Republic of Iraq
Ministry of Higher Education
And Scientific Research
Al-Mustansiriyah University
College of Dentistry

- Microbiology -

Culture Media II

Classification and Types of Culture Media:-

A. Based on consistency (Physical forms):

B. Based on Nutritional Components:

C. Based on Application:

1. Basal Media:

2. Selective media:

3. Differential (indicator) media

4. Enriched media

5. Special media

6. Transport media

By: Assistant Lecturer Sulaiman Dawod

Lab 5: Culture Media II

3- Differential or indicator media: It contains certain indicators like dyes or metabolic substrates in the medium composition which gives different colors to colonies of different microbial species when they utilize or react with these components. It allows the growth of more than one microorganism, however, the bacterial colonies are differentiated based on their color when a chemical change occurs in the indicator, such as neutral red, phenol red, methylene blue.

(it is basal medium +differential substance) these are capable of differentiating between two organisms by their characteristic reactions towards the medium.

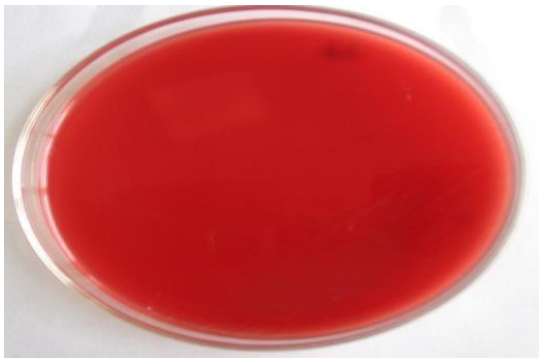
Mostly ,Differential media are contained an indicators, their color changes due the activity (ies) of the microorganism such (oxidation or fermentation of the specific carbohydrates or other c-sources which presents in the medium

Examples are:

1. **Blood Agar:** In blood agar, three types of blood cell lysis or hemolysis are observed: alpha, beta, and gamma hemolysis It allows the growth of many microorganisms, however, their ability to lyse blood cells differs, and this helps to distinguish the bacterial colonies.

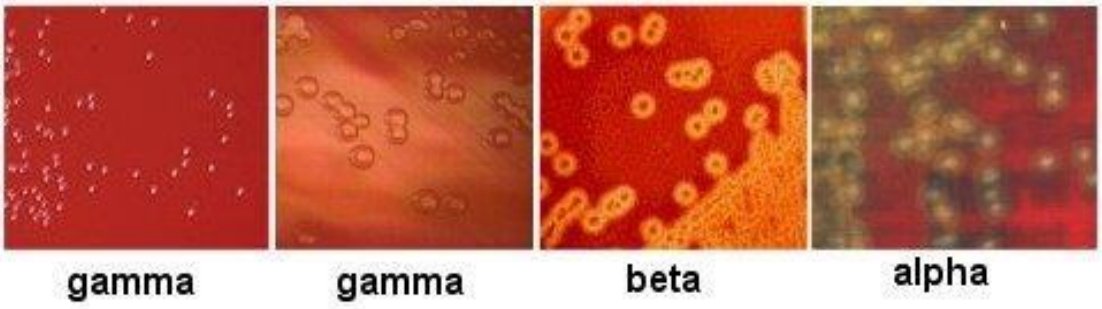
Blood Agar – Differentiation between hemolytic and nonhemolytic

- **a)- Blood agar plate** differentiates between different types of bacteria depending on their haemolytic patterns, as follows:
- **1. Beta- haemolytic**: this has a clear zone around the colony (complete haemolysis or destruction of RBCs) such as *Streptococcus pyogenes*
- **2. Alpha- haemolytic**: this has a green colour around the colony (partial haemolysis or destruction of RBCs) such as *Streptococcus pneumonia*
- **3. Gamma haemolytic(Non- haemolytic):** this shows no haemolysis or destruction of RBCs such as *Streptococcus bovis.*
- Differentiates between different organisms growing on the same plate
- Example: Blood agar



By: Assistant Lecturer Sulaiman Dawod

Fig. (10) : Blood Agar



2. **MacConkey Agar:** (Differentiation between L-F lactose fermenter & non lactose fermenterN.L.F.) It differentiates the Gram-negative bacteria based on their lactose metabolism. The lactose fermenting bacteria, such as *Escherichia coli, Klebsiella spp, Citrobacter,* and *Enterobacter* forms pink-red colonies, while lactose non-fermenters, like *Salmonella, Shigella, Proteus, Providencia, Pseudomonas,* and *Morganella* form pale or colorless colonies.

- **3. Thiosulfate citrate bile salts sucrose (TCBS) agar:** The media contain sucrose, which is utilized by ferment microbes and helps to distinguish them from non-ferment microorganisms.

  Based on this characteristic, different colored bacterial colonies are formed on the media that help to identify and distinguish them from each other.

  For example, *V. cholerae* ferment the sucrose and form slightly flattened yellow colonies having opaque centers and translucent peripheries.
  Whereas, *V. parahaemolyticus* can't ferment the sucrose and forms green to blue-green colonies.

.

3. Eosin – Methylene Blue (EMB) PH 7.2.
4. Sugar Broth – Differentiation between sugar fermenter and nonfermenter.

4 . Enriched Media

In these media substance like blood, serum or egg is added to basal medium e.g., **Blood agar**, **Chocolate agar**, Most of the oral bacteria isolated on enriched media such as brain heart infusion agar or broth, blood agar and tryptic soy agar or broth.

A. Brain – Heart Infusion Agar (PH 7.4)

Calf brains, infusion of beef heart, infusion from proteose peptone, Dextrose, NaCl, Na2 PO4, agar, Dist. Water

BHI broth has the same above material amounts excluding the agar.

Lab 5: Culture Media II

B. Blood Agar Prepare by adding 10% of sterile blood to sterile Nutrient agar at 45-50°C used human blood or sheep blood. These media are considered as enriched and differential for Beta (B) and Alpha (α) hemolytic bacteria.

Prerаration of Blood Agar medium

Dissolve the above materials by heating and sterilize it by autoclaving at 121°c and 15 lbs pressure for 15 minutes, cool it to 50 °c, then add a definite ml of sterile defibrinated blood . Mix thoroughly then pour into plates while it in a liquid phase

C. Chocolate agar

Figure (10) Blood Agar

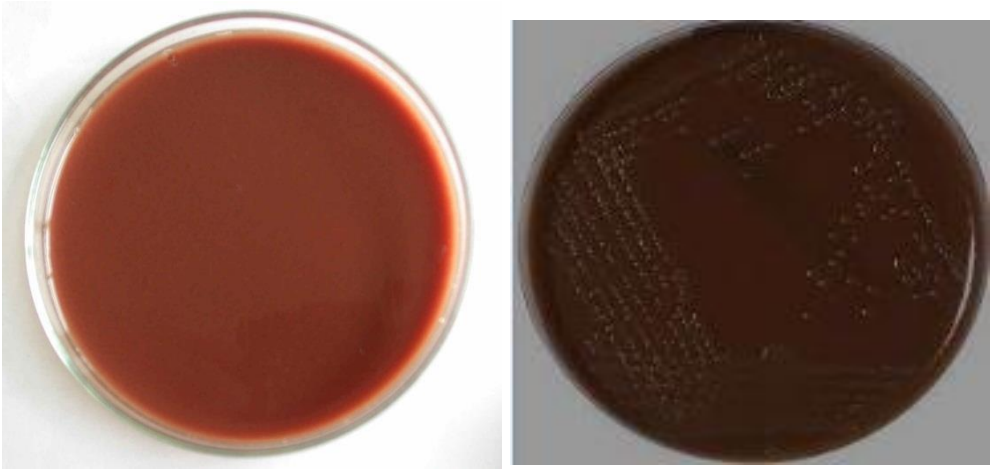


Fig.(11) Chocolate Agar

Chocolate Agar

- Prepare by :
- 1-Adding 10% of sterile blood to the heated Nutrient agar (more than 50°C).and the R.B.C. will be lysis and V&X factors are released to the media.

2- By putting the blood agar in oven or in incubator at 55°C or

more

for 1-2 hours. Notify the figure 13

5-Special Media

- Used for one type of bacteria
- For example: Lowenstein — Jensen medium used for the cultivation of Mycobacterium tuberculosis
- This contain Malachite green which inhibition all groups of bacteria except Mycobacterium Spp. and contain glycerol inhibit M.bovis and allow to growth Mycobacterium tuberculosis

Figure (12) Lowenstein – Jensen Medium

6-Transport media: used to sustain sensitive organisms for a short period. They are inhibit self destruction and multiplication of microorganism. Various media are used for transporting different types of specimens like:

- **Stuart transport medium**: is used for CSF and cervical specimens, this medium preserves both *Neisseria* species as well as *Trichomonas vaginalis*

-**Cary-Blair** and **Amies transport media**: are used for transporting faecal material on rectal swab, and are effective in preserving *Salmonella, Shigella* and *Vibrio*.

THERE IS ANOTHER CLASSIFICATION OF MEDIA ACCORDING TO REQUIREMENT OF $O_{2(OXYGEN)}$

- Role of Oxygen. Bacteria may be classified into four groups on oxygen requirement :

1. **Aerobes.** They cannot grow without oxygen, e.g. *Mycobacterium* tuberculosis. In laboratory cultures of **aerobic organisms** are maintained in Petri dishes or test tubes under aerobic conditions by using ordinary incubators (Aerobic media ,all types of media, which incubate in incubator in aerobic condition)
2. **Facultative anaerobes.** These grow under both aerobic and anaerobic conditions. Most bacteria are facultative anaerobes, e.g. Enterobacteriaceae.

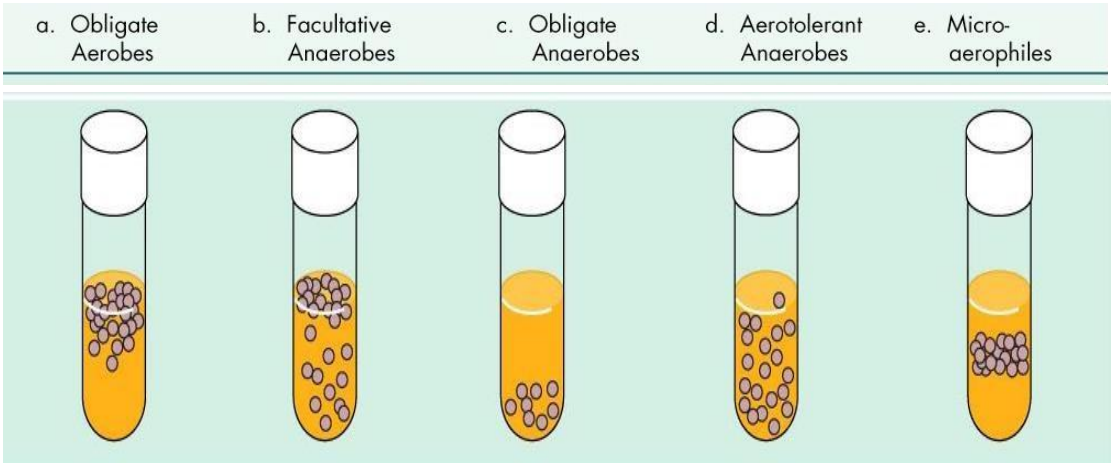
By: Assistant Lecturer Sulaiman Dawod

Lab 5: Culture Media II

3. Anaerobes . They only grow in absence of free oxygen, e.g. Clostridium, Bacteroides. For anaerobic cultures **two main methods** may be used such as **thioglycollate broth method**, this is one of the most common methods of producing an anaerobic environment for microbes by introducing a reducing agent (sodium thiogLycollate) into a liquid medium. The reducing agents remove oxygen from the liquid medium through a chemical reaction. The other method by using the **Brewer anaerobic jar method and gas- pak system**, the anaerobic condition of the Brewer jar is created by physically evacuating the air out with the help of a vacuum pump and avacuated space is filled with an inert gas like hydrogen, nitrogen or carbon dioxide whereas in gas pak system, oxygen is chemically removed from the chamber.

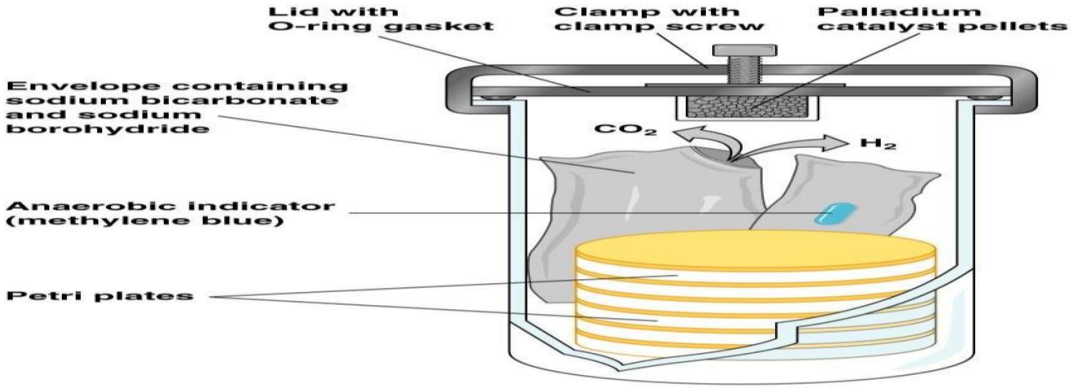
So, the **Anaerobic media:** This media is for anaerobic bacteria which require low oxygen levels, extra nutrients, and reduced oxidation-reduction potential. It is supplemented with hemin and vitamin K nutrients and oxygen is removed by boiling it in a water bath and sealing it with paraffin film Examples are: Thioglycollate broth and Robertson Cooked Meat (RCM) medium which is commonly used to grow Clostridium *spp*

4. Microaerophils grow best in oxygen less than that present in the air, with carbon dioxide (5-10% CO2) e.g. *Campylobacter*. **microaerophiles** cultures are incubated in candle jar or CO2 incubator



By: Assistant Lecturer Sulaiman Dawod

Lab 5: Culture Media II

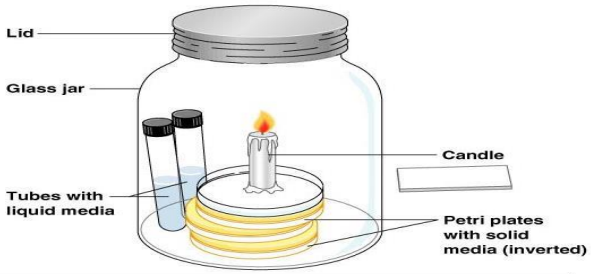


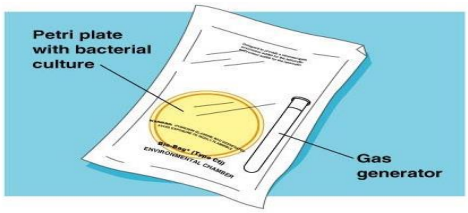
Anaerobic Culture Methods

2) Anaerobic chamber

3) Candle jar

4) CO_2-packet





By: Assistant Lecturer Sulaiman Dawod