Aim 18

To Study the Aseptic Techniques

Introduction

Microbes are present everywhere, So during microbiological work, a special care should be taken to prevent the entry of unwanted microorganism/contamination from air current, hands, contaminated surface etc. Laminar airflow chamber (LAF) is used surface/ chamber for microbiological work and hands should be cleaned or sterilized with 70% alcohol before starting work in LAF cabinet.

Aseptic technique is the precautionary measure that is taken to prevent contamination or it is the procedure which is carried out in laminar airflow chamber (aseptic area) in order to avoid contamination/entry of unwanted microbes by any means.

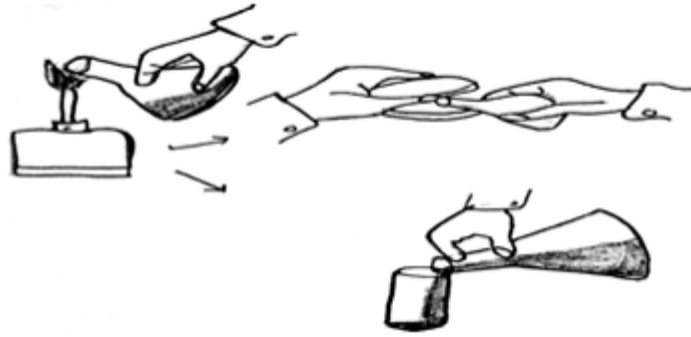
Aseptic transfer of sterile nutrient agar into test tubes, petriplate for preparing nutrient agar deep tubes, nutirent agar slants and nutrient agar plates.

1. Hold the flask containing sterile nutrient agar molten media in the right hand and remove the cotton plug of the flask.
2. Flame the open neck of the flask with the burner.
3. Pour media into sterile test tubes, heat the tube and replug the tube.
4. Put it in an upright position (for the preparation agar deep tubes) or slanted position (for preparing agar slants).

5. For agar plates, lift the half of the lid of sterile Petri plate and pour media into the Petri plates.

burner

Petriplate



Transfer of agar into petriplates and test tubes

(a) (b) (c)

a- Agar petriplates
b- Agar slant
c- Agar deep tube

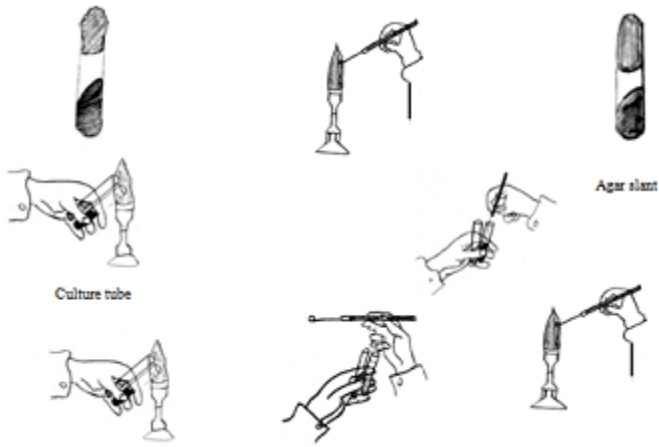
Aseptic transfer of microorganism from culture tube broth slant

1. In the left hand take culture tube and broth/slant.
2. Then in right hand take the inoculating loop and on flame heat it until the nichrome wire is hot red.

3. Allow it to cool.
4. Cotton plugs are removed for unplugging the tubes and heat the neck/rim of the tube in the flame.
5. Introduce the sterile inoculating loop into the stalk culture tube and seize out a few inoculums.
6. This inoculum is transferred to the broth/slant.
7. Reflame the neck/rim of the test tubes and replug the tubes with respective cotton plug.
8. After inoculation flame the inoculating tube loop.

Aseptic transfer of broth

Aseptic transfer of culture to slant or inoculation on agar slant



Similarly, inoculation can be done from broth culture/liquid culture to the slant.

Aseptic transfer of liquid culture to Petriplate/flask/tube

Sometimes, it is necessary to transfer a measured amount of liquid for enumeration of microorganisms from a soil sample, water analysis etc. Sterile micropipettes or serological pipettes are used for transfer of a measured volume of the microbial culture.

The microbial culture should be transfer in one step. Suppose, we have to transfer 1.5 ml than 1.5 ml of sterile pipette should be taken to transfer in one step. For this never use 500 µL capacity micropipettes and transfer 500 µL three times, as it increase chances of contamination.

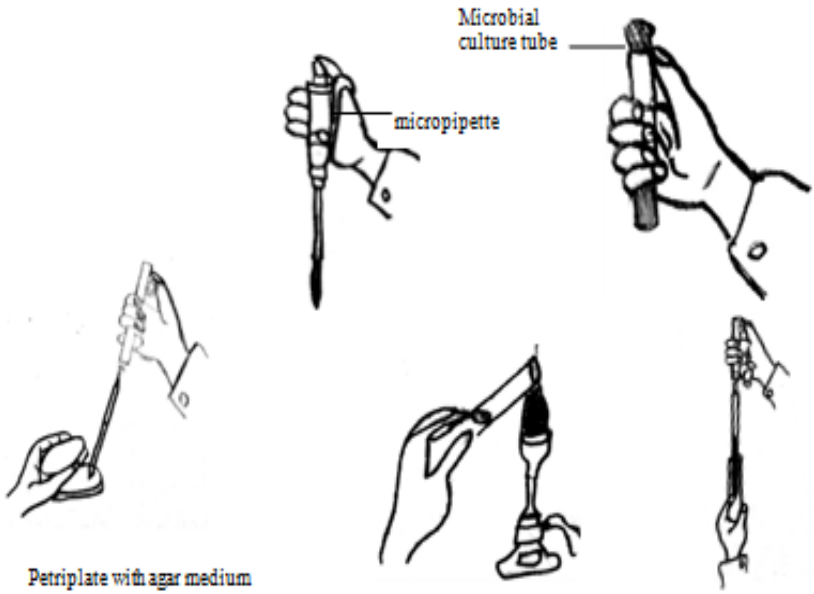
Steps for transfer of microbial culture to petri plate/flask/tube.

1. In the laminar airflow cabinet, insert the pipette into the lob of the pipette pump.
2. Hold the culture tube, then pipette pump (or micropipette with microtip)
3. With the little finger, remove the cotton plug from the culture tube.
4. Heat the neck of the open tube.
5. In the pipette fill up the required volume of culture.
6. Reflame the neck of the opened culture tube and replug it.
7. Lift the half lid of the Petri plate by pressing the pipette pump, pour the culture into the Petri plate. With the help of spreader spread the culture onto the Petri plate.
8. Close the lid of the Petri plate.

Steps 1 to 6 is same for transfer of culture into tubes /flask.

1. Remove the cotton plug from tube/flask.
2. Flame the neck/rim of open tube/flask.
3. Pour the culture in tube/flask containing sterile medium by pressing the pipette pump
4. Again heat the neck/rim of tube/flask.
5. With respective cotton plug replug the culture tube/flask.


Test tube
with medium
Flask with medium
Petriplate with
agar medium
pipettes


Microbial
culture tube
micropipette
Petriplate with agar medium