# PRACTICAL 4 TECHNIQUES OF CULTURING

**Structure**



## 4.1 INTRODUCTION

In the last practical we learnt how to prepare culture media. This practical has been introduced to make you aware of the technique of sub-culturing i.e., the technique used for maintenance and transferring the culture for various microbiological test procedures. It is a must for every microbiology student to know and practice it.

**Objectives**

After undertaking this practical and conducting the exercises given herewith, you will be able to:

- describe the technique of sub-culturing, and
- transfer the culture for various microbiological test procedures.

## 4.2 SUB-CULTURING: A BASIC INTRODUCTION

Microbiological studies require microorganisms in pure form, i.e., a single type of microorganisms should be present in a culture. Maintenance of these pure cultures in laboratory is must. Preparation and maintenance of these pure cultures can be achieved by transfer of culture from one medium to another or from their parent growth source to another. The procedure is called *sub-culturing*. This technique is of basic importance and should be carried out by practicing aseptic technique. The term *picking off* is used when transfer is made from solid to liquid media.

*Aseptic technique* is a series of procedures used to prevent contamination of cultures and sterile culture media. It is one of the first methods to be learned by the students of microbiology. As microorganisms are ubiquitous, unwanted microorganisms or contaminants can enter into pure cultures from air, working bench, hands or surroundings. Therefore, it is essential to adopt microbiological techniques that are designed to avoid contaminants. We shall learn about these techniques now in this practical and then practice these techniques. So lets get started.

## 4.3 TRANSFER OF CULTURE

Transfer of culture can be done from one liquid media to another liquid media or from liquid to solid media or vice versa. The steps followed for aseptic transfer of microorganisms from one liquid to another liquid, from liquid to solid media and from solid to liquid media are enumerated herewith.

### *I. From one liquid media to another liquid media*

Figure 4.1 illustrates the process of transfer of culture from one medium to another medium. The steps to be followed in this technique are also included herewith:

**Figure 4.1: Procedure for subculturing and transfer of microorganisms**

1. Inoculating loop is to be held in the right hand. It is sterilized by incineration in the inner blue portion (hottest) of the flame until it becomes red hot. Upper steel portion of the handle is also passed rapidly through the flame. The loop is cooled by holding for 10-20 seconds in air near the flame or by dipping in fresh medium. Never place the sterilized loop on working bench.

2. The broth culture tube is thoroughly mixed by thumping the bottom of the tube. Both the culture tube and the fresh broth tube are grasped in left hand.

3. Cotton plugs or caps of both the tubes are removed by holding one cap with the little finger and the second with the next finger of right hand (Hand carrying the inoculating loop). The caps should be kept in the hand with the inner aspect of the caps or plugs pointing away from the palm of the hand. Never place caps or cotton plugs on the working bench to avoid contamination.

4. The mouth of both the tubes is passed through the flame and then the loopful of the culture is transferred from the culture tube to fresh medium.

5. Again the mouth is flamed to avoid contamination and the caps/plugs are replaced to respective tubes.

6. Inoculating wire is reflamed immediately after transfer, to destroy adhering microorganisms.

7. The tubes are incubated in the incubator or shaker at $37^{o}$C overnight.

***II. Transfer from liquid medium to solid media***

Here the steps involved include:

1. Inoculating loop is sterilized by incineration and allowed to cool, as described earlier.

2. Liquid culture tube is held in left hand and cotton plug or cap is removed with the little finger of the right hand that holds the sterile inoculating loop.

3. Neck of the tube is flamed briefly and a loopful of culture is taken by inoculating loop for inoculating agar plate or slants.

4. Cap or Cotton plug is replaced on the tube after reflaming the mouth.

5. Agar plate is held in the palm of the left hand near the flame and lid is opened slightly with the thumb. The agar plate is inoculated either in the centre by the culture on the inoculating loop or by the streak method and lid is replaced on the plate.

6. For agar slants, cotton plug or cap is removed from the slant tube. The neck is flamed and then slant is inoculated by inserting and drawing inoculating loop in a slant tube lightly over hardened surface in a zig-zag/straight line. The tube neck is reflamed and cap is replaced. For deep agar tubes, inoculation is done with sterilized inoculating needle. It is inserted straight into the agar upto the bottom (stab inoculation).

***III. From solid culture to liquid media***

The steps involved in this technique are included herewith:

1. Sterilized inoculating loop or needle is touched carefully to the microbial growth in agar plates or in agar slant.

2. After removing the cap/plug from the broth tube and sterilizing its neck, the loop or needle is shaken slightly in sterile broth medium to dislodge the organisms.

After inoculation of the medium (liquid/solid), plates and tubes are kept in the incubator at 37°C or at specific temperature required by microorganism for its growth. For liquid culture, "shake water baths" can be used at appropriate constant temperature.

Now that we have studied all the three techniques of sub-culturing, let us practice what we have learnt by undertaking the exercise given herewith. But first answer the questions given in review questions to consolidate your understanding on this topic.

## 4.4 REVIEW QUESTIONS

1. What is meant by:

   (i) Pure Cultures

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   (ii) Sub-culturing

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2. Why do we require pure culture?

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3. Why aseptic techniques are needed for sub-culturing?

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4. What precautions should be taken during sub-culturing?

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Let us move on to the exercise.

# EXERCISE 1

## SUB-CULTURING OF A GIVEN CULTURE

Date : .....

**Aim** : To perform the sub-culturing of a given culture.

**Requirements**

*Culture* : 24 hr liquid culture of *E. coli* and *Bacillus*

*Media* : Nutrient broth tubes, Nutrient slant and deep tubes and Nutrient agar plates.

*Equipments and Glassware* : Inoculating loop and Inoculating needle, Bunsen burner, Glass marking pen, incubator.

**Principle:**

Write the principle behind sub-culturing in the space provided.

**Procedure:**

Now carry out the exercise following the steps given herewith:

1. Sterilize the inoculating loop and needle by heating to red hot.
2. Label all the tubes and nutrient agar plates.
3. Following the protocol given in section 4.3, transfer the *E. coli* and *Bacillus* sp. culture to:
   (a) Nutrient agar tube,
   (b) Nutrient slant and deep tube, and
   (c) Nutrient broth.
4. Incubate the culture at 37°C and observe the growth after 24 hours.

**Precautions:**

1. Sterilization of inoculating wire before and after the transfer is a must. Sterilized wire should not be kept on laboratory bench.
2. Never place the closure of tubes or lid of the plates on working bench or touch it with any surface.
3. Neck of the tubes should be flamed before and after the inoculation to avoid contamination.

(4) Culture transfer should be done near the flame.

(5) Do not dip the needle into the agar while inoculating slant or agar plate.

(6) Never leave the culture tube open any longer than the amount of time needed to transfer the culture.

**Observations and Results:**

Record your observations in the format given herewith.

| Observations | Results |
|---|---|
| *Comment on the growth of E. coli and Bacillus* In Nutrient Slant Tube | (Draw what you see) |
| In Nutrient deep agar tube | |
| In Nutrient broth | |
| In Nutrient agar plate | |

**Inference/Conclusion:**

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**Now submit the exercise for evaluation.**

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**Counsellor Signature**