

Microbiology Laboratory

Lab 8: Preparation of culture media

What is Culture Media?

The media is a source of nutrients to support the growth of the micro-organisms in-vitro. The media helps in the growth and counting of microbial cells, selection of microorganisms, and survival of microorganisms. The culture medium can be liquid or gel.

Microorganisms have varying nature, characteristics, habitat, and even nutritional requirements; thus, it is impossible to culture them with one type of culture media. However, there are also microorganisms that can't grow on a culture media at all in any condition – these are called obligate parasites. Culturing microorganisms is essential for diagnosing infectious diseases, obtaining antigens, developing serological assays for vaccines, genetic studies, and identification of microbial species.

Common ingredients of culture media

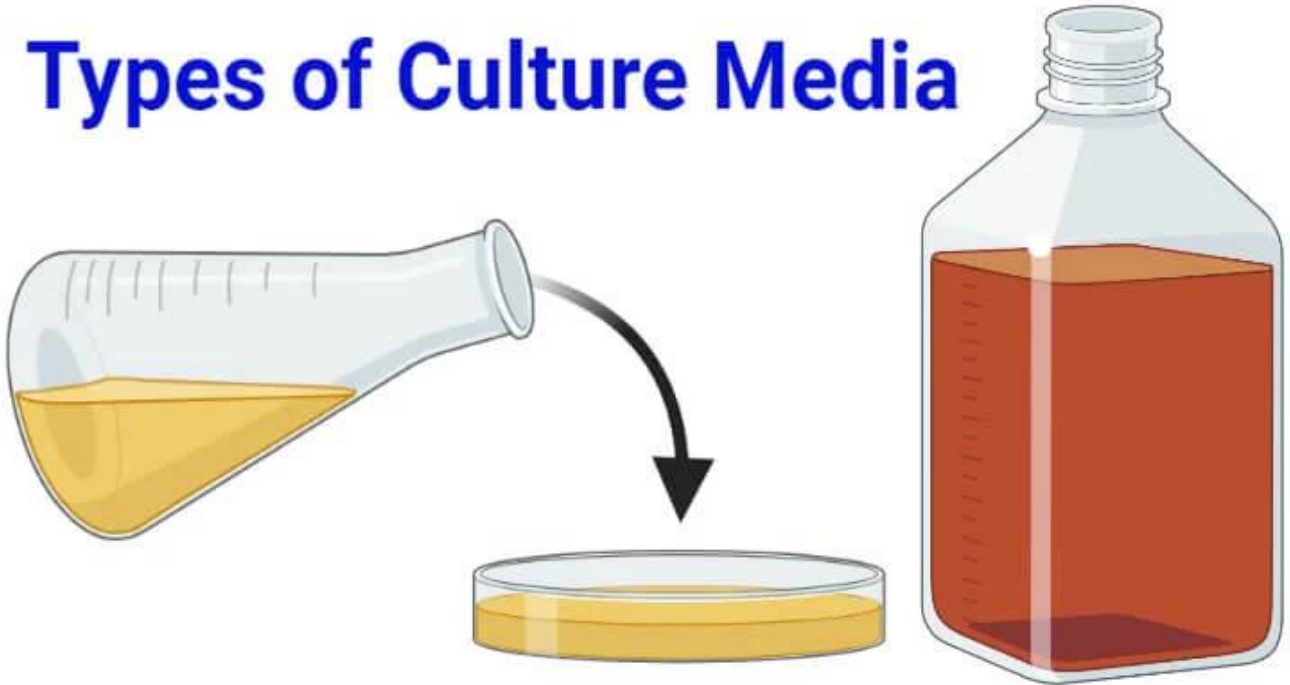
- **Peptone**- source of carbon and nitrogen.
- **Beef extract**- source of amino acid, vitamins, minerals.
- **Yeast extract**- source of vitamin, carbon, nitrogen.
- **Distilled water**
- **Agar**- solidifying agent.

How to prepare culture media?

1. Weigh the amount of ingredients powder on weighing machine.
2. Dissolve the ingredients in distilled water.
3. Adjust PH of the medium if needed.
4. Add agar and boiled it to dissolve.
5. Pour the media into flask.
6. Autoclave the media when ingredients fully dissolve.
7. Sterilization is done in autoclave to prevent from contamination, at 121°C for 15 min at 15lbs.
8. After the autoclave place the media flask in laminar air flow.
9. Sterilize the laminar air flow with 70% alcohol.
10. A bit cools down the media and pours into sterile Petri-plates for solidification.
11. Then sample is ready to spread(spreaders) / streak
12. (Inoculation loop) on the medium for identification or isolation of microbes.

13. Sealed the Petri plates with paraffin, label them.
 14. Keep them inverted in incubator at 37°C for 24hrs.
 15. Observe the result next day colonies formation is visible on the media.
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Types of Culture Media

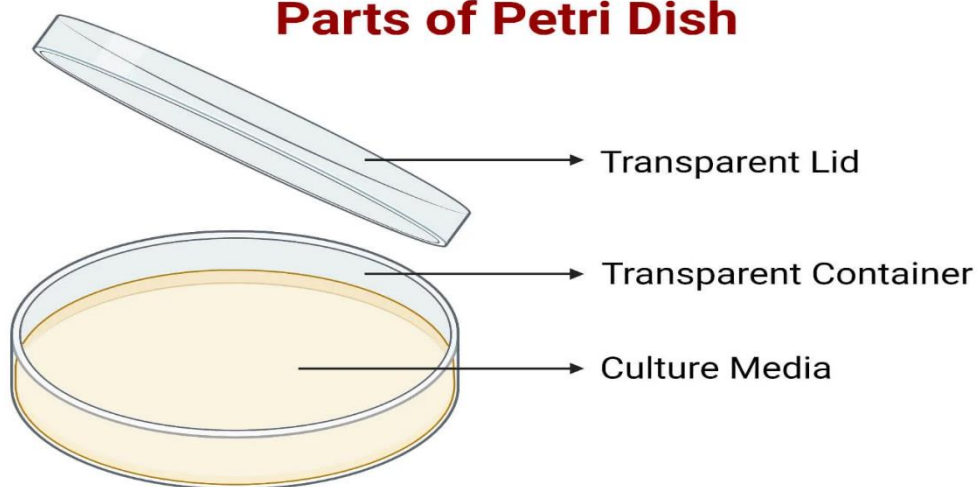


Petri Dish

A petri dish is a small shallow transparent dish with a lid that is mainly used in biological experiments for the culture of cells.

For instance, in microbiological experiments, a **Petri dish** is used as a container to grow microbes with growth media in it. It is derived from the name of its inventor, German bacteriologist Julius Richard Petri. It is also called a Petri plate or culture plate.

Parts of Petri Dish



Types of culture media based on consistency/ physical state

1. Solid medium

2. Semi-solid medium

3. Liquid medium

1. Solid media

It is for the isolation of bacteria as a pure culture on a solid medium. Robert Koch realized the use of solid media. Agar is used to hardening the media at 1.5- 2.0% concentration. Solid media allows the growth of bacteria as colonies by streaking on the medium. It solidified at 37 degrees Celsius. Agar is an un-branched polysaccharide extracted from red algae species like Gelidium. Colonies identification is done on this medium. **Examples of Solid Media:** Nutrient agar, MacConkey agar, Blood agar, Chocolate agar. Growth of bacteria on solid medium **appear as smooth, rough, mucoid, round, irregular, filamentous, punctiform.**

2. Semi-solid media

This media shows the motility of bacteria and the cultivation of microaerophilic bacteria. This media has agar at a concentration of 0.5% or less. It has a jelly consistency. **Examples of Semi-solid media:** Stuart's and Amies media, Hugh and Leifson's oxidation fermentation medium, and Mannitol motility media. The growth of bacteria in semi-solid **appears as a thick line in the medium.**

3. Liquid media

This media shows the growth of a large number of bacteria. It is called Broth that allows bacteria to grow uniformly with turbidity. The growth occurs at 37°C in an incubator for 24hrs. Liquid media don't have the addition of agar; it is for fermentation studies. **Examples of Liquid media** Nutrient broth, Tryptic soy broth, MR-VP broth, phenol red carbohydrate broth. Growth of bacteria in liquid media- **Turbidity is seen at the end of the broth.**

Types of culture media based on chemical composition/application: There are seven routine laboratory media.

1. Basal media
2. Enriched media
3. Selective media
4. Enrichment media
5. Indicator media or differential media.
6. Transport media
7. Storage media.

Application of culture media

1. To culture microbes.
2. To identify the cause of infection.
3. To identify characteristics of microorganisms.
4. To isolate pure culture.
5. To store the culture stock.
6. To observe biochemical reactions.
7. To test microbial contamination in any sample.
8. To check antimicrobial agents and preservatives effect.
9. To observe microbe colony type, its color, shape, cause.
10. To differentiate between different colonies.
11. To create antigens for laboratory use.
12. To estimate viable count.
13. To test antibiotic sensitivity.

Limitations of culture media

1. Risk of cross-contamination.
2. High skill required for optimal results.
3. Increased drying out of media can occur.