

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



العنوان

# **Method of culture media Seven modular unit**



# *The method or types of inoculation (culturing)*

## **I-SOLID MEDIA**

### **A-Plate cultured methods**

Streak plate method

Carpet culture method

Power plate method

### **B-Tube culture methods**

Slope (Slant) culture stock

Deep culture

Stab culture

Roll tubes

## **II-Liquid media**

## **I-SOLID MED**

### **A-Plate cultured methods**

To isolate single colonies and pure culture . This method is designed for culture from broth, agar plate or slopes. After preparing the solid media in Petri-dish must be stored in the refrigerator until inoculation, the agar surface should be free of water of condensation before the streaking is done. The drying of the plates is performed by placing the surface of the lid on to an incubator shelf (at 37 oC) and angling the media-containing dish (media down ward) either within or on the edge of the lid.



## **Streak plate method** (streak culture, plate inoculation)

- **To isolate single colonies ( Obtaining Isolated Colonies )**
- **Goal is to get Isolated Colonies from Food and/or Cultures**
- **Colonies can be Identified and Further Evaluated**

The medium in the Petri dish should be inoculated as follows:

-**Using a sterile loop**, doing a smear over **area A** by loopful of the **clinical specimen** or **broth culture** or **charged loop touching colony** (figure1). **Sterilize the loop** in the Bunsen flame, and when cool streak over **area B**. **Repeat over area C, D and E**. **Incubate the plates at 37 °C**. The maximum available area should be used but care must be taken not to cross a previously inoculated area.

An **alternative method** is to use a **sterile spreader**. This is a **glass rod, 3 mm in diameter**, bent at right angles and sterilized either by boiling, or by wrapping it in Kraft paper and placing in the **hot-air oven at 160 °C for 1 h**. A **small amount** of the specimen is **placed on the medium**, and **smeared over the whole surface, using a sterile spreader**. Any of the specimen remaining from the first inoculation will be transferred to the second Petri dish, and single colonies should be obtained.



## 2-Carpet culture (lawn culture, whole surface, spreading methods).

These methods are prepared by flooding the surface of plate with suspension of bacteria it provides uniform surface growth of bacteria; it is **useful for bacteria phage typing** and **antibiotic sensitivity test**. And this method of streaking may be used either **for culture from solid media** or **for heavy broth culture** and smeared over the whole surface, **using a sterile spreader or loop or swabs**



### 3-Pour plate method

About 15-20 ml of agar media are melted and left to cool in water bath at 45°C-50°C. Appropriate dilution of inoculum are added in 1 ml volume to molten agar and mixed well. Contents of tube are poured in Petri dish. They are allowed to set and after incubation colonies will be seen distributed throughout the depth of the medium. The colonies growing in medium. **This method gives viable bacteria count** in suspension it is recommended and on the method for food microbiology.



## B- TUBE CULTURE METHOD

**1- Slope (Slant) culture stock.** Many tests devised to **differentiate organisms require solid cultures**. It is not always necessary to grow an organism on a whole Petri dish of medium, and slope cultures often suffice. **=Slops'** or **=slants'** are **tubes** or **bottles** containing a small quantity of medium, that has been allowed to solidify with the bottles slightly raised at one end. Such slopes **are used only for maintenance or biochemical tests** once the organism has been isolated in pure culture, they cultured by streaking the surface of slope media

**2- Deep culture.** **Anaerobic organisms require an oxygen-free atmosphere.** For cultivation of these organisms „shake“ or „deep“ cultures are sometimes made. The medium is distributed in 150mm x 20mm tubes to a depth of 6-7cm and allowed to solidify. For use, the medium is melted, cooled at about 45 C°, inoculated with organism, and mixed by rotation between the palms of the hands. When it has solidified, the culture is incubated and the **anaerobic organisms grow at the bottom of the tube. These shake, or deep, tubes can also be used for counts of viable organisms.**

**3- Roll tubes.** The „roll tube“ method **is also useful for counting viable organisms**. The medium is distributed in to 6 x (5/8) in tubes, 1-2 ml per tube, and stored. For use, the medium is melted, cooled to approximately 50 C°, and a known dilution of the test sample is added. The tube is then titled and rolled between finger and thumb, allowing the medium to run all rounds the sides of the tube just bellow the half way mark. This rolling is carried out under cold tap water. A thin film of agar solidifies around the sides of the tube, which is inverted for incubation.

**4- Stab culture.** It is prepared by puncturing charged long **straight wire** (4.5-5 cm). Stab culture are employed mainly for **demonstration of gelatin liquefaction and motility test and for maintaining stock cultures**

## II-Liquid culture

Media prepared in **tubes or bottles or flasks** and inoculated by touching with a charged loop. Liquid culture is preferred when large and quick yield is required. The major **disadvantage of Liquid culture** is that **it does not provide pure culture from mixed inocula**. . This method constitutes a simple technique and used for liquid culture largely but it may **also be used for culture from solid media**.





## ***Cultural characteristic***

Bacteria grown artificially (in vitro) on agar plates are described as colonies vary in size, shape, pigment production and hemolysis on blood agar, depending on the type of media.

### **A. Description of colonies on solid culture**

**Colonies of bacteria are described as follows:**

- a. **Shape:** circular, irregular, radiating or rhizoid.
- b. **Surface:** Bacterial colonies are frequently shiny and smooth in appearance. Other surface descriptions might be: **veined, rough, dull, wrinkled (or shriveled), glistening.**
- c. **Color** – It is important to describe the color or pigment of the colony. Also include descriptive terms for any other relevant optical characteristics such as: **opaque, cloudy, translucent, iridescent.**
- d. **Size:** Surface colonies are measured in millimeter, they are 2-3 mm in diameter. Smaller ones may be less than (about 0.5-1 mm)
- e. **Elevation:** may be raised, low convex, implicated or dome
- f. **Edges:** mostly edges are entire, sometimes crenate, fimbriated or effuse.
- g. **Color(pigmentation):** some organism may produce pigmented colonies (*Staphylococcus, Pseudomonas*)
- h. **Opacity:** colonies on nutrient agar may be transparent, translucent or opaque.
- i. **Consistency:** **Mostly** soft and butyrous and may be hard, firm, mucoid, tenacious, dry, adherent to medium, friable and membranous.

**j. Contiguity:** may be discrete or swarming.

**k. Changes in the medium:** colonial growth may bring about color changes in the media themselves produce soluble pigment that diffuse in to the medium and some organism haemolysis the blood of medium around the colony.

**l. Emulsifiability:** Growth of some bacteria is easily emulsifiable (like *E. coli*, *salmonella*) where as growth of *N. catarrhalis* is not emulsifiable and form granules.

**i. Odor**



## B. Description of growth in liquid culture

Growth in liquid medium is described as:

1. **Turbidity:** Clear or turbid
2. **Deposit :** Growth of *Streptococcus pyogenus* is characterized by deposit at the bottom of the tube
3. **Surface growth:** Surface growth is related to aerobic nature of organism.
4. **Color changes:** Some organisms produce water soluble, pigment which after diffusion change the color of medium e.g. *Pseudomonas pyocyneous*.

### Methods for anaerobic culture

Oxygen is required for the growth of many, but not all, microorganism. Growth of the obligatory anaerobe depends on the state of oxidation or reduction in its environment. One explanation as to why anaerobe organisms do not grow in the presence of oxygen is that many organisms form hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) when incubated in the presence of oxygen. Most aerobic organism produce an enzyme, called catalase, which catalyses  $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \frac{1}{2} \text{O}_2$ . Anaerobes do not have this enzyme, and are therefore destroyed by the peroxide ( $\text{H}_2\text{O}_2$ ) and when grown in the absence of oxygen can be performed in several ways as fellows.



# **1. Growth in special media**

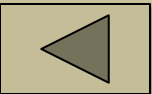
a-GLOCOSE AGAR DEEP

b-THIOGLYCOLLATE BROTH

## **2. The anaerobic jar**

3. Jar with gas bag system







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