# Microbiology Laboratory

# Lab 9: Types of culturing

To study bacteria and other microorganisms, it is necessary to grow them in controlled conditions. Microbes are grown in substances that provide the nutrients necessary to sustain their metabolic activities and reproduction called growth media or simply media. **Growth media** can be either liquid or solid or semi-sold.

**1- liquid medium (diffuse growth)** is called a broth. Broths can be used to determine growth patterns in a liquid medium, and for certain types of inoculations and metabolic tests. They are also the method of choice for growing large quantities of bacteria, bacteria grow producing general turbidity. **Disadvantages:** It does not provide a pure culture from mixed inoculum and Identification of bacteria is not possible

**2-Solid media** (discrete colonies) usually contains agar, which is a mixture of polysaccharides derived from red algae. It is used as a solidification agent because it (1) is not broken down by bacteria, (2) contains no nutrients that can be used by bacteria and (3) melts at high temperatures, and yet is solid at temperatures used for most bacterial growth. Solid growth media is used in the following forms: agar plates, agar slants and agar deeps. Melted agar is poured into a test tube and then allowed to solidify vertically for an agar deep, or at an angle for an agar slant. Agar plates are made by pouring melted agar into a petri dish.

**3-Semi-solid media (motility medium)** soft media are useful in demonstrating bacterial motility and separating motile from non-motile strain.

**Types of culturing for isolating and counting the viable microorganisms present in a liquid sample:**

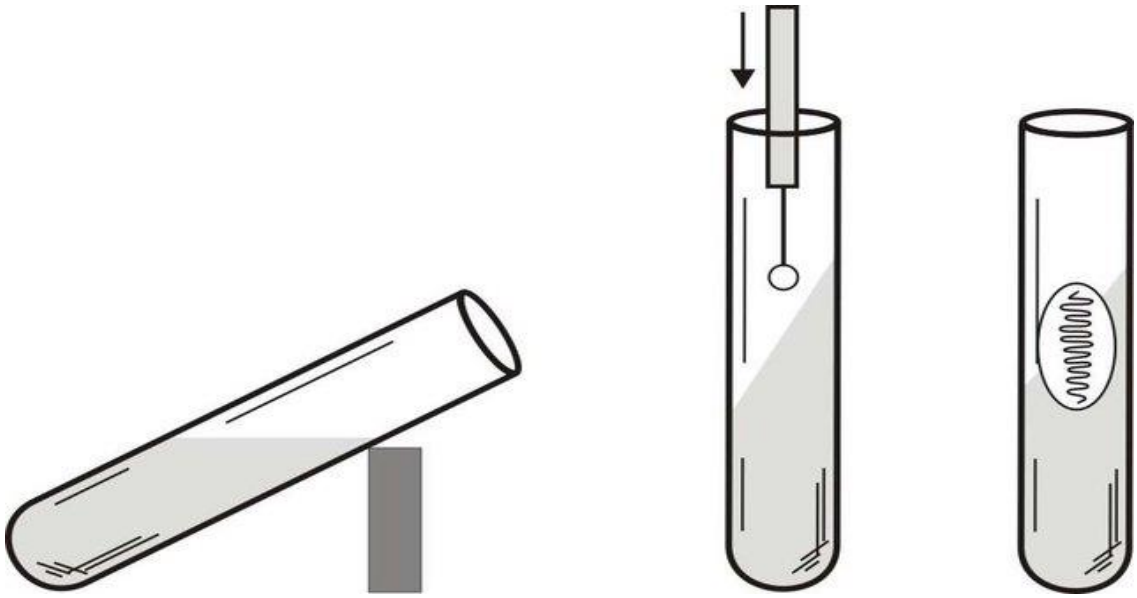
1. **Streak Culture (Surface Plating)**

**In this method a microbial inoculum is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns**. This method is routinely employed for the isolation of bacteria in pure culture from clinical specimens. A platinum loop is charged with the specimen to be cultured. Owing to the high cost of platinum, loops for routine work are made of nichrome resistance wire. The loop is flat, circular, and completely closed with a 2-4 mm internal diameter mounted on a handle. One loopful of the specimen is smeared thoroughly over an area, on the surface of a well-dried plate, to give a well-inoculum or ‘well’. The loop is re-sterilized and drawn from the well in two or three parallel lines onto the fresh surface of

the medium. This process is repeated several times. At each step, the inoculum is derived from the most distal part of the immediately preceding strokes. Plates are incubated in the inverted position with the lid underneath. On incubation, growth may be confluent at the site of original inoculation (well) but becomes progressively thinner, and well-separated colonies are obtained over the final series of streaks.

**2. Stroke Culture**

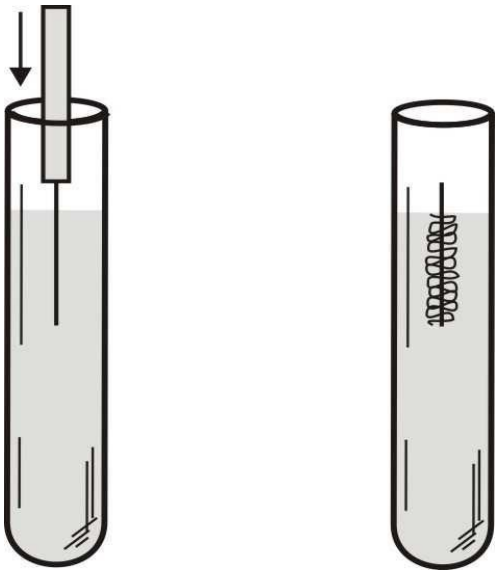
Stroke culture is made in tubes containing agar slope or slant. Slopes are seeded by lightly smearing the surface of agar with a loop in a zig-zag pattern taking care not to cut the agar. It is employed for providing pure growth of the bacterium for slide agglutination and other diagnostic tests.



**3. Stab Culture**

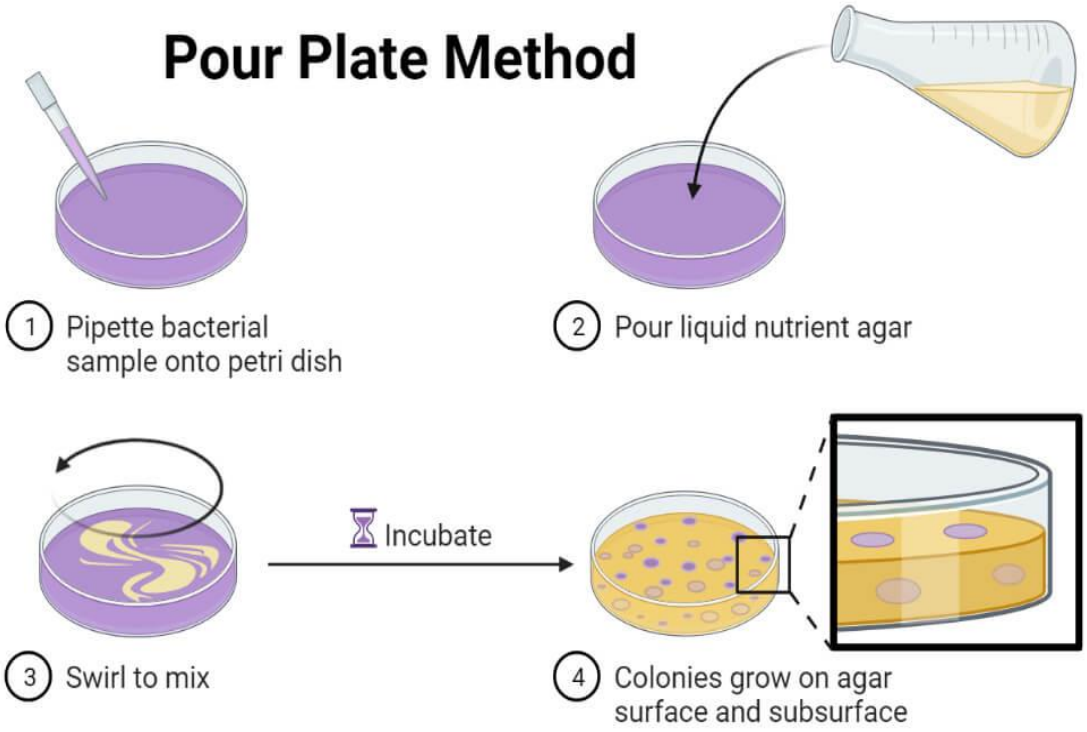
In the preparation of the stab cultures, a suitable medium such as nutrient gelatin or glucose agar is punctured with a long, straight, charged wire into the center of the medium and withdrawing in the same line to avoid splitting the medium. The medium is allowed to set, with the tube in the upright position, providing a flat surface at the top of the medium. **Uses for:**

- Mainly for demonstration of gelatin liquefaction.
- Demonstration of oxygen requirement of the bacterium under study.
- For the maintenance of stock cultures.
- To study the motility of bacteria in semisolid agar.



### 4. Pour-Plate Culture

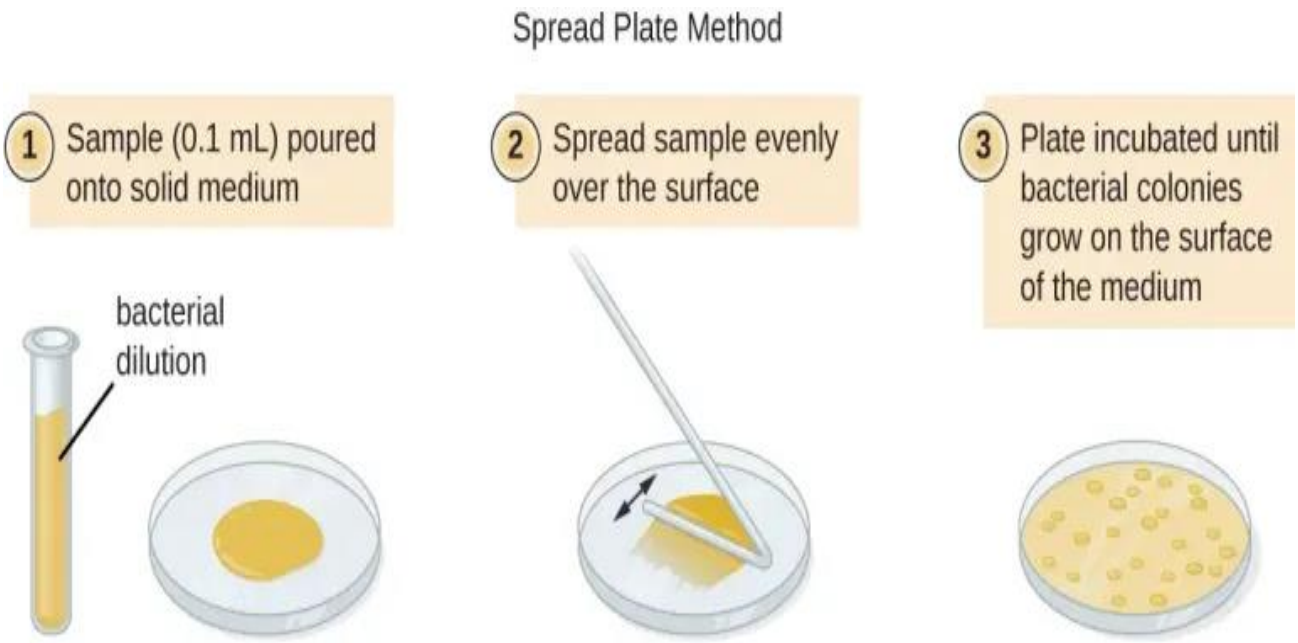
I**n the pour plate the original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies after plating**. This method is used for counting the number of living bacteria or groups of bacteria in liquid culture or suspension. A measured amount of the suspension is mixed with molten agar medium in a Petri dish. Either 1.0 ml or 0.1 ml of dilutions of the bacterial suspension are introduced into a Petri dish. The nutrient medium, in which the agar is kept liquid by holding it in a water bath at 45-50°C, is poured over the sample, which is then mixed into the medium by gentle agitation of the plate. When the agar solidifies, the plate is incubated inverted at 37°C for 48 hours, or most suitable for the species examined. After incubation, colonies will grow within the nutrient agar (from cells suspended in the nutrient medium as the agar solidifies) as well as on the surface of the agar plate and can be enumerated using colony counters. **Uses for**: Gives an estimate of the viable bacterial count in a suspension & the recommended method for quantitative urine cultures**.**




### 5. Spread Plate Culture

**The spread plate method is a microbiological laboratory technique for isolating and counting the viable microorganisms present in a liquid sample by spreading a certain volume of the sample, so is transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-glass rod.** The spread plate technique is a viable counting method employed to plate a liquid sample to isolate or count the bacteria in that sample. A perfect spread plate technique will

result in visible and countable colonies of bacteria evenly distributed on the plate. **uses**: the spread-plate technique can be performed quantitatively to determine the number of bacteria present in a sample. The spread plate technique is most commonly applied for microbial testing of foods or any other samples or to isolate and identify a variety of microbial flora present in the environmental samples




**Benefits compared to pour plate technique:**

1- Only surface colonies develop

2- The organisms are not required to withstand the temperature of liquid agar.