

**Practical Microbiology**

**Cihan University  
Medical Laboratory Analysis**

**Lab 3: Culture Media, preparation, and their physical, chemical and functional types**

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**MSc: Microbiology & Animal Cell Culture**

**Second stage (1<sup>st</sup> semester)**

**2023-2024**

# Objective

- Students should get the detailed information about the types and composition of culture media regarding:
  - ✓ Physical state.
  - ✓ Chemical composition.
  - ✓ Functional type.
- Methods of their preparation, sterilization and pouring them into petri dishes & test tubes.



# Culture media

- Culture medium or growth medium is a liquid, semi-solid or solid substance designed to support the growth of microbes (bacteria, yeast, fungus, algae, protozoa & virus [animal cells]).
- Culture media contains the nutrients needed to support the growth of microbes.
- ❖ **Culture media provide seven factors:**
  - ✓ Water (Substrate for all reactions).
  - ✓ Carbon (for synthesis of all cell materials).
  - ✓ Energy (to perform cell activities).
  - ✓ Nitrogen (to build cells).
  - ✓ Minerals (for normal growth).
  - ✓ Growth factors.
  - ✓ pH (affects the growth of microbes).



# Physical state

## ➤ Three types:

**1) Liquid media or broth:** does not contain any solidifying agents (agar), remain as liquids even at room temperature, nutrient broth, Tryptic soy broth, and MacConkey broth.

**2) Semi-solid media:** is jelly like media, exhibit a clot like consistency at room temp. because contain an amount of solidifying agent (0.3-0.5 % agar) that thickens them but does not produce a solid. Sulfide Indole Motility Medium (SIM).

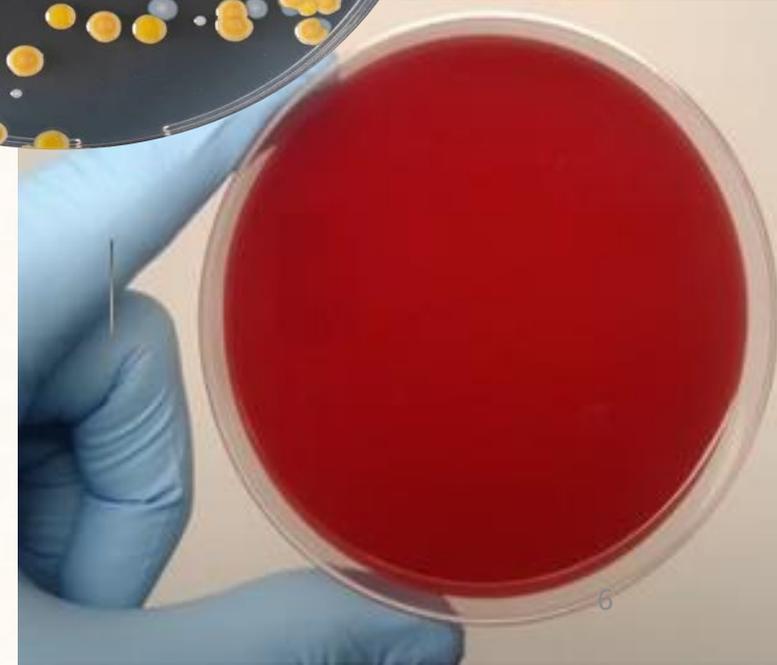
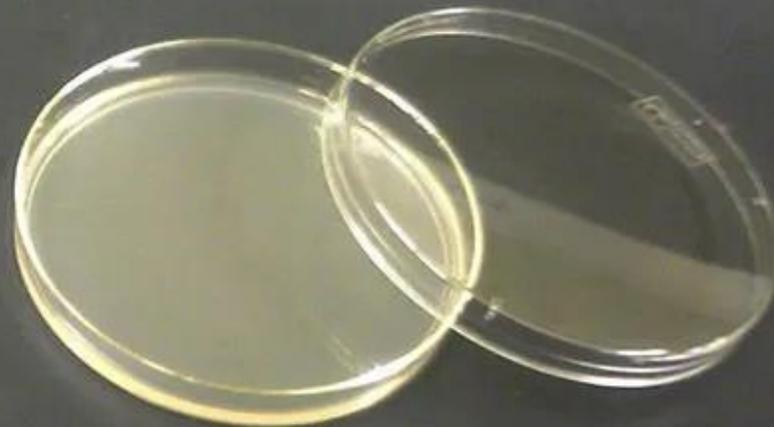


# Physical state

**3) Solid media:** contain solidifying agent (1.5- 2.0% agar), provide a firm surface on which cells can form discrete colonies. Nutrient agar & Mannitol salt agar.

➤ Used for:

- ✓ Isolation of pure culture (bacteria & fungi).
- ✓ To observe colony structure.
- ✓ For observe specific biochemical reactions.



# Agar

- Agar is a polymer of Galactose and sulfur-containing carbohydrates isolated from seaweeds (red algae), used in preparing solid & semi-solid media.
- **Agar is a good solidifying agent:**
  - ✓ That is not utilized by microorganisms.
  - ✓ It is liquid at boiling temperature.
  - ✓ Does not inhibit microbial growth.
  - ✓ Does not liquefy at room temperature.
  - ✓ It solidified below 42 °C.



➤ In the liquid state, solid media can be poured into either a test tube or petri dish:

If the medium in the test tube is allowed to harden in a slanted position, the tube is designated an agar slant.



If the tube is allowed to harden in an upright position, the tube is designated an agar deep tube.



If the agar is poured into a petri plate, allowed to harden, the plate is designated an agar plate.



# Chemical composition

**1) Chemically defined OR synthetic media:**  
the exact formula or ingredients is known both qualitatively and quantitatively.  
Minimal media.

**2) Chemically nondefined or non synthetic media:**  
even one component of a given medium is not chemically definable. Extracted from animal & plant.  
Nutrient agar, Blood agar, MacConkey agar.

0.25 Grams Each of These Amino Acids	0.5 Grams Each of These Amino Acids	0.12 Grams Each of These Amino Acids
Cystine	Arginine	Aspartic acid
Histidine	Glycine	Glutamic acid
Leucine	Isoleucine	
Phenylalanine	Lysine	
Proline	Methionine	
Tryptophan	Serine	
Tyrosine	Threonine	
	Valine	
<b>Additional ingredients</b>		
0.005 mole nicotinamide	}—Vitamins	
0.005 mole thiamine		
0.005 mole pyridoxine		
0.5 micrograms biotin		
1.25 grams magnesium sulfate	}—Salts	
1.25 grams dipotassium hydrogen phosphate		
1.25 grams sodium chloride		
0.125 grams iron chloride		
Ingredients are dissolved in 1,000 milliliters of distilled water and buffered to a final pH of 7.0.		

**TABLE 3.6B**

**Brain-Heart Infusion Broth: A Complex, Nonsynthetic Medium for Growth and Maintenance of Pathogenic *Staphylococcus aureus***

27.5 grams brain-heart extract, peptone extract  
2 grams glucose  
5 grams sodium chloride  
2.5 grams disodium hydrogen phosphate  
Ingredients are dissolved in 1,000 milliliters of distilled water and buffered to a final pH of 7.0.

# Functional types

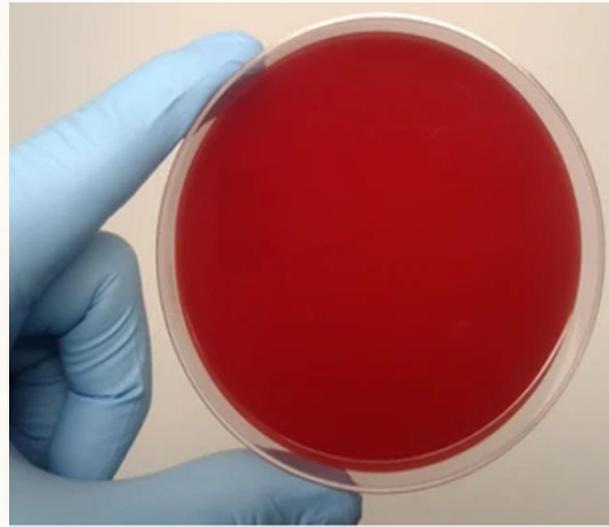
**1) General purpose (basal) media:** are designed to grow broad spectrum of microbes that do not have special growth requirements.

For primary isolation of microbes & enumeration.

Ex: Nutrient agar and Trypticase soy agar.



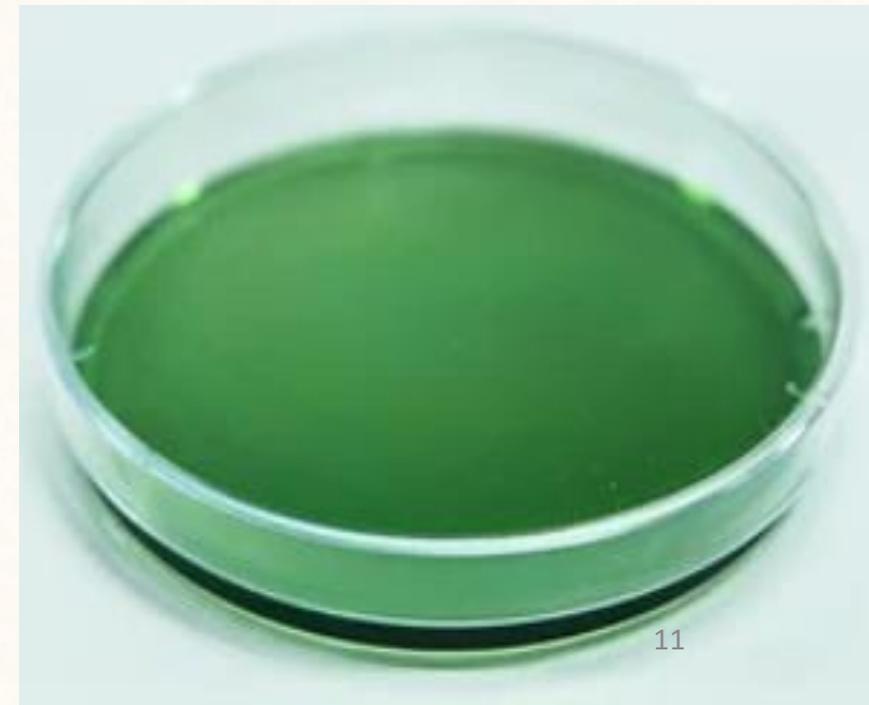
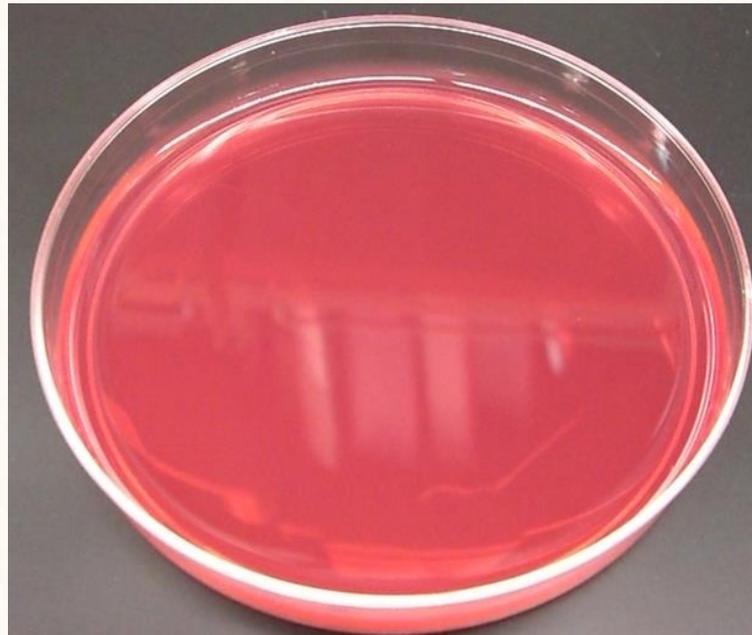
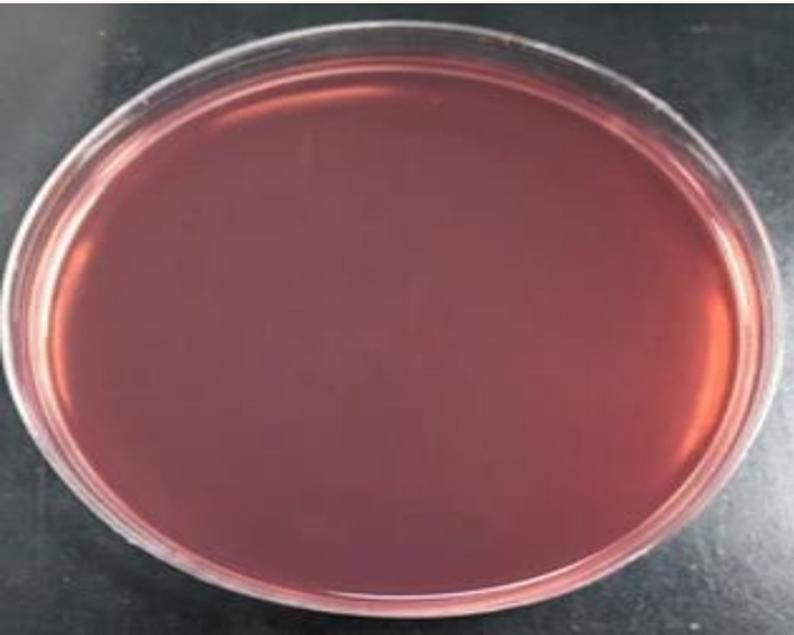
**2) Enriched media:** is a basal media, a complex organic substances such as blood, serum, or growth factors are added to certain species in order to grow. Used for cultivation of fastidious microbes (streptococci) & to detect hemolytic activity. Ex: Blood agar, chocolate agar.



# Functional types

**4) Selective media:** These media contain one or more inhibitory agents that allow the growth of a specific bacterium (desired) by inhibiting the growth of undesired bacteria. For isolation of a specific type of microorganism from samples containing mixtures of different species.

➤ MacConkey agar, Polymyxin-Lysozyme-EDTA-Thallos acetate (PLET) agar, Mannitol salt agar, TCBS Agar, Ceftrimide agar.



# Functional types

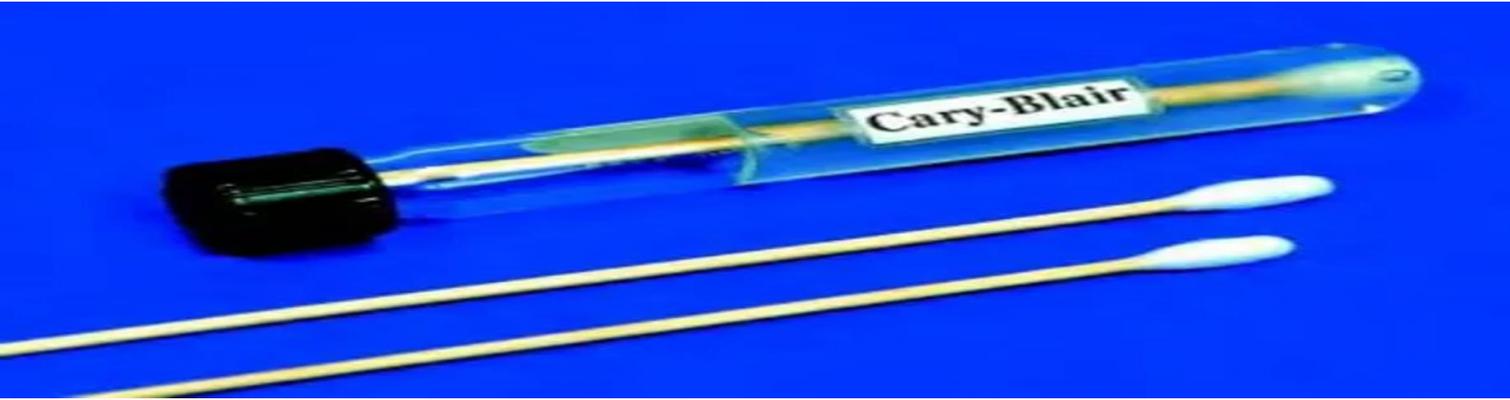
**5) Differential (indicator) media:** these media can differentiate between two groups or two microbes according to characteristic of the colony color or media color changes.

Ex: MacConkey agar, Mannitol salt agar (MSA), Triple-sugar iron agar (TSIA).



# Functional types

**6) Transport media:** These media are used when specimen cannot be cultured soon after collection. Ex: Cary-Blair medium, Amies medium, Stuart medium.



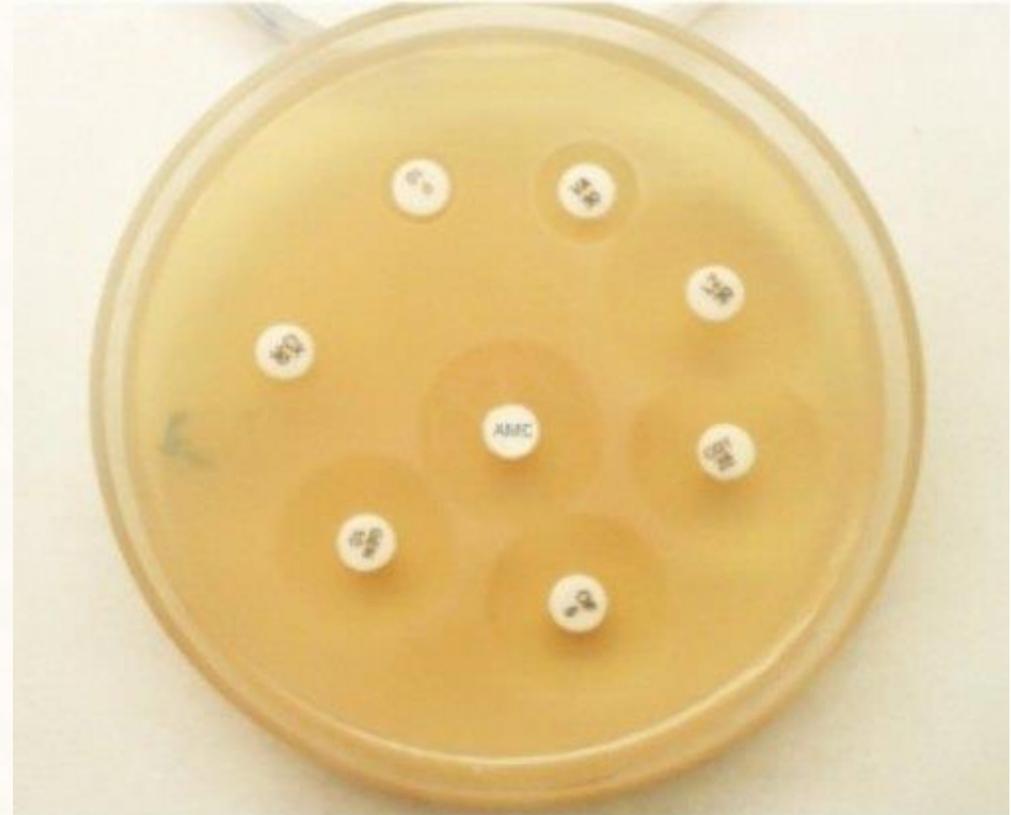
**7) Reducing (anaerobic) media:** contains a substance that absorbs oxygen or slows penetration of oxygen into medium; used for growing anaerobic bacteria. Ex: Thioglycolate broth.



# Functional types

**8) Assay media:** used in some microbiological tests such as antibiotic sensitivity assay, antimicrobial assay (plant extract) and disinfectant assay.

Ex: Mueller Hinton Agar.



# Practical part

## Nutrient agar preparation

- Look at the box to know about the procedure of medium preparation.
- ✓ Suspend 28 gm of nutrient agar powder in 1 liter of distilled water (DW).
- ✓ Heat this mixture while stirring to fully dissolve all components.
- ✓ Autoclave the dissolved mixture at 121 °C for 15 minutes.
- ✓ Once the nutrient agar has been autoclaved, allow it to cool but not solidify.
- ✓ Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.
- ✓ Store the plates in a refrigerator.

# Practical part

➤ **Each subgroup must prepare one of the following media:**

✓ **Nutrient agar (plate, slant and deep tube).**

✓ **Nutrient broth.**

✓ **Mannitol salt agar.**

✓ **MacConkey agar.**

✓ **Blood agar.**

✓ **Mueller Hinton Agar.**

# Report

- **Prepare a report regarding selected medium:**
- ✓ Medium's general description (physical, chemical & functional type).
- ✓ Medium's composition.
- ✓ Protocol for its preparation, sterilization & pouring.
- ✓ Usefulness of a medium.

# References

- ✓ Bauman, R.W. and Primm, T.P. (2018). Microbiology with diseases by body system. Pearson. USA.
- ✓ Talaro, K.P. and Chess, B. (2012). FOUNDATIONS IN MICROBIOLOGY. 8thed. McGraw-Hill Companies, Inc. New York, USA.
- ✓ Leboffe, M.J. and Pierce, B.E., 2011. A photographic atlas for the microbiology laboratory. Morton Publishing Company.
- ✓ Valls. J. S, Nacente . B and Coll. S (2001). Hand book of microbiological culture media. 6th ed. Bercelona, Scharlau Chemie.
- ✓ [http://www.dalynn.com/dyn/ck\\_assets/files/tech/TS58.pdf](http://www.dalynn.com/dyn/ck_assets/files/tech/TS58.pdf)
- ✓ [https://microbeonline.com/types-of-bacteriological-culture-medium/#google\\_vignette](https://microbeonline.com/types-of-bacteriological-culture-medium/#google_vignette)
- ✓ <https://sharebiology.com/minimal-media-davis-formulation/#gs.f1ok1y>
- ✓ <https://microbiologyinfo.com/nutrient-agar-composition-preparation-and-uses/>
- ✓ [https://www.brainkart.com/article/Types-of-Culture-Media\\_17850/](https://www.brainkart.com/article/Types-of-Culture-Media_17850/)