

SPECIMEN

General principle

Proper collection and transport of specimens is critical to the quality of results produced by the microbiology laboratory. The validity of all diagnostic information produced in the lab is contingent on the quality of the specimen received. Consequences of poorly collected and/or poorly transported specimens include failure to isolate the causative organism, and recovery of contaminants or normal flora which could lead to improper treatment of the patient.

Collection of Specimens for Microbiology

1. Collect specimen before administration of antimicrobial agents when possible.
2. Collect a sufficient quantity of specimen. Too little may yield false negative results.
3. Collect with as little contamination from normal flora as possible to ensure that the specimen represents the infected site. If collection is through intact skin, cleanse the skin first.
4. Use appropriate collection devices, sterile equipment, and aseptic technique. Use only the standard equipment recommended by the laboratory.

Labeling

Proper transport and storage of specimens are prerequisites for reliable culture results. Ensure that the requisition and specimen are labeled with:

- a. Patient name and Date of Birth (this must be on each specimen)
- b. Identification number
- c. Source
- d. Date
- e. Time of collection

Transport of Specimens

1. Transport all specimens to the laboratory promptly (within 30 minutes of collection).
2. All specimens for culture must be transported in a suitable container or placed in a biohazard bag for safe transport to the laboratory.

Methods of cultivation and Isolation of pure cultures

Introduction

Bacterial cultures are the ideal method for multiplying and reproducing microorganisms in laboratory conditions. Culturing bacteria is mainly used for infectious diseases of sick patients and is also an extremely important tool in microbiology labs for research and for teaching students of microbiology. The identification process of an unknown microbe relies on obtaining a pure culture of that organism .

Pure culture: is a culture that contains only one kind of microorganisms , Isolation of pure cultures is very important in a clinical microbiology laboratory .

mixed culture: is a culture that contains more than one kind of microorganisms.

- There are three methods of cultivation and obtaining a pure culture

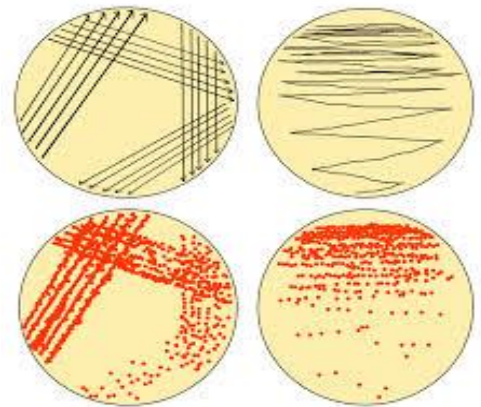
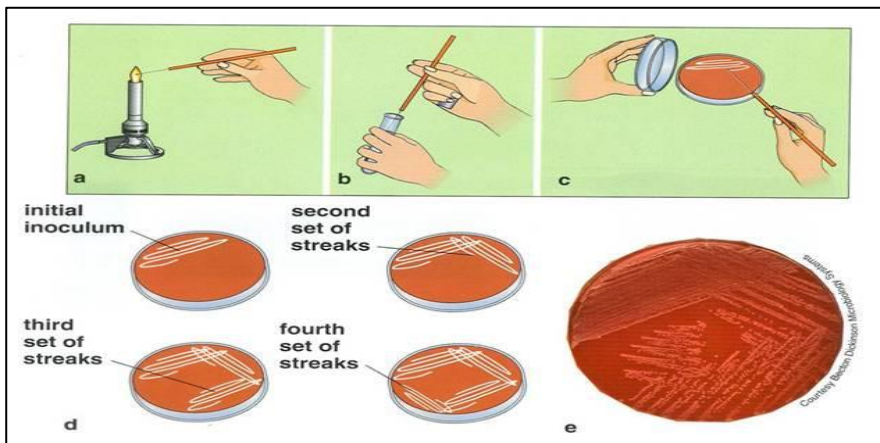
A- Streak plate method:

The streak plate method produces individual colonies on an agar plate. A portion of an isolated colony then may be transferred to a sterile medium to start a pure culture. In the streak plate method of isolation, a bacterial sample (always assumed to be a mixed culture) is streaked over the surface of a plated agar medium .During streaking, the cell density decreases, eventually leading to individual cells being deposited separately on the agar surface. Cells that have been sufficiently isolated will grow into colonies consisting only of the original cell type.

Method :-

- 1- Take a sterile agar plate and label on the back of the agar surface 1,2,3,4 at four corners, approximately 1 cm away from the edge of the plate .
- 2- Take an inoculating loop and flame to deep red and cool.
- 3- Charge the inoculating loop with the specimen to be cultured .

- 4- Streak very gently with a loop on the surface of agar (area 1) over a small area at periphery near the flame .
- 5- Re flame and cool the loop .
- 6- Turn the Petri dish 90° and touch the loop to a corner of the culture in the area 1 and drag it several time across the agar in area 2 .
- 7- Re flame and cool the loop .
- 8- Repeat the same in area 3 and area 4 .
- 9- Incubate plate in an inverted position for up to(24-48) hours at 37 °C .
- 10-Carefully observe the colony morphology of different colonies .

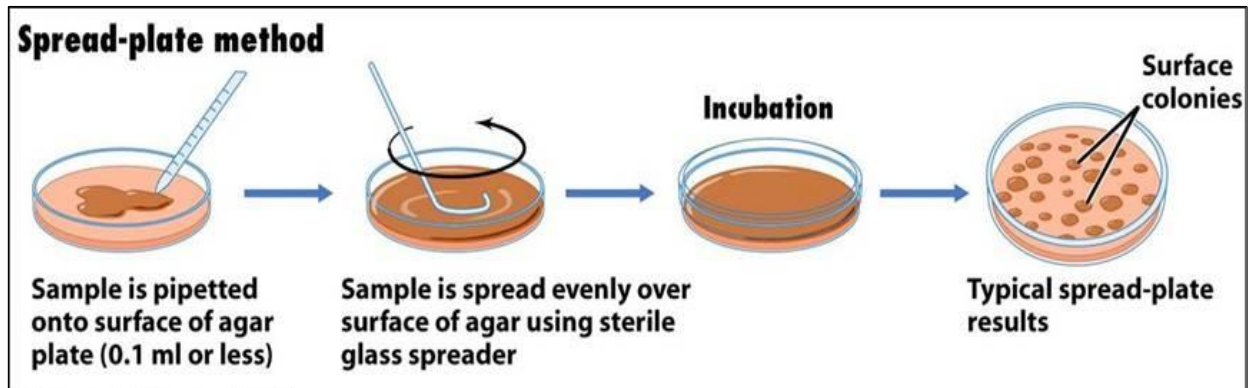


B- Spread plate method :

method of distributing bacteria evenly over the surface of an agar plate medium is commonly referred to as the spread plate method. Classically a small volume of a bacterial suspension is spread evenly over the agar surface using a sterile bent glass rod as the spreading device. The goal in evenly distributing the bacterial suspension is typically to permit the growth of colonies that can subsequently be enumerated (see Serial Dilution Protocols) and/or sampled following incubation. Each plate is spread with a single inoculum of the bacterial suspension.

Method :-

- 1- Take a sterile agar plate and label on the back of the agar surface .
- 2- With a sterile pipette, transfer(0.1) ml of culture to the agar plate .
- 3- Take L-shape glass rod , dip the lower bent portion in to 70% ethanol .
- 4- Remove the glass rod from ethanol and pass it through the Bunsen burner flame , with the bent portion of the rod pointing downward to prevent the burning alcohol from running down to arm . Allow the alcohol to burn off the rod completely .Cool the rod for 10-15 seconds .
- 5- Remove the Petri dish cover and lightly touch with sterile bent rod the culture on the surface of the agar and move it back and forth .(instead of spreader we can use swap).
- 6- Replace the cover on agar plate .
- 7- Remove the glass rod , dip in alcohol and re flame .
- 8- Incubate plate in an inverted position for up to(24-48) hours at 73 °C .
- 9- Carefully observe the colony morphology of different colonies .



3- Pour plate method :

The Pour plate method is not widely used but may be more convenient in some instances and some experienced workers prefer the pour plate method for isolating microaerophilic species . Some bacteria swarm over the surface and do not form colonies ,trapping them inside agar via the pour plate method is one way to get colonies of them .

Method :-

- 1- Liquefy agar media by autoclave or by boiling and cool the molten agar and maintain in a water bath at 45°C .
- 2- Take a sterile empty Petri dish and label on the back of the plate .
- 3- Aseptically add 1 ml of culture in the empty Petri dish .
- 4- Pour the agar media on the culture in the Petri dish and rolling the plate carefully to mix the contents well .
- 5- Allow the agar to solidify .
- 6- After solidifying, incubate plate in an inverted position for up to (24-48) hours at 37 °C .
- 7- Carefully observe the colony morphology of different colonies .

