

Lab3 : Culture Media

Culture media, also known as growth media, are specific mixtures of nutrients and other substances that support the growth of microorganisms such as bacteria and fungi (yeasts and molds).

Requirements for the growth of microorganisms :

- 1)Water
- 2)Source of energy
- 3)Source of carbon and nitrogen
- 4)Mineral elements
- 5)Vitamins



Objective of using culture media

- 1- Growth and isolation of microorganisms in vitro.
- 2 – Isolated colonies of bacteria and identify pathogen.
- 3 – Study the characteristics of microorganisms for the purpose of diagnosis.
- 4 –Preservation of microorganisms for a long period.
- 5 – Preparing vaccines.
- 6 – Genetic engineering experiments.

Culture Media Preparation :

- Tools , Glassware , and instruments :



Conical flask



Petri dish



Cotton



Electronic balance



Graduated cylinder



Bunsen burner



Autoclave

Preparation steps :

First step : calculation and weighing of media powder using calculator and sensitive balance .

Second step : Mixing media with heating using hot plate/ magnetic stirrer.

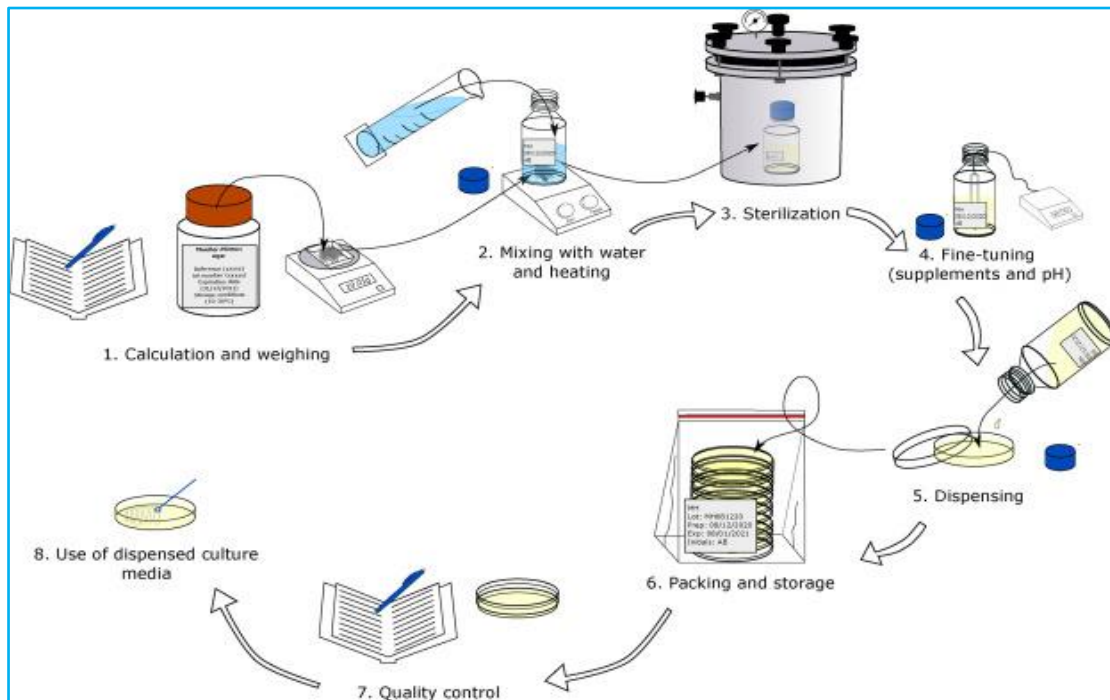
Third step : Sterilization of culture media using autoclave .

Fourth step : Fine- tuning , such as pH .

Fifth step : Pouring petri dishes.

Sixth step : packing and storage .

Seventh step : Quality control.



The culture media are classified according to the **physical state of the media**, into:

A – Solid Media : It is similar in shape and clear texture. The reason for its stiffness is the addition of agar to it that hardens the media.

B – Liquid Media : This media contains the same contents as the solid media without agar.



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Semi solid media C- Semisolid media : are prepared with agar at concentrations of 0.5% or less. It is useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

ROUTINE LABORATORY MEDIA

These are classified into six types: (1) Basal media, (2) Enriched media, (3) Selective media, (4) Indicator media, (5) Transport media, and (6) Storage media.

1. BASAL MEDIA; Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.

2. ENRICHED MEDIA; The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar. Streptococci grow in blood agar media.

3. SELECTIVE MEDIA; These media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: **MacConkey agar, Lowenstein-Jensen media, tellurite media** (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli). Antibiotic may be added to a medium for inhibition.

4. INDICATOR (DIFFERENTIAL) MEDIA; An indicator is included in the medium. A particular organism causes change in the indicator. Examples: **Blood agar and MacConkey agar** are indicator media.

5. TRANSPORT MEDIA;

Transport media these criteria should fulfill:

- ❖ Temporary storage of specimens being transported to the laboratory for cultivation
- ❖ Maintain the viability of all organisms in the specimen without altering their concentration
- ❖ Contain only buffers and salt
- ❖ Lack of carbon, nitrogen, and organic growth factors so as to prevent microbial multiplication
- ❖ Transport media used in the isolation of anaerobes must be free of molecular oxygen.

Examples of transport media:

- Thioglycolate broth is for strict anaerobes.
- Stuart transport medium.

6. STORAGE MEDIA; Media used for storing the bacteria for a long period of time.

Examples: Egg saline medium, chalk cooked meat broth.

COMMON MEDIA IN ROUTINE

Blood Agar; Most commonly used medium. 5- 10% defibrinated sheep or horse blood is added to melted agar at 45-50°C. Blood acts as an enrichment material and also as an indicator. e.g. *Streptococcus pyogenes* is a beta haemolytic streptococci, (b) Alpha (a) haemolysis, (the colony is surrounded by a zone of greenish discolouration due to formation of biliverdin), e.g. *Viridans streptococci*, (c) Gamma haemolysis, or, No haemolysis. There is no change in the medium surrounding the colony.

MacConkey Agar; Most commonly used for enterobac-teriaceae. It contains agar, peptone, sodium chloride, bile salt, lactose and neutral red. It is a selective and indicator medium: (1) Selective as bile salt does not inhibit the growth of enterobacteriaceae but inhibits growth of many other bacteria. (2) Indicator medium as the colonies of bacteria that ferment lactose take a pink colour due to production of acid. Acid turns the indicator neutral red to pink. These bacteria are called 'lactose fermenter', e.g. *Escherichia coll.* Colourless colony indicates that lactose is not fermented, i.e. the bacterium is non-lactose fermenter, e.g. *Salmonella*, *Shigella*, *Vibrio*.

Mueller Hinton Agar; Disc diffusion sensitivity tests for antimicrobial drugs should be carried out on this media as per WHO recommendation to promote reproducibility and comparability of results.

