Mi2 : Aseptic work : transfer of medium, preparation of an agar plate ; streak plate and pour plate technique

Requirements for one experiment

Two sterile Petri dishes
Two cottoned tubes of 25 mL of sterile melted agar (in a water bath at 80°C) } for parts 2 -3

5 mL of mixture of (E.coli + Staphylococcus aureus) broth
One cottoned tube containing 5 mL of sterile water
One sterile transfer pipette } for part 2

One agar slant with E.coli
One tube of 5 mL of sterile water } for part 3

One broth 5 mL with E.coli
One sterile agar slant } for part 5

1. Introduction

Page 1 : the general forms of media found in a microbiology laboratory : broth, agar slant, agar deep, agar plate ; study the properties of broth culture.

2. Broth transfer : page 2

Insert the loop into the broth of mixture and introduce the inoculum into the sterile water : this preparation is kept for a streak plate technique

3. Agar slants as Source of inoculum : page 3

Remove a small portion of the culture of E.coli and immerse the inoculum in the sterile water, and gently shake : this preparation is kept for a streak plate technique

4. Preparation of agar plate and streak plate technique

- Preparation of the plate : remove a melted nutrient agar deep from the water bath, cool to 45°C ; pour the content of the tube into the bottom of a labelled Petri dish, allow the agar to harden (10min)
- streak plate technique : page 4 ; faster technique : streak the inoculum (mixture and pure suspension of Ecoli) on the agar surface from side-to-side in parallel lines covering one half of the plate (do not dig into the agar) ; don't flame the loop ; rotate the Petri plate one quarter, make a second set of streaks covering one quarter of the plate, without skimming the first set of streaks ; rotate the plate one quarter and make a last set of streak, more spaced. Incubate the plate in an inverted position, at 37°C.

This technique can be used to isolate colonies : it is the pour plate technique, in which the melted agar is mixed with a culture and then poured in a Petri dish ; after incubation, isolated cells grow into colonies and can be used to establish pure culture : page 5

After incubation : observe the colonies where they are isolated : page 6 ; above all :
- the number of types of colonies
- diameter

- elevation : flat or convex
- shape : irregular or circular
- surface properties : smooth or wrinkled
- pigmentation

5. Inoculation of an agar slant : page 7

Obtain an inoculum by removing loopful of the broth of E.coli ; place the loop into sterile agar slant surface at its bottom ; move the loop from side to side as you pull it upward out of the tube : incubate at 37°C.
After incubation, observe the culture on the agar slant.

Report :

Description of the isolated colonies for :
- the mixture Ecoli + Staphylococcus aureus
- the pure culture of Ecoli

Aspect of the culture on the agar slant.