Lecture-6: Cultivation and Isolation of Viable Pathogen

Laboratory media

Culture media are required to isolate the bacteria from the clinical specimens; following which the appropriate biochemical tests can be performed to identify the causative agent.

Constituents of culture media:

The basic constituents of culture media are:

1- **Water**: Distilled water or potable water with low mineral content is used for media preparation.

2- **Electrolytes**: sodium chloride or other electrolytes.

3- **Peptone**: it is a complex mixture of partially digested proteins.

• Source of peptone: It is obtained from lean meat or other protein material, such as heart muscle, casein or fibrin, or soya flour usually by digestion with proteolytic enzymes, such as pepsin

• Constituents: 1t contains proteoses, amino acids, inorganic salts (phosphates, potassium and magnesium), accessory growth factors like nicotinic acid and riboflavin.

- **Agar**: it is used for solidifying the culture media. It is commercially available in powder form; melts in water after boiling and jellifies after cooling also called 'agar-agar' is prepared from the cell wall of variety of **seaweeds (red algae of species *Gelidium* and *Gracilaria)***

Preparation of agar media: The appropriate amount of agar powder is added to water and the mixture is dissolved and then sterilized by placing it in an autoclave. When the temperature of the molten agar comes down to 45°C, it is poured to the Petri dishes and then allowed to set for 20 minutes.

- **Meat extract:** It is a commercial preparation of highly concentrated **meat stock, usually made from beef.** It contains protein degradation products, inorganic salts, carbohydrates and growth factors.

- **Yeast extract:** It is prepared commercially from **washed cells of Baker's yeast.** It contains amino acids, inorganic salts (potassium and phosphates) and carbohydrates. (Figure-1)

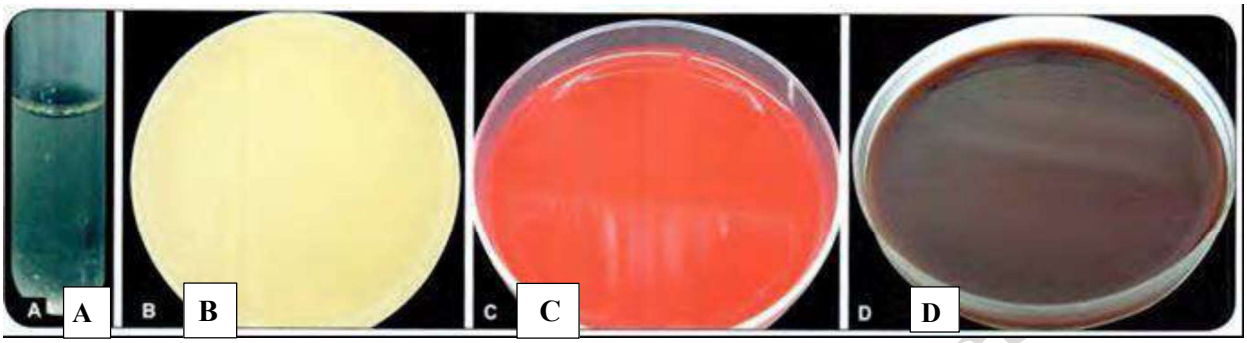

Figure (1): A. Peptone water; B. Nutrient agar; C. Blood agar; D. Chocolate agar.

- **Meat extract**: It consists of maltose (about 50%), starch, dextren, glucose and 5% of protein products.
- **Blood and serum**: The are important components of enriched media provide extra nutrition to fastidious bacteria. Usually 5- % of sheep blood is used. Horse, ox or human blood can also be used.

Types of culture media:

Bacteriological culture media can be classified in <u>two ways</u>:

A. Based on consistency, culture media are grouped into:

1. Liquid media (or broth).
2. Semisolid media.
3. Solid media.

B- Based on the growth requirements, culture media are classified as:

1. Routine laboratory media: They are prepared from nutrients, such as aqueous extract of meat, peptone, etc. **They can further be classified into various types based on functional use or application**, as **Simple/basal media; Enriched media; Enrichment broth; Selective media; Differential media; Transport media; Anaerobic media.**

2. Defined or synthetic media: They are prepared from pure chemical substances and the **exact composition of the media is known**, **this type of media either simple synthetic media and Complex synthetic media.**

Simple media

Many bacteria will grow in or on simple media such as nutrient broth/nutrient agar that contains 'peptone' (polypeptides and amino acids from the enzymatic digestion of meat) and 'meat extract' (water-soluble components of meat containing mineral salts and vitamins).

Enriched media

These contain **additional nutrients** for the **isolation of more fastidious bacteria** that require special conditions for growth like agar containing whole blood (blood agar) or agar containing lysed blood **(chocolate agar**). (Figure-2).

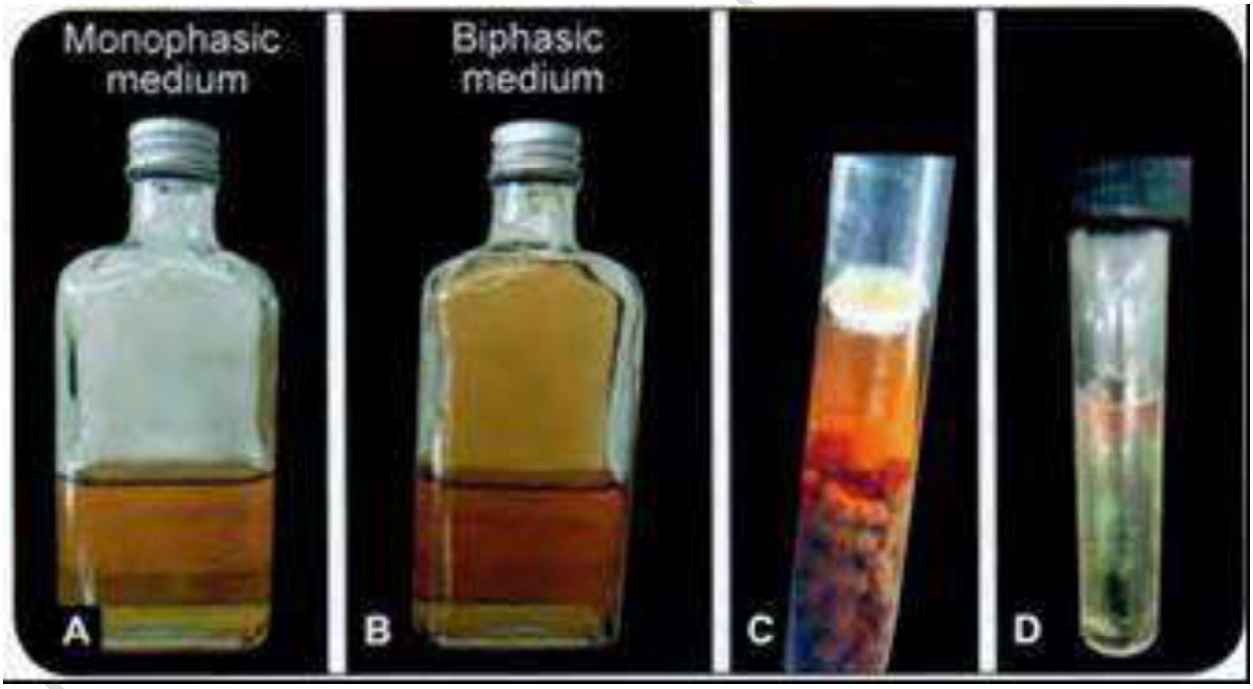

Figure (2): A. Brain-heart infusion broth; B. Biphasic medium (Brain-heart infusion broth/agar); C. Robertson's cooked meat medium; D. Thioglycollate broth.

Selective media

These are designed to facilitate growth of some bacteria, while suppressing the growth of others, and include:

Mannitol salt agar which include increases NaCl (salt) concentration for the recovery of staphylococci.

MacConkey agar which contains bile salt and allows the growth of bile-tolerant bacteria only (such as gram negative bacteria).

Antibiotics, which are frequently added to the media to allow only some bacteria to grow while suppressing or killing others type of bacteria.

Indicator media

These are designed to aid the detection and recognition of particular pathogens. **They are often based on sugar fermentation reactions that result in production of acid and the subsequent color change of a pH indicator, such as MacConkey agar contains lactose and a pH indicator (neutral red); lactose-fermenting bacteria (*Escherichia coli*) produces acid and form pink colonies,** whereas non-lactose fermenting bacteria (***Salmonella spp***.) do not produce acid and form pale yellow colonies. This property facilitates the recognition of possible Salmonella colonies among normal bowel flora. Note that indicator media may also contain selective agents including antibiotics or substances such as bile salts and crystal violet to suppress growth of most Gram- positive microorganisms. MacConkey agar is therefore both a selective medium and an indicator medium.

……..