EXPERIMENT 8 INOCULATION TECHNIQUES AND INCUBATION OF CULTURE

Structure

8.0 OBJECTIVES

After going through this experiment, you will be able to:

- inoculate microbes into both fluid and solid culturing media;
- incubate the media containing microbes; and
- take aseptic precautions need to be followed while going for inoculation.

8.1 INTRODUCTION

Inoculation means introduction of micro-organism to media to culture them. The microbes getting a favourable environment (inculcations) and nutrient (from media) grow to form colony. Each and every micro-organism has the capability to form a colony. So for quantitative purpose it is necessary that micro-organism should be well spread in medium so that colony formed by each organism can be clearly observed. For this we are adopting dilution technique before inoculation. However for qualitative purpose i.e. just to produce the culture of micro-organism which can be further utilized for quantitative purpose simple streaking is followed. Here we will deal with qualitative aspect only.

8.2 ASEPTIC PRECAUTIONS

Aseptic techniques are important to protect the worker from infection from the clinical specimen and to prevent contamination of the material under process.

Aseptic conditions can be achieved by following steps:

1) Sterilize wire loops, straight wires, and metal forceps by flaming before and after use. Whenever possible, use a hooded Bunsen burner.

2) Flame the necks of specimen bottles, culture bottles, and tubes after removing and before replacing caps, bungs, or plugs.

3) When inoculating, do not let the tops or caps of bottles and tubes touch a surface which is not sterile. This can be avoided by holding the top or cap in the hand.

4) Always use racks to hold tubes and bottles containing specimens or media.

5) Make slide preparations from specimens after inoculating the culture media.

6) Decontaminate the workbench with 70% alcohol before starting the day's work and after finishing.

7) Use a safety cabinet of appropriate class while working with hazardous pathogens.

8) Wear protective clothing, wash hands after handling infected material, never resort to mouth pipetting and, avoid eating, drinking, or smoking in the laboratory.

8.2.1 Making a Wire Loop

Loops must be made correctly to ensure inoculums are well spread, and to prevent the release of aerosols from long and springy loops or loops that are not completely closed. Wire loops are usually made of nichrome wire because it cools quickly, is not too rigid and is less expensive than platinum wire.

The method of making a wire loop is as follows:

1) Cut a piece of wire about 125 mm in length and thickness of standard wire gauge (swg) 26 or 27. Wind it around a loop holder.

2) Using a pair of scissors, cut off one arm of the wire leaving the loop and about 50 mm of wire. Bend the loop back to make it central using a pair of forceps. The length of wire from the loop to the loop holder should be about 50 mm.

3) Insert the wire in a loop holder. Make sure the loop is completely closed.

Note: When sterilizing a wire loop, hold it in the blue part of a Bunsen burner flame. Allow the loop to cool before using it.

8.3 INOCULATION OF MEDIA

8.3.1 Seeding a Culture Plate

The technique used to inoculate media in Petridishes (plates) must provide single colony for identification and to see whether a culture is pure or mixed, i.e. consisting of a single type of organism or several different organisms. A pathogen must be isolated in pure culture before it can be identified.

The inoculation of media in Petridishes is referred to as 'plating out' or 'looping out'. It is not necessary to use whole plates of media. Considerable savings can be made by using a half or even a third of a plate (especially if the medium is a selective one). The area of medium used must be sufficient to give separate colonies.

There are three commonly employed techniques for seeding culture plates. The most common is shown in Fig 8.1 The inoculum from the clinical material or another plate is

first spread out in the form of a primary inoculum (as at A in Fig. 8.1) which is also called as 'well-inoculum' or only 'well'. The successive series of strokes B, C, D and E are made with the loop sterilized between each sequence. At each step the inoculum is derived from the most distal part of the immediately preceding strokes so as to gradually reduce the number of bacteria. This helps in obtaining isolated colonies.

In an alternative plating procedure, one edge of a large loop is used to make a secondary well (see B in Fig. 8.1). The other edge is then used to make succession of strokes across the remaining unseeded area. When the inoculum is small or the medium is selective it can be more heavily inoculated (Fig. 8.2). Several loop-full of the specimen are used to spread the primary inoculum (see A in Fig. 8.2). After sterilizing the loop, it is recharged by rubbing it over area A and the plate is seeded in parallel strokes.

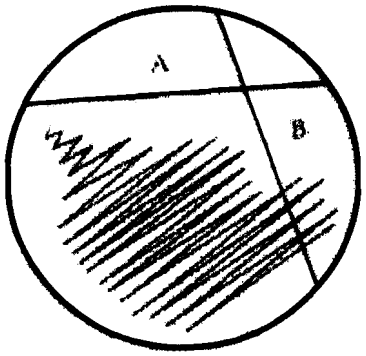

Fig. 8.1: Seeding a Culture Plate

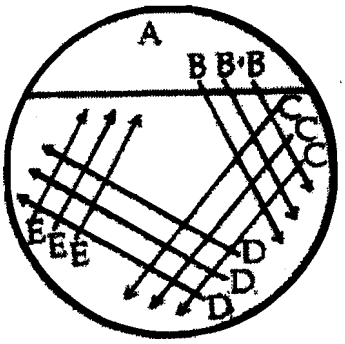

Fig. 8.2: Seeding with Heavy Inoculum

Other plating techniques such as pour plate method, drop plate and spread plate methods are described in experiment 9

8.3.2 Inoculation of Slopes

To inoculate slopes such as Dorset egg medium or Loeffler serum use a sterile straight wire to streak the inoculum down the center of the slope and then spread the inoculum in a zig-zag pattern similar to seeding with heavy inoculum.

To inoculate a slope and butt medium, such as Kligler 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.

8.3.3 Inoculation of Stab Media (Deeps)

Use a sterile wire to inoculate a stab medium, for example motility indole urea (MIU) medium. Stab through the center of the medium taking care to withdraw the wire along the line of inoculum without making further stab lines.

8.3.4 Inoculation of Fluid Media

Broths and other fluid media are inoculated using a sterile wire loop, straight wire, or Pasteur pipette depending on whether the inoculum is colonial growth or a fluid culture or specimen. If using a wire loop to subculture colonies, hold the bottle or tube at an angle and rub the loop against the side of the container below the level of the fluid.

8.3.5 Precautions

i) Hot loop should not be touched directly to culture. It can be judged by touching the tip to a agar which get melted if it's too hot.

ii) The plates with media should be firmly held so that while streaking the unwanted line should not be touched.

iii) Streaking should be performed in proximity of flame (less than 15 cm.)

iv) Always handle the specimen carefully and after taking out for innoculation the stock should not be left over table.

8.4 INCUBATION OF CULTURES

Inoculated media should be incubated as soon as possible. A delay in incubation can affect the viability of pathogens. It can also increase the risk of plates becoming contaminated from small insects and dust especially in the dry season and from fungal spores in rainy season. Uninoculated and inoculated media must be protected from sunlight.

While keeping for incubation the Petridishes must be kept in inverted position. There are two basic reasons behind it.

i) The water droplets formed after condensation should not fall on media and disturb the culture.

ii) If the loose upper lid is kept upper side there is chances while lifting the base plate get uncovered.

Microorganisms require incubation at the temperature and in the humidity and gaseous atmosphere most suited to their metabolism. The length of time of incubation depends on how long an organism takes to develop the cultural characteristics by which it is recognized.

8.4.1 Temperature of Incubation

The temperature at which a microorganism grows best is referred to as its optimum temperature. The temperature below which growth stops (not necessarily resulting in death) is called the minimum temperature, and that above which growth stops and death occurs is called the maximum temperature.

The temperature selected for routine culturing is 35 to 37°C while most microbiologists recommending 35°C in preference to 36°C or 37°C. In general, the growth of microorganisms is more affected by slight rises above their optimum temperature than by reductions below it.

8.5 ACTIVITY

After going through this experiment you take a meat sample and inoculate and incubate the sample for microbial analysis.