**Microbiology Lab.**

**Bacterial Culture Media**

Culture media contains nutrients and physical growth parameters necessary for microbial growth. Organisms that cannot grow in artificial culture medium are known as **obligate parasites**. *Mycobacterium leprae, rickettsias, Chlamydias,* and *Treponema pallidum* are obligate parasites.

* **Classification of media according to consistency**

1. **Solid medium.**

Solid medium contains agar at a concentration of 1.5-2.0%. Solid medium has physical structure and allows bacteria to grow in informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

1. **Semisolid media**.

They are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of[microaerophilicbacteria](https://microbeonline.com/oxygen-requirements-for-pathogenic-bacteria/)or for [determinationofbacterial **motility.**](https://microbeonline.com/tests-bacterial-motility-procedure-results/)

1. **Liquid (Broth) medium**.

These media contains specific amounts of nutrients but don’t have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests.

* **Classification of Media based according to function**

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

1. **General media**

Basal media are basically simple media that supports most non-fastidious bacteria. These media are generally used for the primary isolation of microorganisms, such as Peptone water, nutrient broth and nutrient agar.

**2. Enriched medium**

Addition of extra nutrients in the form of blood, serum, egg yolk etc, to medium makes them enriched media, like [blood agar](https://microbeonline.com/blood-agar-composition-preparation-uses-and-types-of-hemolysis/)\*\*\* and chocolate agar.

**3. Selective media**

Selected media are designed to inhibit unwanted bacteria and support the growth of some other microorganisms. Any agar media can be made selective by addition of certain inhibitory agents that don’t affect the pathogen of interest, like antibiotics, dyes, chemicals, alteration of pH or a combination of these.

**Examples of selective media include:**

1. [**Thayer Martin Agar**](https://microbeonline.com/thayer-martin-agar-composition-preparation-uses-colony-characteristics/) used to recover *N. gonorrhoeae* contains antibiotics; vancomycin, colistin and nystatin.
2. [**Mannitol Salt Agar**](https://microbeonline.com/mannitol-salt-agar-msa-composition-uses-and-colony-characteristics/) and Salt Milk Agar used to recover *S. aureus* contain 10% NaCl.
3. **Potassium tellurite medium** used to recover *C. diphtheriae* contains 0.04% potassium tellurite.
4. [**MacConkey Agar**](https://microbeonline.com/macconkey-agar-mac-composition-preparation-uses-and-colony-characteristics/) used for [Enterobacteriaceae](https://microbeonline.com/seven-common-characteristics-family-enterobacteriaceae/) members contains bile salt that inhibits most gram positive bacteria.
5. **Cetrimide Agar** used to recover *P. aeruginosa* contains cetrimide (antiseptic agent).
6. **Crystal Violet Blood Agar** used to recover *S. pyogenes* contains 0.0002% crystal violet.
7. [**Lowenstein Jensen Medium**](https://microbeonline.com/preparation-uses-lowenstein-jensen-lj-medium/) used to recover *M. tuberculosis* is made selective by incorporating malachite green.
8. **Wilson and Blair Agar** for recovering *S. typhi* rendered selective by the addition of dye brilliant green.
9. [**TCBS Agar**](https://microbeonline.com/thiosulfate-citrate-bile-salts-sucrose-tcbs-agarcomposition-uses-colony-characteristics/) used for isolating *V. cholerae* from fecal specimens has elevated pH (8.5-8.6), which inhibits most other bacteria.
10. **SS agar (Hopkins media)** used to grow Salmonella and Shigella bacteria.
11. **Staph 110** containing high concentration of salt and used to grow *Sthaphylococcus aureus* bacteria.

**4. Differential medium**

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony color. Various factors include dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently colored colonies. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

**Examples of differential media include:**

1. [**Mannitol salts agar**](https://microbeonline.com/mannitol-salt-agar-msa-composition-uses-and-colony-characteristics/) (mannitol fermentation = yellow)
2. [**Blood agar**](https://microbeonline.com/blood-agar-composition-preparation-uses-and-types-of-hemolysis/) (various kinds of hemolysis i.e. α, β and γ hemolysis)
3. [**MacConkey agar**](https://microbeonline.com/macconkey-agar-mac-composition-preparation-uses-and-colony-characteristics/) (lactose fermenters, pink colonies whereas non- lactose fermenter produces pale or colorless colonies.
4. [**TCBS**](https://microbeonline.com/thiosulfate-citrate-bile-salts-sucrose-tcbs-agarcomposition-uses-colony-characteristics/)(*Vibrio cholerae* produces yellow colonies due to fermentation of sucrose)
5. **Eosin methylene blue (EMB)** (containing methylene blue dye).
6. **Transport media**

Clinical samples must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Most of these media are semi-solid in consistency.

1. **Anaerobic media**

Anaerobic bacteria need special media for growth because they need low oxygen content and extra nutrients like hemin and vitamin K. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

**\*\*\* Hemolytic Reactions on Blood Agar**

Observation of the hemolytic reactions on blood agar is a very useful tool in the preliminary identification of bacteria. The types of hemolysis are defined as follows:

**alpha (α) hemolysis**: An indistinct zone of partial destruction of red blood cells (RBCs) appears around the colony, often accompanied by a greenish to brownish discoloration of the medium. *Streptococcus pneumoniae* and many oral streptococci are α hemolytic.

**beta (β) hemolysis**: A clear, colorless zone appears around the colonies, in which the RBCs have undergone complete lysis. *Streptococcus pyogenes*, *S. agalactiae*, and several other species of streptococci are β hemolytic. Many other bacteria besides streptococci can be β hemolytic, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, etc.

**gama (γ) hemolysis**: No apparent hemolytic activity or discoloration is produced (also called gamma hemolysis).