Aim 21 Subculturing of Microorganisms

Introduction

Subculturing is the transfer of microorganisms from one medium to another. The term picking off is used for the transfer of microorganism from solid medium to liquid medium (broth).

The well isolated/separated colonies obtained by spreading, streaking or pour-plating that can be subculture by picking some cells from single colony onto the agar plate/slants or in broth with the help of needle or loop. From this number of culture can be prepared and used for maintaining stock culture.

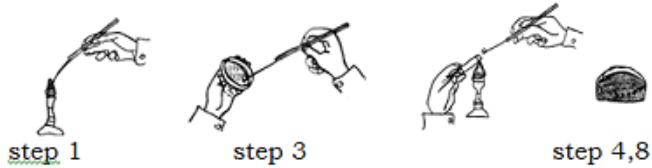
Requirements

1. Colonies obtained from streaking, spreading or pour plating.
2. Nutrient broth
3. Nutrient agar slants
4. Nutrient agar plates
5. Inoculating loop/ needle

Procedure

1. By flaming sterilize the inoculating loop/needle.
2. Allow the loop to cool.
3. Touch the tip of the loop/needle to the surface of the specific colony.
4. Unplug the agar slant and flame the rim/neck of the tube.

5. By inserting the loaded loop into the test tube, inoculate the slant and drawing it upward in a zig-zag manner over the surface of the agar.
6. Heat the rim/neck of the tube and replug it.
7. Lift the lid of the nutrient agar plate, inoculate it and replace the lid of petri plate.
8. Cells from the colony can be transferred to the broth (liquid medium) by removing the plug of the tube/flask, flame the neck of the tube/flask than insert the loop into the liquid medium.
9. Reflame the rim/neck of the tube/flask and replug it.
10. Heat the loop/needle to destroy the microorganisms on it.
11. At 28°C incubate the cultures in the slants / petri dish/liquid medium for 48 - 72 hours.
12. Examine the slants/ plates/broth/ for the growth of pure cultures. Similarly inoculation can be done from nutrient broth cultures to nutrient broth/ slants.


step 1

step 3

step 4,8


step 5,7,8


step 6,7,8


step 9

Precautions

1. Before and after each inoculation the loop/ needle must be flamed.
2. All transfer work should be done under aseptic conditions.
3. Always heat the neck of the test tube/flask before inserting the loop and after withdrawing the loop.