Microbiological culture media

Microorganisms that are grown in the lab are referred to as "cultures". Culture media are solutions containing all of the nutrients and necessary physical growth parameters necessary for microbial growth. The survival and growth of microorganisms depend on available nutrients and a favorable growth environment. These environments contain energy source, fundamental units & necessary contents for build and conformation the cell compartments. These fundamental units are the sources of carbon (C) & Nitrogen (N) the materials that all the microorganisms that need them), and these culture media contain high percentage of H_2O & other metals such as: Cu, Zn, metallic salts sometimes vitamins and gases such as O_2.

The most important reasons for culturing M.O. *in vitro* are its utility in diagnosing infectious diseases, isolating a bacterium from sites in body normally known to be sterile is an indication of its role in the disease process, and culturing bacteria is also the initial step in studying its morphology and its identification.

Classification of culture media

Generally, culture media can be distinguished on the basis of composition, consistency and purpose.

A- *Classification of culture media based on the basis of composition:*

1) Natural media: contain natural materials such as meat extract & peptone. (Used to grow large numbers of microorganisms).
2) Synthetic media (a.k.a. chemically defined media): are composed of ingredients of known chemical composition and concentration.
3) Semi synthetic media: resemble synthetic media in containing a known set of ingredients, but differ in that at least some of the ingredients are of unknown or variable composition (e.g. adding of yeast extract and blood serum for synthetic media).

B- *Classification of culture media based on consistency:*

1) Solid medium: It's media containing agar (at a concentration of 1.5-2.0%) or some other, mostly inert solidifying agent. Solid medium is useful for isolating bacteria or for determining the characteristics of colonies. Solid media that is provided in tubes often has a slanted surface and are referred to as "slants". Solid media that is provided in Petri- dishes are referred to as "plates" (e.g. Blood agar).
2) Semisolid media: It's prepared with agar at concentrations of 0.5% or less. They have a soft jelly-like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility (e.g. motility media).
3) Liquid (Broth) media: These media contain specific amounts of nutrients but without solidifying 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 (e.g. nutrient broth, brain heart infusion broth).

Note: Agar (complex polysaccharide) is derived from seaweed (red algae) and used as a solidifying agent.

C- *Classification of culture media based on purpose (functional use):*

1) **Basic (Ordinary) media**: Basal media are basically simple media that support most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar considered basal medium. These media are generally used for the primary isolation of microorganisms.
2) **Enriched medium**: Addition of extra nutrients in the form of blood, serum, egg yolk etc., to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum.
3) **Enrichment Medium**: Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. It is typically used as broth medium and incorporated with inhibitory substances to suppress the unwanted organism (e.g., Selenite F Broth- for the isolation of *Salmonella*, *Shigella* and Alkaline Peptone Water- for the isolation of *Vibrio cholera.*
4) **Selective media**: They are solid media in which some special substances are incorporated which will allow the growth of some bacteria and prevent the others.

   Examples of selective media:

   • MacConkey agar: Used for selective isolation of intestinal G-ve rods and to detect lactose fermentation, lactose-fermenting bacteria appear in red pink colony while non-lactose fermenting appears as pale colony. Bile salts, in combination with crystal violet, will also inhibit most gram-positive organisms.

   • T.C.B.S. (Thiosulphate Citrate Bromothymole blue Sucrose) medium, for selective isolation of *V. cholerae*.

   • Lowenstein Jensens medium: For selective isolation of mycobacteria by incorporating Malachite green.

   • S-S agar (Salmonella-Shigella agar): For isolation of *Salmonella* and *Shigella*. These two species of bacteria grow on S-S agar in pale colonies with (black center) in *Salmonella* spp. growth.

5) **Differential media:** They are incorporated with some substances that help in differentiation one type of microorganism that is able to grow in this media. The colonies of some microorganism on these media possess culture characters that are morphologically distinguished from other M.O. e.g. MacConkey agar, S-S agar, blood agar.

According to the hemolytic activity of the bacteria, there are three types of hemolysis:
1. β-hemolytic (complete hemolysis): A clear zone around the colony.
2. α-hemolytic (partial hemolysis): A greenished coloration of the medium.
3. γ-hemolytic (no hemolysis).

6) **Indicator media:** Contain pH indicator that change the color of the medium according to the pH e.g. mannitol salt agar, which uses for selective isolation of *Staphylococcus aureus*. The indicator is phenol red.

7) **Special media:** Media that cannot be easily grouped under one of the foregoing heading will be discussed here. Most of these are used to ascertain one or more biochemical characteristics. For ex.:

* **Triple sugar iron agar (TSI):** This medium let to solidify in slant tubes, will detect an organism's ability to ferment glucose, lactose, sucrose, or combination of these, and to produce H_2S and gas.

8) **Transport media:** are devised to maintain the viability of pathogen and to avoid overgrowth of the contaminants during transit from the patient to the Lab. (e.g., Stuart's medium & Amie's medium).

9) **Anaerobic media**: These media are used to grow anaerobic organisms (e.g., Robertson's cooked meat medium, Thioglycolate medium).

10) **Assay media:** These media are used for the assay of vitamins, amino acids and antibiotics (e.g., Mueller Hinton Agar that used for determining antibiotic potency).

Preparation of Agar Plate:

Most of agar are present in powder form. They dissolved in distilled water as per their instructions as follow: -

1- In a conical flask media dissolved in distilled water.
2- It usually necessary to gently boil the mixture to facilitate dissolving by hot plate or over gently flame burner (Bunsen burner).
3- Sealed the top mouth of flask with a cotton, and finally covering the cotton with loose layer of aluminum foil.
4- To sterile the media autoclaved for (15) minutes, (121 ^{o}C) and 15 bar/Inch^2 in autoclave.
5- The sterile media is then allowed to cool to (45 ^{o}C), pouring at this temperature prevent condensation forming on the lid.
6- Pour the media into a sterilized Petri dishes or tubes (in plane or slant surface).
7- Allow to solidify.
8- Storage plates (upside down) or tubes into refrigerator at 4 ^{0}C until use.

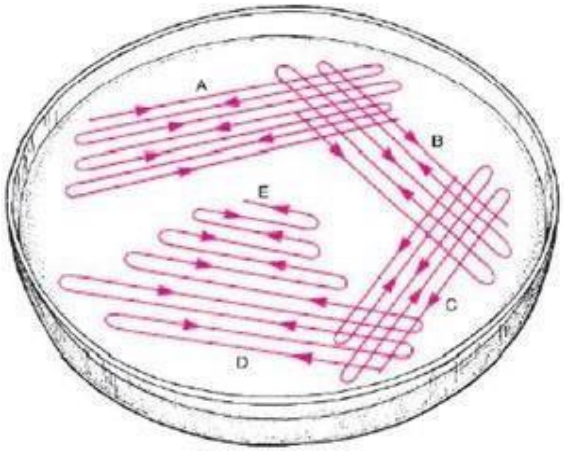
***Inoculation of Culture Media*:-**

One of the first requirements to study specific microorganisms is to separate them from the mixed microbial populations in which they are found in the environment. To achieve this goal, microbiologists use culture media and aseptic transfer techniques. The **aseptic (sterile) technique** is a technique designed to keep the working environment as free of contaminants as possible.

To cultivate, or culture**,** microorganisms, one introduces a tiny sample (the inoculum) into a container of nutrient **medium** (pl. media), which provides an environment in which they multiply. This process is called **inoculation.** The observable growth that later appears in or on the medium is known as a culture. The first inoculation on culture medium is called **a primary culture.** The transfer of microorganisms from one culture medium to another is called "**Subculturing".** The nature of the sample being cultured depends on the objectives of the analysis. Clinical specimens for determining the cause of an infectious disease are obtained from body fluids (blood, cerebrospinal fluid), discharges (sputum, urine, feces), or diseased tissue.

A **pure culture** is a culture that contains only one species of bacteria. **A mixed culture** encompasses more than one species. When isolating bacteria from the environment the microbiologist always starts with a mixed culture. A pure culture can be obtained from the mixed culture by sub-culturing and streaking for isolation. Many techniques can be used to isolate different microorganisms:

1- <u>Streak plate technique:</u>



An inoculating loop is used to spread the inoculated M.O. over the agar plate to produce **isolated colonies**, colonies not touching each other. This is accomplished by streaking all four quadrants of a plate (by using the **Quadrant streak method)**, decreasing the amount of culture material as the streaking proceeds into each successive quadrant. Care must be taken to avoid tearing the surface of the agar.

Procedure:

a- Put a sterile wire loop over the flame of the burner until be red-hot and allow to cool.
b- Remove very small amount of bacterial culture or clinical materials by sterile wire loop, and then streak the first quadrant.
c- The loop is sterilized, cooled, and then is used to spread the material from the first quadrant into the second quadrant.

d- The loop is again sterilized, cooled, and is used to spread material from the second quadrant into the third quadrant, and then from the third into the fourth.

e- In the fourth quadrant, the streaking procedure (by a zigzag motion) is done carefully to produce isolated colonies of the bacteria.

f- Incubate the plate in an inverted position at 37°C for 18-24 hr.

2- Spread plate (or Lawn culture) technique:

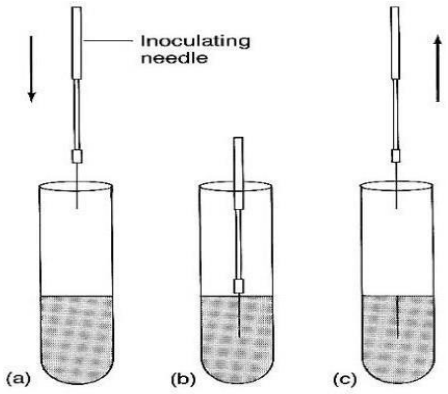
This technique is used for counting bacteria, antimicrobial susceptibility testing, and for swabbing from clinical lesions such as throat swab, ear swab, vaginal swab and others. In this method, take 0.1 ml of sample of bacterial culture by pipette place over the surface of agar medium. Spread the bacteria culture over the surface by glass spreader or by cotton swab, and then incubate the plate at 37°C for 18-24hr.

3- Inoculation of an agar slant (*Stroke method*):

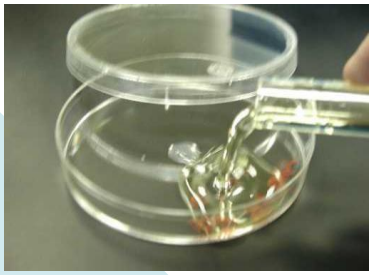
To inoculate slants such as Loeffler serum medium, use a sterile straight wire to streak the inoculum down the centre of the slope and then spread the inoculum in a zig-zag pattern.

Note: To inoculate a slant and butt medium, such as Kigler iron agar, use a sterile straight wire to stab into the butt first and then use the same wire to streak the slope in a zig-zag pattern.

4- Inoculation of Stab Media (deeps) (Stab method):



This technique is used to differentiate between motile and non-motile bacteria. Use a Sterile straight wire to inoculate a stab medium, for example mannitol motility medium. Inoculate with a straight wire (needle) making a single stab down of center of the tube to about half the depth of media. Incubate in 37°C for 1-2 days.



5- Pouring plate technique:

This technique is used for counting bacteria (Variable count). Procedure of this technique is: a) Inoculate a sterilized empty Petri dish with 0.1ml of bacterial culture or sample by using sterile pipette. b) Pour the media into the Petri dish and allow to solidify. c) Incubate the plate at 37°C for 18-24 hr.

6- <u>Cultivation of anaerobic bacteria:</u>

Obligate anaerobic grow only in absent of O_2, because the bacteria lack mechanism of oxidation through respiratory enzymes (like cytochrome oxidase, catalase, peroxidase) resulting in H_2O_2 accumulation. The H_2O_2 is toxic for Bacterial growth. The methods described for achieving anaerobic conditions are:

1. Exclusion of O_2 by heating or vacuum.
2. Absorption of O_2 by chemical means.
3. Reduction of O_2 by candle or Gas Pak Jar.

Gas Pak system is now the method of choice for isolation of anaerorbes. It generates CO_2 and H_2 in moist condition. H_2 combine with O_2 to form H_2O in present palladium pellets, the pellets act as catalyst for combination, methylene blue strip act as indicator, it is colorless in anaerobic conditions, and remain blue in aerobically.

The procedure:

1. Place inoculated agar plates media into the Jar in an inverted.
2. Open the indicator strip and place it in the Jar.
3. Open the Gas Pak envelop and introduce 10 ml D.W. into the envelop and put it in the Jar.
4. Place the lid and tighten the screw clamp.
5. Incubate the Jar at 37°C for 24-48 hr.