Practical Work N° 5

Inoculation Techniques

1. Introduction

Inoculation is the process of depositing microorganisms from a parent culture (natural environment or pure culture) onto a sterile culture medium using a platinum loop or a Pasteur pipette.

2. Objective

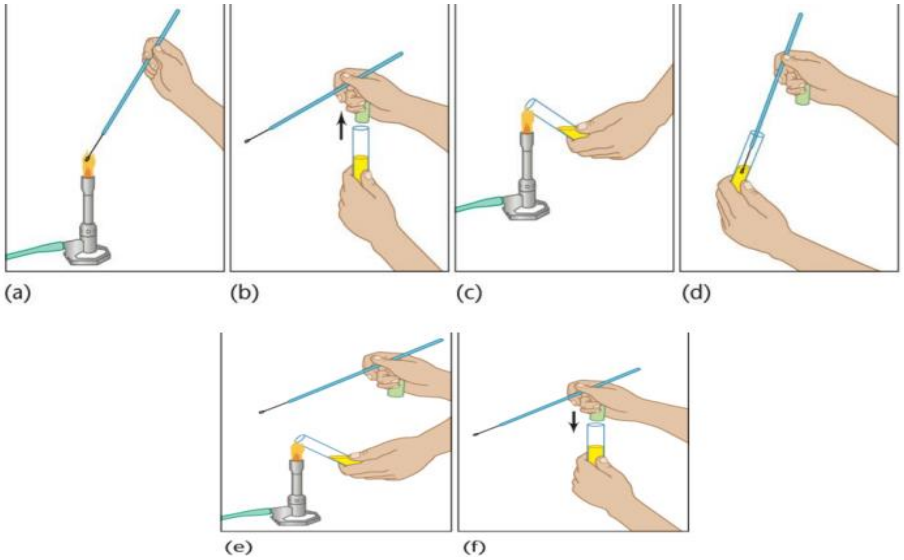
- To learn and apply various inoculation techniques for microbial growth, enumeration, and isolation.
- To master aseptic handling of microbial cultures.

3. Materials

- Platinum loop (straight and looped)
- Pasteur pipettes
- Sterile swabs
- Test tubes
- Petri dishes
- Sterile tubes and racks
- Culture media (broth and agar)
- Incubator
- Bunsen burner
- Sterile water or sterile diluent
- Sterile spreaders ("rakes" made from Pasteur pipettes)
- Bleach (for disposal of microbial waste)

4. Methods

Generally, for a right-handed operator, the right hand—which holds the platinum loop or Pasteur pipette in the aseptic area—should remain still. All necessary materials (culture medium tubes, dilution tubes, sterile tubes, or slides) must be brought toward the right hand using the left hand.

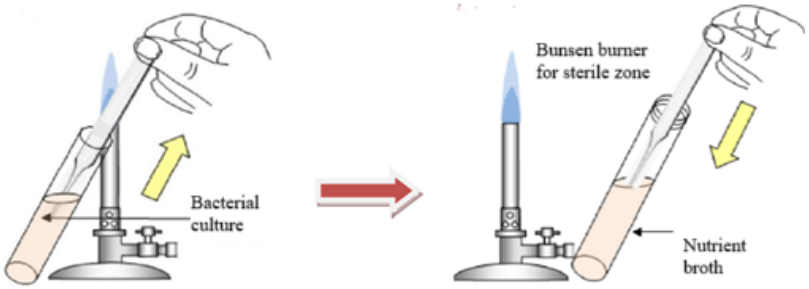


4.1. Main inoculation techniques

Depending on the type of culture medium, its intended use, and the desired objective, different inoculation methods are employed.

4.1.1. Inoculation of liquid culture media

Liquid culture media are usually placed in test tubes. Inoculation is performed by simply depositing microorganisms, from either a liquid or solid culture, using a platinum loop, a Pasteur pipette, or a glass pipette.

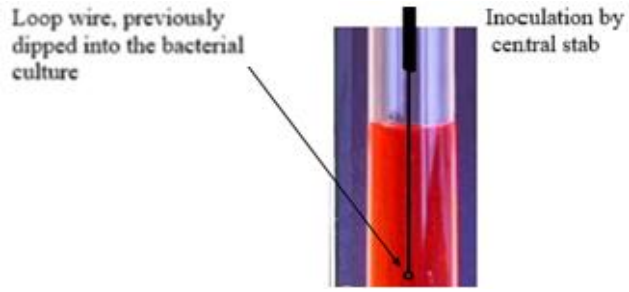


Inoculum sampling **Inoculation of the nutrient broth**

4.1.2. Inoculation of solid culture media

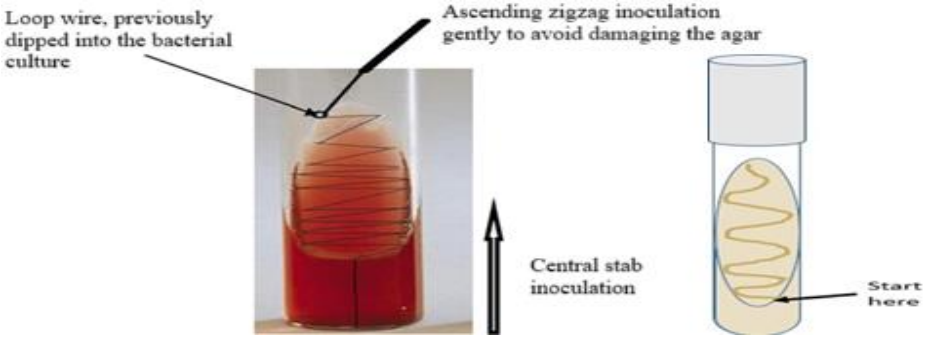
A. In tubes

- Agar deep tube: The medium is inoculated by a central stab along the agar surface using a straight platinum wire loop or a Pasteur pipette previously dipped in the bacterial culture.



- Slant agar: Two areas of the agar must be inoculated:

- The butt is inoculated with a central stab.
- The slant is inoculated by streaking (in a zigzag motion) from bottom to top using a looped platinum wire or a Pasteur pipette.



B. In Petri dishes

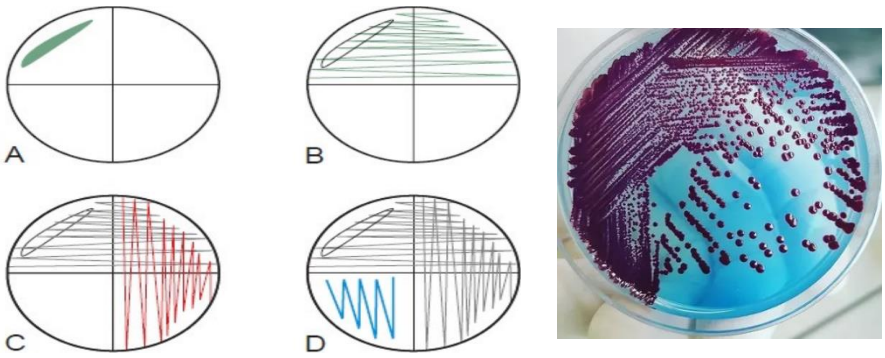
B.1. Surface inoculation

Depending on the type of analysis, surface inoculation can be performed by:

a. Streaking ("quadrant method") (used for purification, etc.):

- Hold the Petri dish in the left hand and the inoculating loop in the right hand.

- Deposit the microbial suspension at the edge of the first quadrant.
- Use the loop (or a Pasteur pipette) to make tight streaks across the first quadrant, applying gentle pressure to avoid damaging the agar.
- Flame the loop and allow it to cool.
- Rotate the dish to the second quadrant and repeat the streaking.
- Rotate again for the third quadrant, and streak with spaced lines that do not cross the previous streaks.



b. Flooding:

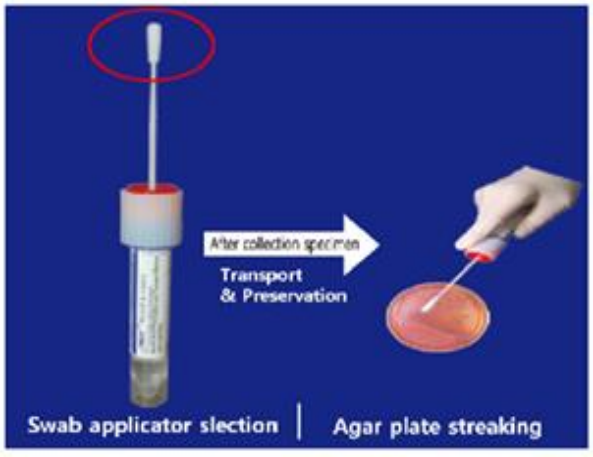
- Pour 1 to 5 ml of the microbial suspension over the solidified surface.
- Ensure the surface is fully covered, then remove and discard any excess suspension into bleach.

c. Spreading (used for microbial enumeration):

- Deposit 100 µl of the microbial suspension onto the solidified surface.
- Use a spreader "rake" (can be made from a Pasteur pipette) to evenly distribute the drop across the surface.

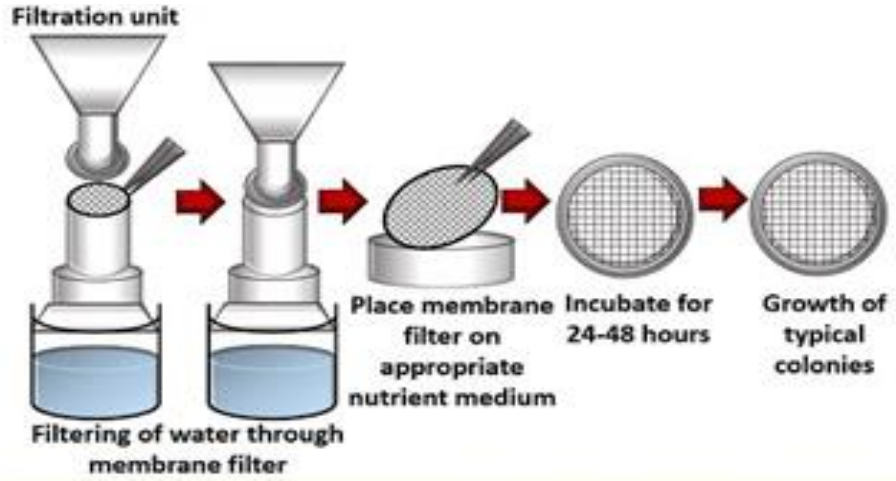
d. Swabbing (used either to sample from a microbial suspension — for example, in antimicrobial sensitivity testing — or to sample a surface):

- For antimicrobial sensitivity testing, use a dry sterile swab to collect the microbial suspension directly.
- For surface sampling, moisten the sterile swab with sterile diluent or broth before use.
- Rub the swab gently over the surface to be tested or dip it into the microbial suspension as appropriate.
- Then streak the swab evenly across the surface of the solidified culture medium.



e. Membrane filtration:

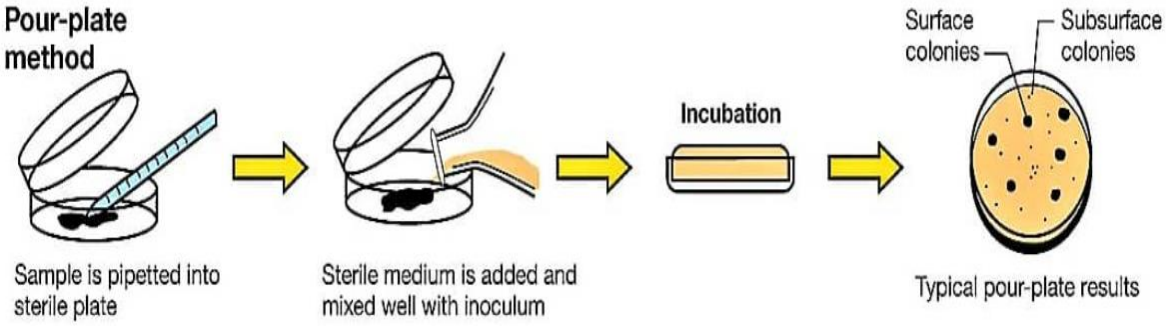
- Filter a large volume of water sample and place the membrane filter onto the surface of a solidified culture medium.
- This technique is used to enumerate bacteria present at very low concentrations in water.



B.2. Deep inoculation

a. Pour plate method (Inoculation into the medium):

- Deposit 1 ml of microbial suspension into an empty Petri dish using a sterile glass pipette or micropipette.
- Pour about 15 ml of appropriate molten culture medium (at 47°C) over it.
- Gently mix by making a figure-eight motion.
- Allow to solidify, then incubate.



b. Double-Layer inoculation :

- Perform a pour plate inoculation as above.
- Once the medium has cooled and solidified, pour a second layer of about 5 ml of molten culture medium (at 47°C) over it.
- This creates a microaerophilic environment.

Note :

- Every inoculation must be followed by incubation in an incubator. The temperature and duration depend on the microorganism to be isolated.

Example: For *Escherichia coli* and many other bacteria, incubation is carried out at 37°C for 24 hours.

- Microbial growth appears as:
  - **Turbidity** in liquid media.
  - **Colonies** on solid agar.