

Preparation of culture media (liquid & solid) for bacterial cultivation

Principle:

Bacterial culture medium is solid or liquid source of nutrients that support growth of bacteria. Although bacteria may vary in their nutritional requirements and some require specialized environment to grow, there are certain media, that can support growth of wide range of bacteria, for example nutrient media.

Part-A: Broth medium:

The liquid culture medium, devoid of agar, used for cultivation of bacteria is called broth medium. The required ingredients are dissolved in distilled water and pH is adjusted before making up the desired volume. Sterilization of culture medium is generally achieved by autoclaving or membrane filtration technique (for heat labile ingredients).

Requirements:

- Required ingredients
- 1 N HCl / 1 N NaOH – for pH adjustment
- pH paper/pH meter
- Distilled water
- Autoclave, heater
- Glassware: Culture tube, Conical flask, Measuring cylinder, Pipette etc.
- Electronic balance

Procedure:

- 1) The required ingredients are weighed in electronic balance accurately and transferred into conical flask.
- 2) The ingredients are dissolved (heated if required) in distilled water of about 70% of the desired final volume.
- 3) pH of the mixture is measured and adjusted to required value by adding acid/alkali when necessary.
- 4) The mixture is taken in a measuring cylinder and the final volume is adjusted by adding distilled water to it.
- 5) This culture media is sterilized by autoclaving (generally it is 15 PSI pressure, 121⁰ C temperature for at least 15 mins.) or by membrane filtration (for heat labile substances).

Part-B: Agar medium

When broth medium is solidified using agar agar (or simply agar), it is called agar medium. For example, Nutrient Agar is used for cultivation of bacteria. Agar is nitrogen free complex polysaccharide possessing 3,6-anhydro-L-galactopyranose units. Agar medium is generally solidified as agar plate, agar slants, agar deep tubes for routine cultivation of microorganisms. Agar medium is used for studying colony morphology, counting CFU (colony forming unit), preservation of pure bacterial culture etc.

Requirements:

- Broth medium
- Agar powder
- Heater, Autoclave
- Glassware- Conical flask. Culture tubes, petridishes etc.
- Electronic balance
- Laminar air flow

Procedure:

- 1) Prepared broth medium (pH adjusted) is transferred to conical flask.
- 2) 1.5% w/v agar is accurately weighed and added to it.
- 3) Contents of the flask is gently heated to dissolve the agar powder.
- 4) Sterilization is done by autoclaving at 15 psi pressure, 121⁰ C temperature for 15 minutes.
- 5) For stab culture, melted agar medium in test tubes are allowed to solidify at room temperature in vertical position in test tube stands.
- 6) For slant preparation, melted agar medium in test tubes are allowed to solidify at room temperature in slanted position so that the media should not touch the cotton plug.
- 7) For agar plate preparation, melted agar medium is poured aseptically to petridishes in laminar air flow hood and allowed to solidify.