

## Culture media

Culture media are environments for microorganism growth. They are considered as the energy source for this M.O., due to their Composition.



### Composition of culture media:

When bacteria culture, it is very important to provide similar environmental and nutritional conditions that exist in its natural habitat. Hence, an artificial culture medium must provide all the nutritional components that a bacterium gets in its natural habitat. Most often, a culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors. Besides these, the pH of the medium must be set accordingly.

### Classification:

Bacterial culture media can be classified in at least three ways; Based on consistency, based on nutritional component and based on its functional use.

#### 1. Classification based on consistency( Physical state):

- A) Liquid media
- B) Solid media
- C) Semi-solid agar

To obtain a solid and semi- sold culture media we can use one of the following solidification agents:

**Gelatin:** it is a protein substance, it is added at the amount of 10 -15 % of the media. It is soluble at incubator temperature degree (30- 36)<sup>0</sup>C, and it's analyzable by the most types of germs. Therefor it usage limited for incubation of viruses and living cells such as cancer cells, human, animal and plant cells.

**Agar –agar:** it is a complex carbohydrate substance obtained from algae (sea weeds). It is added at the rate of 0.3- 0.5 and 1.2- 2% of media to obtain semi-solid and solid media. It is never analyzable by the most types of M.O. because

it has not nutritive value. It is dissolved at 90 -100 °C and remained liquid at 42- 50 °C therefor it is usage in M.O.

## 2. Classification based on nutritional component:

- a. **Natural media:** environment containing fresh natural compounds like peptone and meat extract, they are used for the most types of M. O.
- b. **Synthetic media:** environment containing chemical substance like inorganic salts and organic compounds. They are used to isolate special types of M.O.
- c. **Semi- synthetic media:** they are composed between natural and chemical substances. The most culture media belong to this type.

## 3. Classification based on functional use or application:

A) **Basal media** : are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar considered basal medium.

B) **Enriched media:** Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Such as Blood agar, chocolate agar.

C) **Selective media:** They are prepared by adding chemical substances like crystal violate and methylene blue dyes to the simple culture to prevent the growth of some germs without inhibition the growth of others, for example MacConkey agar and Eosin methylene blue agar.

D) **Enrichment media:** are liquid media that contain substance inhibited the growth unwanted organism but favour the free growth of organisms desired to grow, such as alkaline peptone water and salt meat broth

E) **Differential media:** Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony color. It is simple media with some factors and chemical substances make many

changes in the growth of germs and differentiate between them, such as blood agar which differentiate hemolytic bacteria from non-hemolytic.

**F) Assay media:** they are constant chemical substance used for test some substance such as vitamins, amino acids and antibiotics, for example Mueller Hinton media which used for test the activity of antibiotics against M.O.

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

**H) Maintenance media:** they are simple media used for maintaining bacteria activity and their physiological properties for a long time, like nutrient broth plus 10% glycerol, this media maintained the bacteria growth at 70°C for a long time.

### Filling of culture media

1. Liquid culture media: they are filling before sterilization in the test tube.
2. Solid culture media: they are filling before sterilization in the test tube to obtain a deep agar in slob position, while are filling in petri dish after sterilization and this method know pour plate.

Sterilization of culture media were achieved by autoclaving at 121 °C of 15 lb/inch<sup>2</sup> for 15 minutes, after adjusted pH for (7.2) .

### Methods :

1. Distribute the broth (nutrient broth )in the test tube by pipette as equal of 5 ml for each tube then close it, if the tubes were glass then close them by cotton capped then put them in the special carrier and transport it in the sterilization system (autoclave).

2. Distribute the agar (nutrient agar) in the test tube by pipette as equal of 5 ml for each tube at 45- 50 °C, then close it, then put them in the special carrier and transport it in the sterilization system (autoclave), after sterilization, put closed side of the test tube in high position about 1 cm and put the other a deep position 0 cm, tile solidification.
3. Pour plate method
  1. Clean and disinfect the work table and put petri dish adjacent benzene burner.
  2. Put the flask which contain the culture media in the water path at 50 °C or leave it tile cooled to 45 -50 °C.
  3. Open the flasks which contain the culture media and sterilize the mouth of the flask by the flam, then put 15-20 ml of the media in the petri dish and close it, sterilize the mouth of the flask again and close it.
  4. Remove the air which formed inside or around the media in the petri dish by expose it to the flame directly.
  5. Leave the petri dish to cool then put them in the refrigerator until used.