

## **PREPARATION OF PURE CULTURES OF MICROORGANISMS**

### **BY POUR PLATE METHOD**

The forerunner of the present pour plate method was developed in the laboratory of the famous bacteriologist, Robert Koch. In this technique successive dilutions of the inoculums (serially diluting the original specimen) are added into sterile Petri plates to which melted and cooled (42–45°C) agar medium is poured and thoroughly mixed by rotating the plates which is then allowed to solidify. After incubation, the plates are examined for the presence of individual colonies growing throughout the medium. The pure colonies which are of different size, shape and color may be isolated/ transferred into test tube culture media for making pure cultures. Pour plates are also used as a means of determining the number of viable organisms in a liquid such as water, milk, urine, or broth culture as well as to determine the hemolytic activity of deep colonies of some bacteria, such as the streptococci, via an agar medium containing blood

### **Material Required**

Twenty-four hour nutrient broth cultures of mixed bacterium, nutrient agar medium, sterile 9-ml water blanks, sterile Petri plates, sterile 1-ml pipettes, test tube rack, water bath, Bunsen burner, wax marking pencil

**Procedure** Place the nutrient agar medium into the boiling water bath for melting. Allow this to cool to 45°C. Prepare a serial dilution of the mixed bacterial culture up to 10<sup>-4</sup>. Also label the Petri plates and aseptically transfer 1ml of the bacterial suspension from each dilution to the individual plates that are previously numbered. Remove a nutrient agar medium flask from the water bath (at 45°C), pour the medium (20 ml) into these plates, and rotate the plate gently to ensure uniform distribution of cells in the medium. Allow the medium to solidify. Incubate the inoculated plates for 24–48 hours at 28 ± 2°C in an inverted position

**Observations** Examine the plates for the appearance of individual colonies growing throughout the agar medium. It will be observed that progressively poured plates will have fewer and fewer numbers of colonies that will be distributed more or less sparsely in the plates that may be transferred (subcultured) to other media (fresh plates) or agar slants for further study

**Precautions** The medium to be poured in the Petri plates should have a temperature of 45°C. The plates should be incubated in an inverted position to prevent collection of condensation on the agar surface. Unless the surface is dry it will be difficult to obtain discrete surface colonies.

**BY SPREAD PLATE TECHNIQUE** The spread plate technique is used for the separation of a diluted, mixed population of microorganisms so that individual colonies can be isolated. In this technique microorganisms are spread over the solidified agar medium with a sterile L-shaped glass rod while the Petri dish is spun on a turntable. The theory behind this technique is that as the Petri dish spins, at some stage, single cells will be deposited with the bent glass rod on to the agar surface. Some of these cells will be separated from each other by a distance sufficient to allow the colonies that develop to be free from each other.

**Material Requirements** Twenty-four hour nutrient broth cultures of known bacterial mixture, nutrient agar plates, lazy susan turntable, L-shaped bent glass rod, 95 percent alcohol, beaker (50 ml), Bunsen burner, wax marking pencil.

**Procedure** Label nutrient agar plates with bacterial species with a wax pencil. Pour 95 percent alcohol into a beaker and dip the bent glass rod in it. Aseptically transfer a loopful culture of mixed bacterium in the center of the appropriately labeled nutrient agar plate. Place the inoculated plate on the turntable. Remove the glass rod from the beaker and sterilize the bent portion in the Bunsen burner flame. Cool the rod for 10–15 seconds. Remove the cover of the Petri dish and spin the turntable. Lightly touch the sterile bent rod to the agar surface and move it back and forth while the turntable is spinning for spreading the culture over the agar surface. Replace the Petri dish cover when the turntable stops spinning. Immerse the bent rod in alcohol and re flame to sterilize it. Incubate all the three plates in an inverted position at  $28 \pm 2^{\circ}\text{C}$  for 24–48 hours

**Observations** Observe all the inoculated plates as to the distribution of colonies on each of the agar plates; some of the colonies will be free from each other. Select a discrete colony from first and second plate and record their form, elevation, pigmentation, and size of the colony