is essential for diagnosing infectious diseases, obtaining antigens, developing serological assays for vaccines, genetic studies, and identification of microbial species. Furthermore, it's also essential for isolating pure cultures, storing culture stock, studying biochemical reactions, testing microbial contamination, checking antimicrobial agents and preservatives effect, testing viable count, and testing antibiotic sensitivity.

The culture medium should be sterile and contamination of a culture with other organisms should be prevented.

Classification and Types of Culture Media

Culture media are classified in different methods as follows:

A. Based on consistency (Physical forms):

Media are classified into **liquid media**, **semi-solid media** and **solid media**. By increasing the concentration of agar, consistency of the culture media is variable, so we have:

1. Liquid media (Broth): Liquid media are also called broths, it is the medium that does not contain any solidifying agents (Agar or Gelatin), it is the media without addition of agar. large bacterial growth can be observed in this media. they allow for uniform and turbid growth of bacteria when incubated at 37°C for 24hrs.

This media is used for the profuse growth of microorganisms and fermentation studies.

Examples include (Nutrient broth, mineral salt broth, brain heart infusion broth, tryptic soy broth etc).

❖ Examples:

(Nutrient broth) (PH 7.0) - Peptone, Beef extract, NaCl + Distilled water.

(mineral salts broth) (PH 7.0) - K₂ HPO₄, KH₂ PO₄, Mg SO₄ .7H₂ O, (NH₄)₂ SO₄, Glucose & Distilled water.



Figure (1): Nutrient broth

These types of media are used for:

- Produce large numbers of microbial cells.
- Various biochemical tests.

2. Semisolid media: These media are contained the required nutrients + (5 g) of agar or (150-200 g) of gelatin in one liter of water.

Semi-solid mediums are used for the following purposes:

- Determination of microbial motility.
- Determination of gelatinase enzyme.
- Production and promotion of anaerobic growth.

The medium is heated until boiling to dissolve the components, the prepared media must be distributed in test tubes.

3. Solid media: These types of media are contained the required nutrients + solidifying agent (agar) such nutrient agar.

it is the media with the addition of 1.5-2 % agar (agar does not melt until near boiling point, and when it cools, agar remain molten until just over 40°C, allowing heat sensitive media components such as blood to be added). This media is placed in Petri dishes and test tubes for culturing microbes. Petri dishes (solid media) are used for the isolation of colonies growing on the medium.

Solid media are used to:

- Grow microorganisms in their full physical form.
- Isolate bacteria to study colony characteristics.
- To observe the specific biochemical reactions.
- Prepare bacterial pure cultures.
- Storage of cultures (stock cultures).

All contents must be dissolved by heating with shaking during the heating until boiling. Then sterilized by autoclaving under pressure (15 pps) At (121°C) for (15 minutes). The prepared quantity either divided in test tubes for slants for pouring in Petri-dishes. (See figure 2 and 3).

For pouring plate of the solid media, the container must be transferred to 48-50°C water bath or must be cooled to about 50°C.

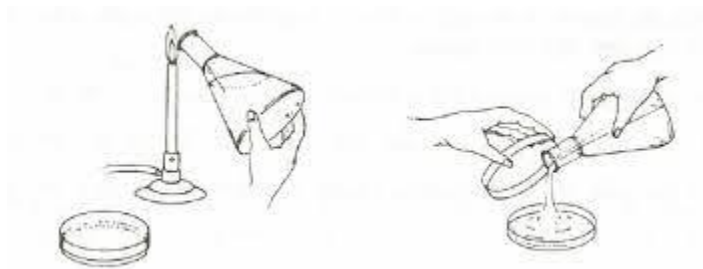


Figure (2) Pouring Agar plates method.

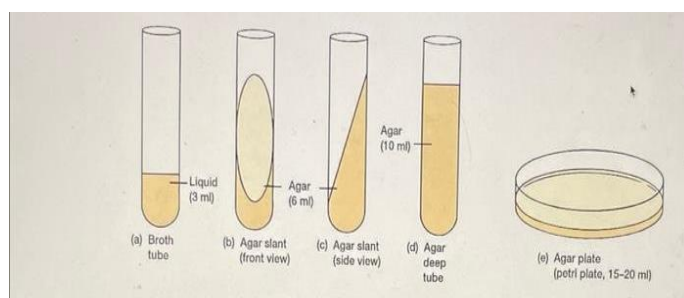


Figure (3) Different forms of culture media

B. Based on Nutritional Components:

1. Simple (General) media: It's a general-purpose media that supports the growth of non-fastidious microbes, and it is primarily used for the isolation of microorganisms. Examples are nutrient broth, peptone water, and nutrient agar.

2. Complex media: These are media containing nutrients in unknown quantities that are added to bring about a particular characteristic of a microbial strain. Examples are tryptic soy broth and blood agar.

3. Synthetic media (defined media): Synthetic media is a type of chemically defined media and is produced from pure chemical substances. A defined media refers to a medium having a known concentration of ingredients, like sugar (glucose or glycerol) and nitrogen source (such as ammonium salt or nitrate as inorganic nitrogen). It is generally used in scientific research, and an example is Czapek Dox Medium, Nutrient broth and Nutrient agar.

C. Based on Application:

1. Basal Media: These are routinely used simple media having carbon and nitrogen sources that boost the growth of many microorganisms. They are also known as general-purpose media and are considered non-selective media. They are generally used to isolate microorganisms in labs or in sub-culturing processes. Examples are nutrient broth, nutrient agar, and peptone water.

2. Selective media: This media allows the growth of certain microbes while inhibiting the growth of others. The selective growth of microbes

is decided by adding substances like antibiotics, dyes, bile salts, or by pH adjustments.

Some of the media contain stains or antibiotics as inhibitors which prevents the growth of some microbial groups and allow others to grow, these specific media are termed **selective**. These types of media are used for isolation of bacterial genera or species or for fungal groups.

There are several examples of the selective media for different bacterial groups such as:

- a. MacConkey Agar and Broth
- b. *Salmonella –Shigella* agar (SS agar)
- c. Eosin Methylene Blue (EMB- Agar)
- d. Mannitol Salt Agar (MSA)
- e. Mitis Salivarius Agar

3. Differential (indicator) media

4. Enriched media

5. Special media

6. Transport media

2. Selective media:

a. MacConkey Agar and Broth

These media are used for isolation the enteric or coliform bacteria, which contains **bile salts** and **crystal violet**, that inhibits all bacterial growth except the coliform or enteric bacteria.

Compositions of MacConkey agar: (PH 7.1), Peptone, Proteose or Polypeptone, Lactose, Bile salts, NaCl, Neutral red. Crystal violet, Agar Dist. Water. MacConkey broth medium constituents are all above materials except the agar.



Figure (4) MacConky agar.

b. *Salmonella* –*Shigella* agar (SS agar)



Selective medium, Inhibition the growth of all bacteria and allow to growth of *Salmonella* and *Shigella*.

Salmonella appears on the agar in brown colony with black center, while *Shigella* appears pale or colorless.

Figure (5) SS agar.

c. Eosin Methylene Blue (EMB- Agar)

EMB is the **selective** and **differential** medium for coliforms. It contains the following ingredients: peptone, Lactose, Dipotassium phosphate, Eosin Y (dye), Methylene blue (dye) and Agar. This medium inhibits the growth of Gram-positive bacteria and provides a color indicator; if *E. coli* is grown it will give a “distinctive green metallic sheen”

with a dark center, *Aerobacter aerogenesa* colonies have a brown center, and non lactose-fermenting Gram-negative bacteria appear pink.



Figure (6) – EMB Agar

d. Mannitol Salt Agar (MSA)

This medium is used for isolation of pathogenic *Staphylococcus* (*S. aureus*), which contains high concentrates of NaCl (7-10%), that inhibits the growth of nonpathogenic staphylococci and other types of bacteria. The colonies of *S. aureus* change to yellow and color of culture medium change from pink to yellow due to the fermentation of mannitol sugar.



Compositions: Beef extrat, Peptone, Sodium chloride (NaCl), D-mannitol, Phenol red and Distilled Water.

Figure (7) – Manitol Salt Agar (MSA)

e. Mitis Salivarius Agar

This medium is selective for oral streptococci.

Composition: Tryptose, Peptone, Dextrose, Succhrose, Dipotassium phosphate Crystal violet, Trypan Blue, Agar and Distilled water.



Figure (8) Mitis Salivarius Agar

Selective media (for fungi. Molds and yeast):

There are several media, which can be used for isolation of the fungi, these media has specific materials as carbon sources, which can be utilized by the fungi or by adding antibiotics or stains that inhibits or kills the bacteria, such as the following media.

1. Sabouraud Dextrose Agar (PH 5.6) for molds and yeast.

Composed of Peptone, Dextrose and Distilled water.

2. Potato Dextrose Agar. (PH 5.6) contains Potatoes infusion, Dextrose, Agar and Distilled Water.

3. Rose bengal Agar. (PH 4-6) for molds. Contains Malt extract, Rose bengal stain, Streptomycin sulfate, Agar and Tap water.

Appendix: Figures of Lab 4:



Figure (1): Nutrient broth

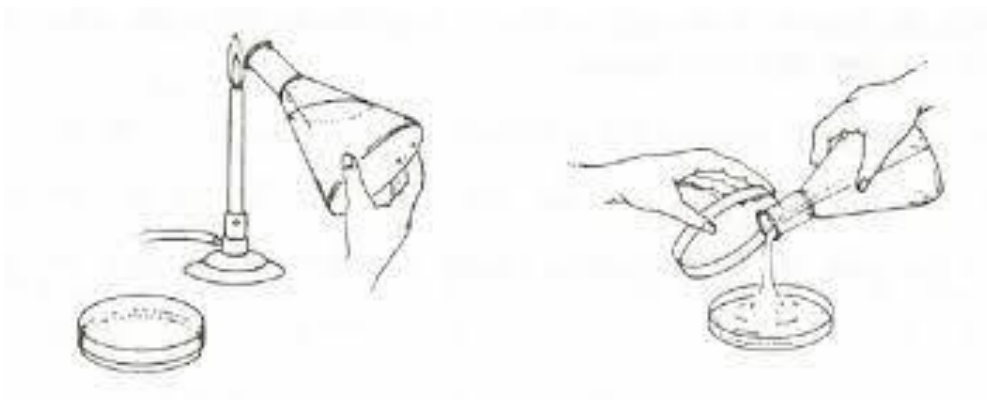


Figure (2) Pouring Agar plates method.

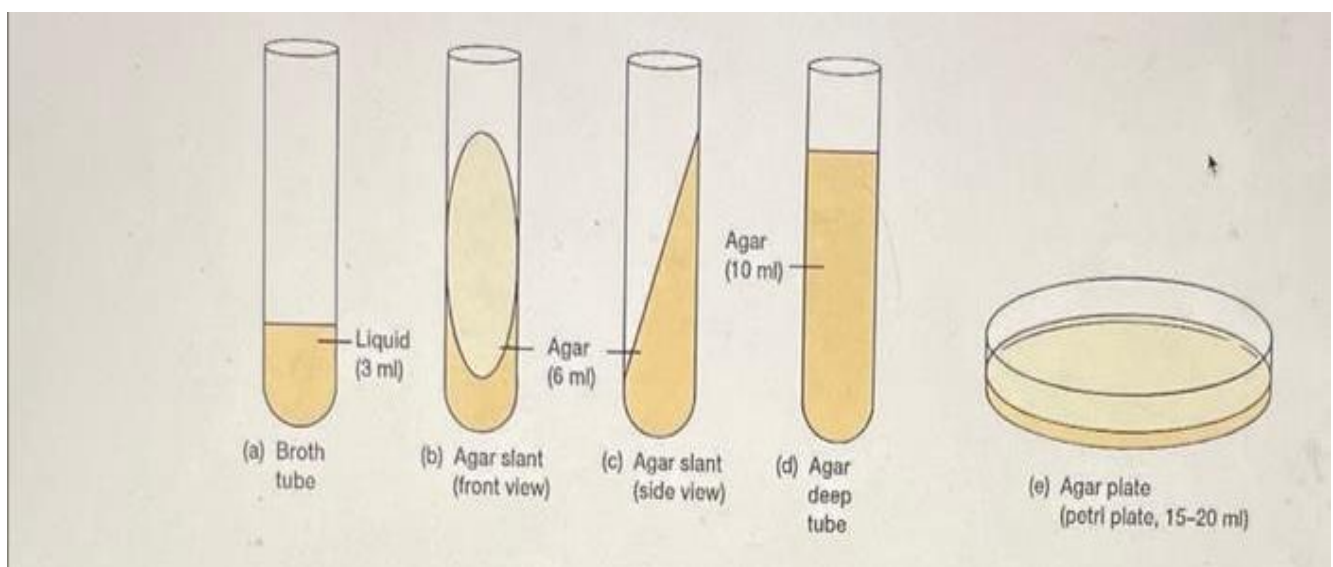


Figure (3) Different forms of culture media



Figure (4-A) MacConky agar.

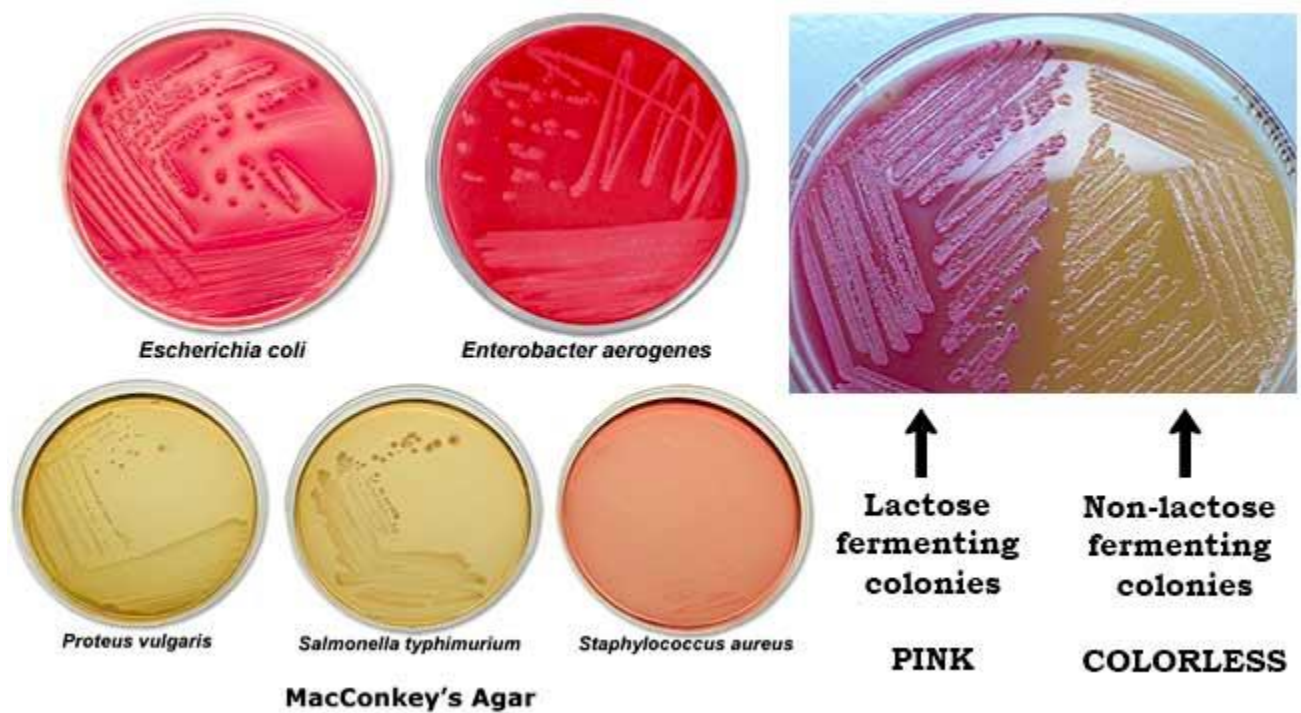


Figure (4-B) MacConky agar.

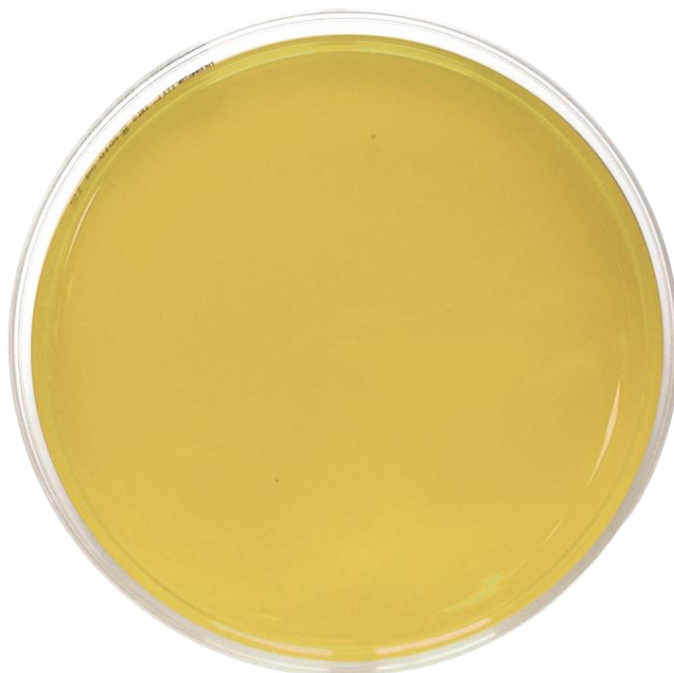
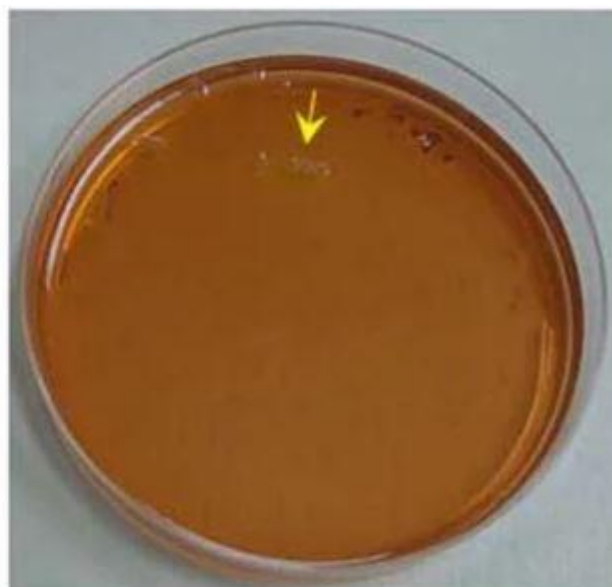


Figure (5-A) Salmonella – Shigella Agar (SS agar)



***Salmonella* on SS Agar**



***Shigella* on SS Agar**

Figure (5-A) *Salmonella* and *Shigella* on SS agar.

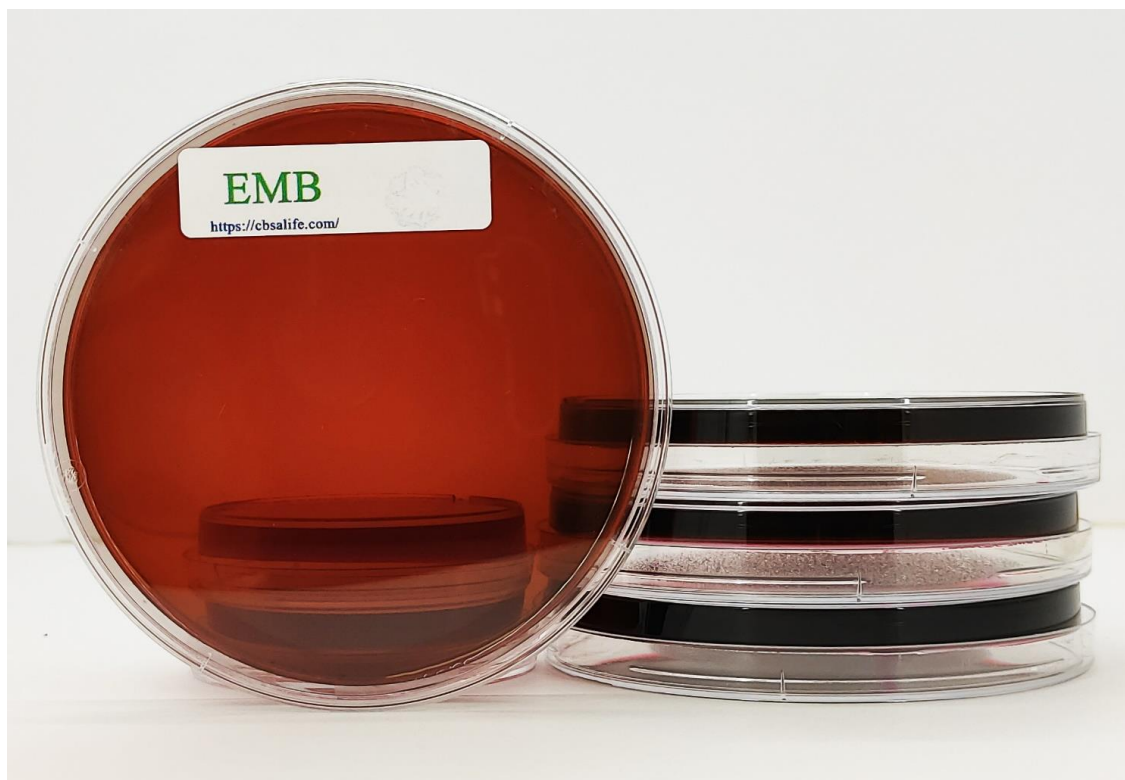


Figure (6-A) – EMB Agar

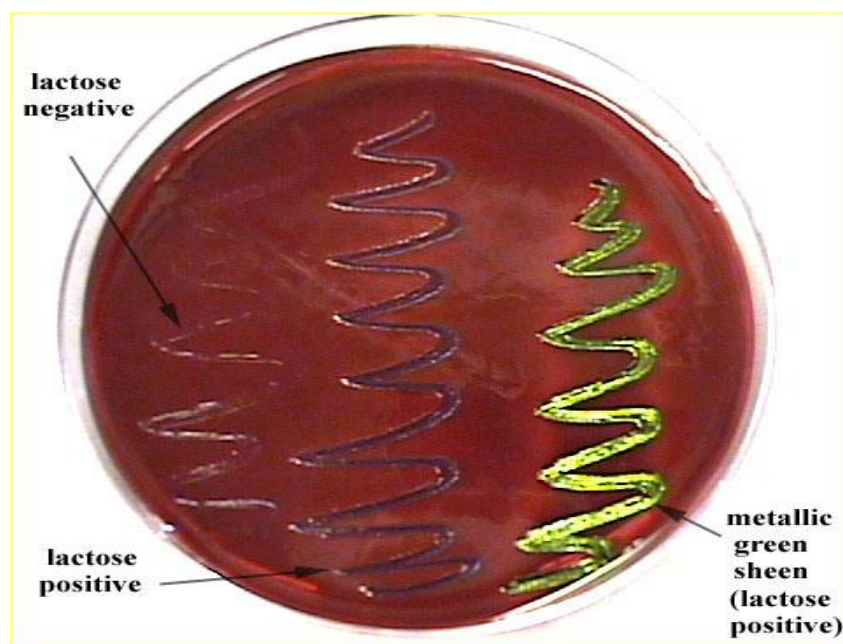


Figure (6-B) – enteric bacteria on EMB Agar



Figure (7-A) – Manitol Salt Agar (MSA)



Figure (7-B) – *Staphylococcus aureus* on MSA.



Figure (8-A) Mitis Salivarius Agar



Figure (8-B) *Streptococcus mitis* on mitis salivarius agar

“Hope you enjoyed the lab”