

Common ingredients of culture media:

- Water: essential for bacterial growth, use deionized or distilled water.
- Peptone: from hydrolysed animal or plant protein, it provides nitrogen and amino acid.
- Meat extract: provides amino acid, vitamins, mineral salts (phosphate and sulphate).
- Yeast extract: used to stimulate the growth of bacteria.
- Mineral salts: traces of magnesium, potassium, iron and calcium which are essential for bacterial enzyme activity.
- Carbohydrates: to provide bacteria with energy and carbon source.
- Agar: inert polysaccharide from sea weed or marine algae, it is solidifying agent with concentration of 1-2%, dissolves at 90-100 °C, solidify at 45 °C.

Forms of media:

- Liquid form (broth): without agar (no solidifying agent), used to grow bacteria in large quantity, the growth appear as turbidity and if no growth it appear clear.
- Solid form: by adding agar, it can be slant or deep agar which is used to keep bacteria for long time (up to 3 months), agar plate can be used to have isolated colonies that help identification.

Pure culture: culture containing only one type of bacteria to study them. It is impossible to study the bacteria when other organisms are present.

Preparation of media:

All constituents of media should be weighed and mixed as indicated in instruction on the bottle.

Example: calculate how many grams needed for 100ml media?

20g in 1000ml (stated in instruction)

So, for 100ml

$100 \times 20 / 1000 = 2 \text{ g}$

- When we want to add material sensitive to heat, we add them after sterilization. Example is the blood that should be added to the cooled media after sterilization.

Pure culture technique

The act of organism culturing into the media is called inoculation or streaking.

- The common method to obtain pure culture (isolated colony) is dry dilution that should be done under septic conditions to prevent growth of contaminants.

Types of media and their functions

- 1- **Basal media:** allows growth of most non pathogenic bacteria. E.g. nutrient agar.
- 2- **Enriched media:** when the basal agar has been enriched through adding blood or serum. To allow the growth of pathogenic bacteria. E.g. blood agar.
- 3- **Selective media:** has certain inhibiting agent to inhibit the growth of some bacteria and allow growth of others.

Example: macconkey agar (Mac): contains bile salt and crystal violet as inhibiting agent. It allows growth of gram negative bacteria and inhibits growth of gram positive ones.

4- Differential media: contains indicator that can differentiate between two types of bacteria.

Examples

- Macconkey(Mac): to differentiate **between lactose fermenting bacteria (LF) and non lactose fermenting ones(NLF)**. The media contains sugar (lactose) and indicator (neutral red).
LF bacteria (such as E.coli) ferment lactose and produce acid + indicator.....pink color.
NLF bacteria (such as proteus) are not able to ferment lactose +indicator.....colorless.
- EMB: **differentiate between LF and NLF**. It has sugar (lactose) and indicator (eosin+methylen blue). E.coli on EMB gives green metallic sheen.
- Mac and EMB are selective and differential media.
- CLED (cystine lactose electrolyte deficient): **differentiate between LF and NLF**. It has sugar (lactose) and indicator (bromo thymol blue). LF appears yellow and NLF appears colorless.
- CLED is only differential but not selective.