isolation , processing of specimen for isolation , isolation in culture , pure culture Techniques

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Specimen collection

Many different specimens are sent for microbiological examination from patients with suspected bacterial infection.

Common specimens include **urine, faeces, wound swabs, throat swabs, vaginal swabs, sputum, and blood**.

cerebrospinal fluid, pleural fluid, joint aspirates, **tissue, bone and prosthetic material** (e.g. line tips).

Methods of isolating pure culture

1. Mixed culture

A culture contains more than one species of microbes.

2. Pure culture

A culture containing only one species of microbes.

The following methods are used to isolate pure culture.

A pure culture is usually derived from a mixed culture by transferring a small sample into new, sterile culture medium by dispersing or thinning the mixed culture multiple times before inoculating them. This technique was developed by German bacteriologist **Robert Koch**.

- **NEED OF PURE CULTURE:**
- To identify and study the characters of microbes.
- To detect the causative agent of particular disease.
- In order to find out antibiotic sensitivity and bacteriophage & bacteria susceptibility.
- Mutation rate in pure culture is low and clone is 99% identical.

Isolation

- Microorganisms occur in huge numbers.
- *Isolation of single species (pure culture) is done by :
  - **Streak plate method**
  - **Spread plate method**
  - **Pour plate method**

*Based on diluting sample out to a point where a single cell will give rise to a single colony

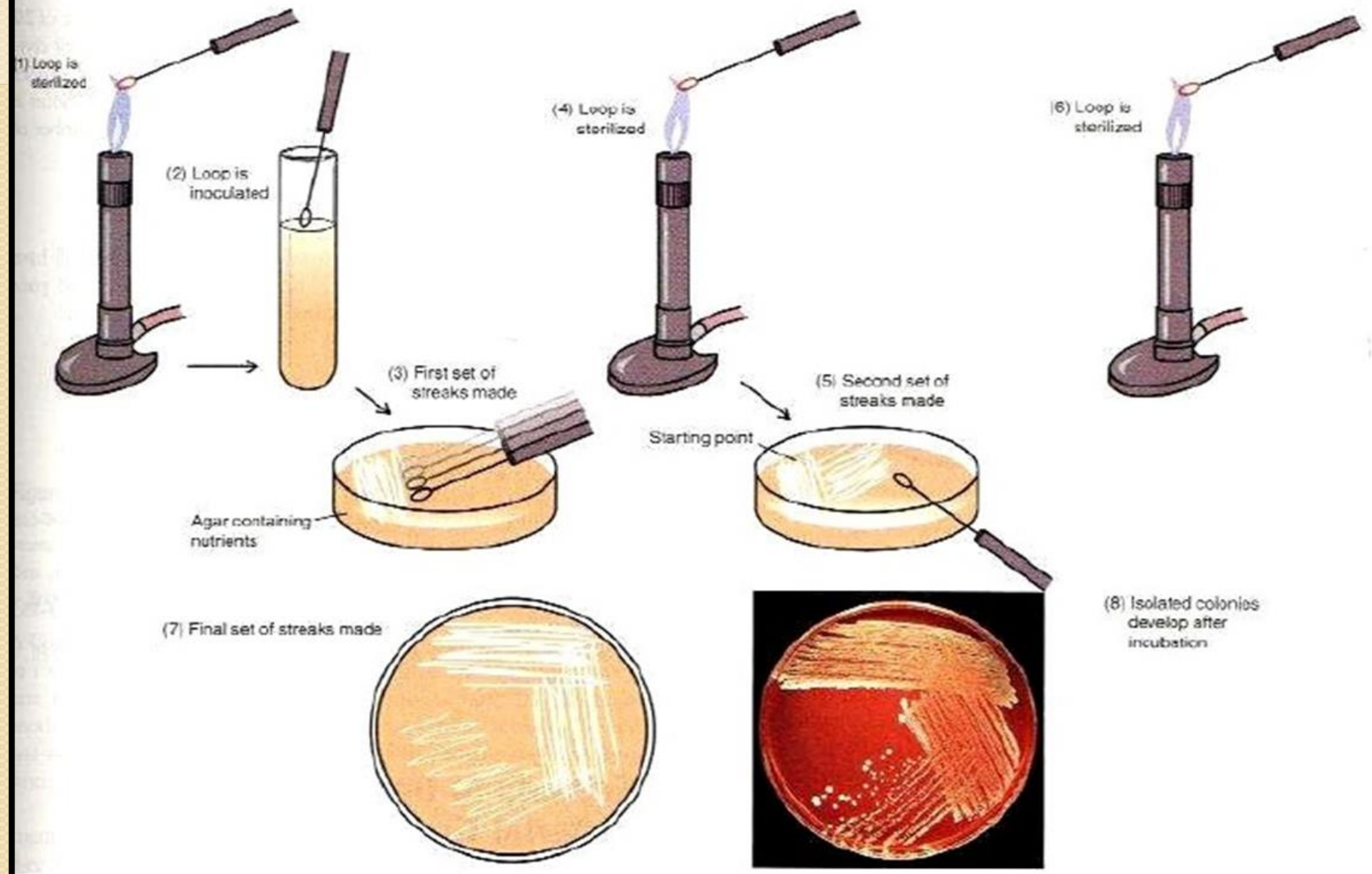
STREAK PLATE METHOD OF ISOLATION

- Most common way of separating bacterial cells on the agar surface to obtain isolated colonies

- After incubation

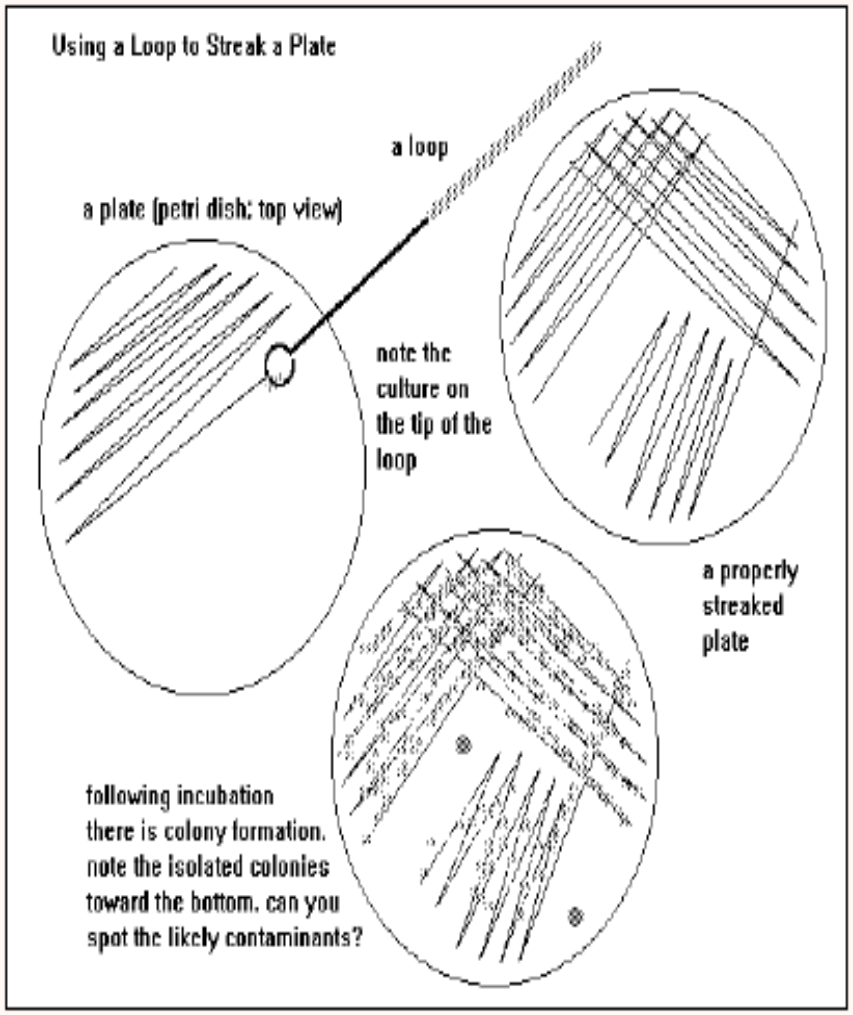
- area at the beginning of the streak pattern -confluent growth

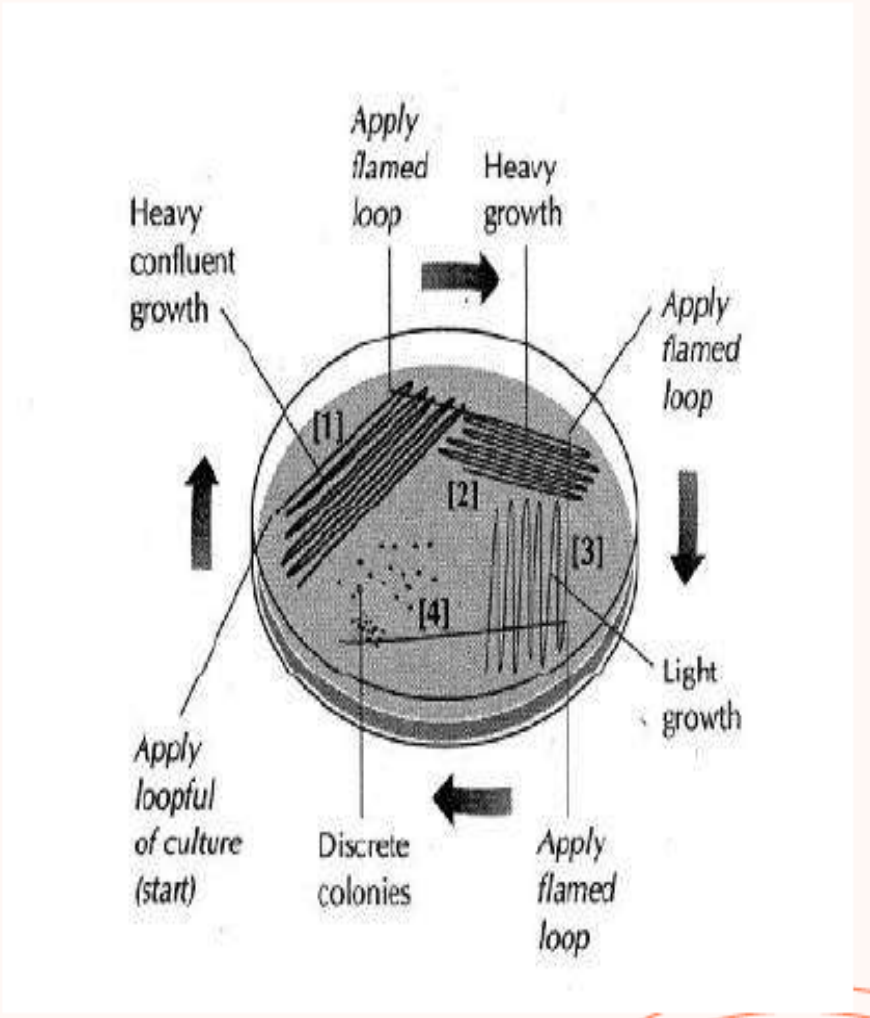
- area near the end of the pattern should show discrete colonies



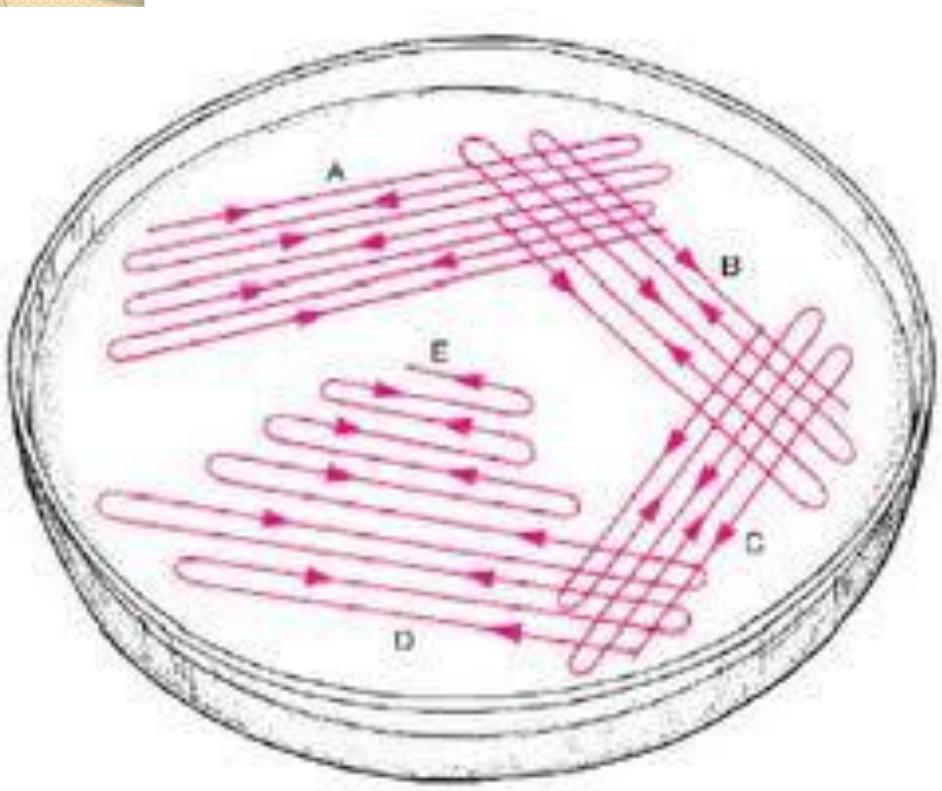
Streak-plate method

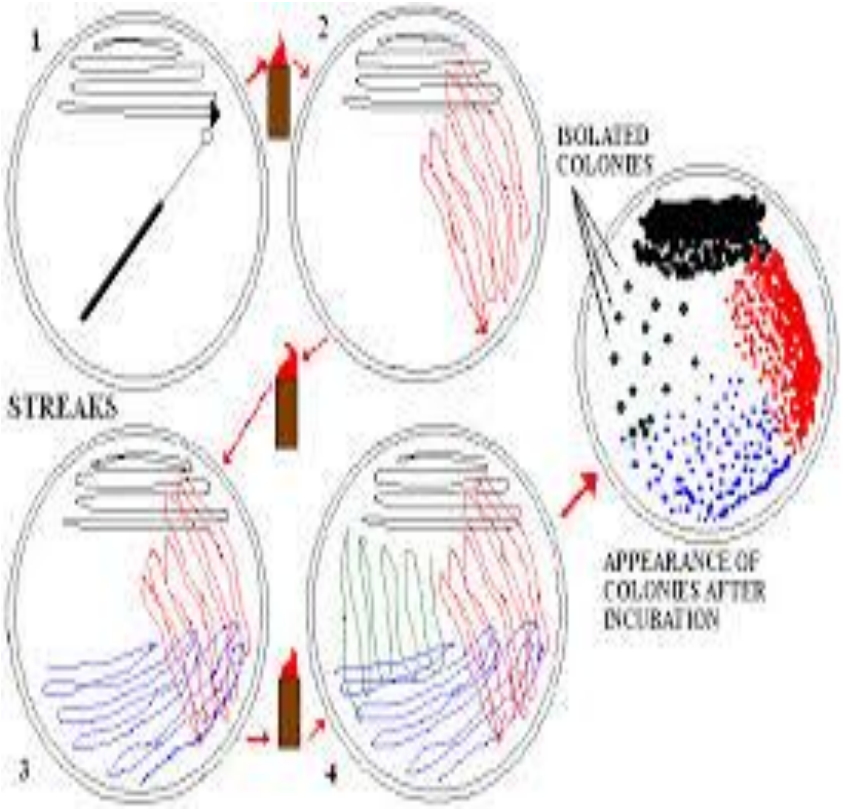
Pure culture – Streak Plates





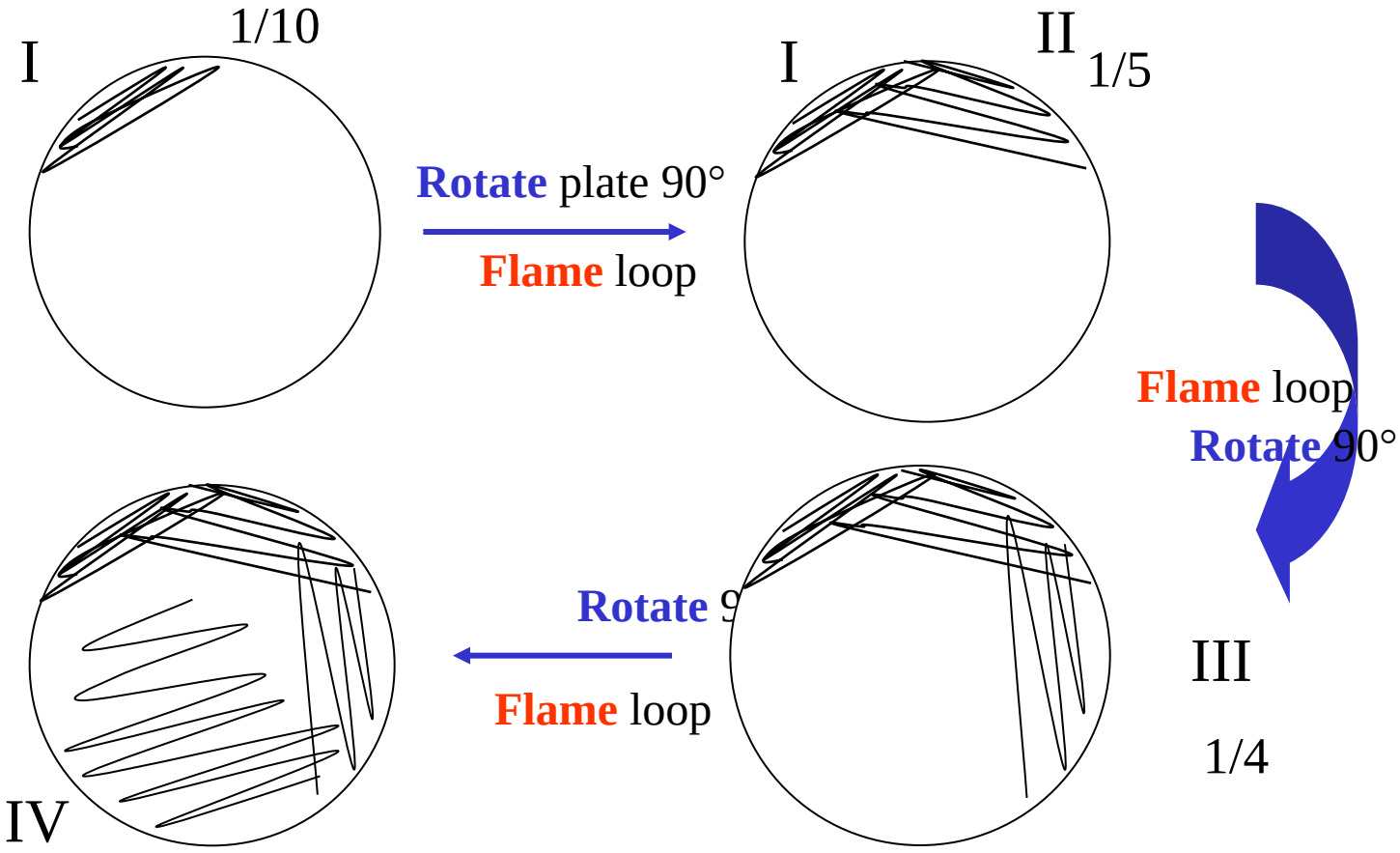
streak – plate technique

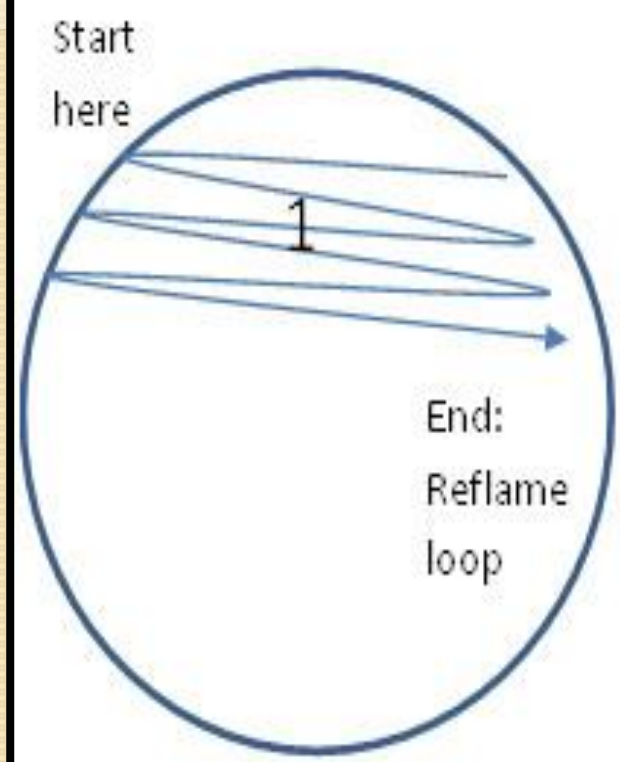




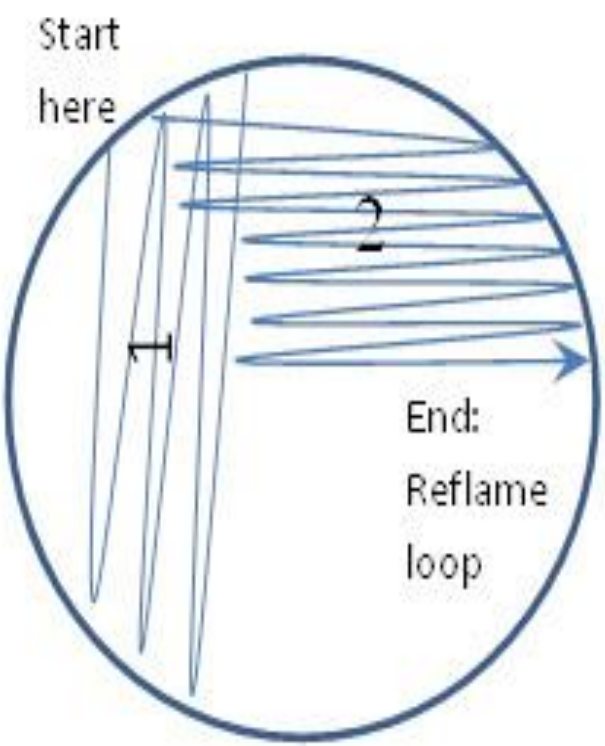
Streak-plate technique

four-area streak plate technique

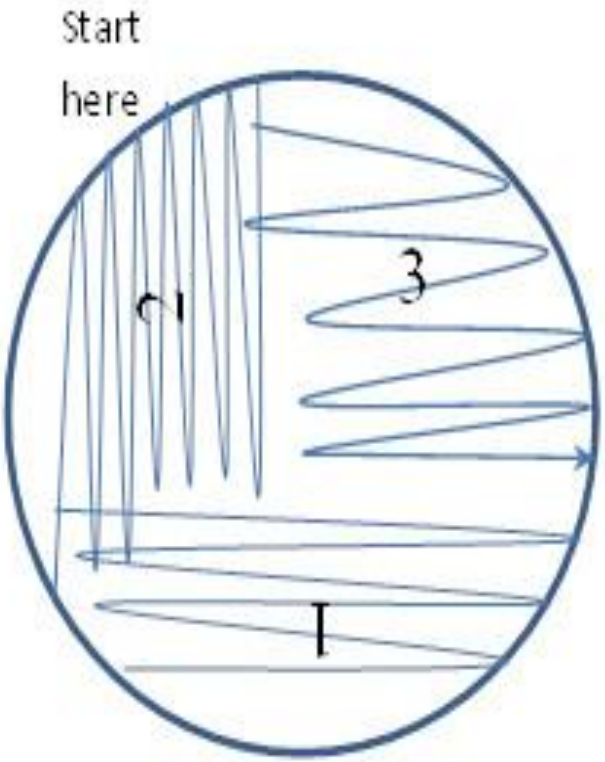




Step 1: Streak plate across the top sector, using continuous motion, but do not cross lines.



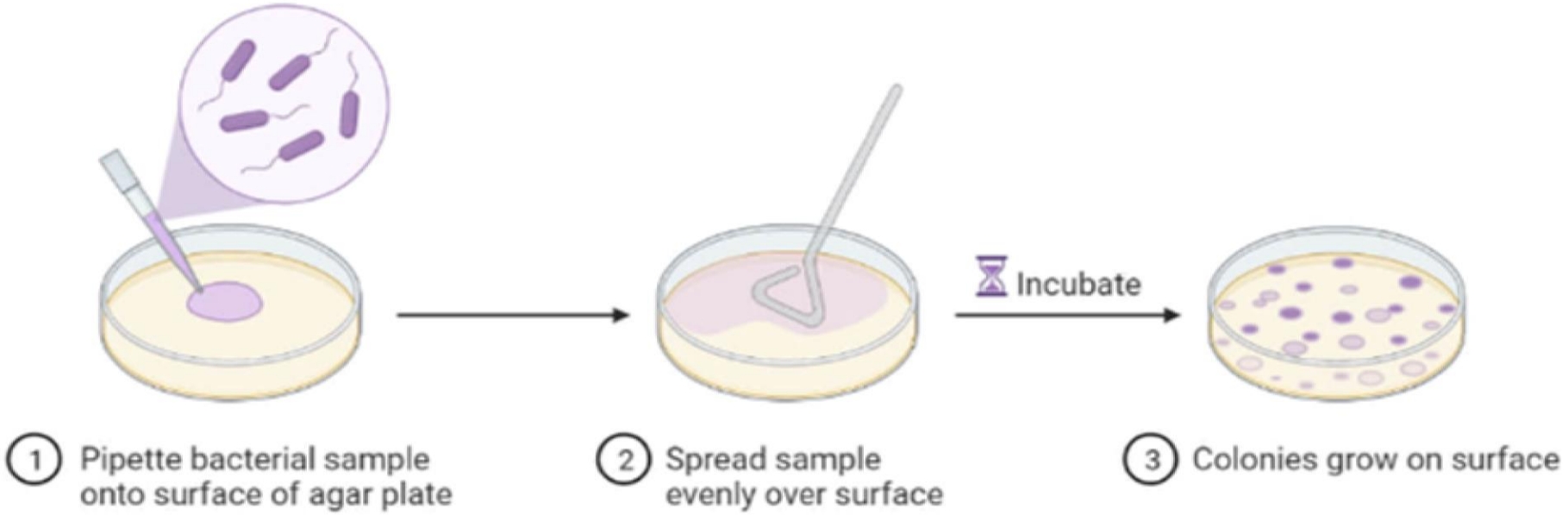
Step 2: Turn plate 90 degrees Streak plate across the second sector, using continuous motion, making sure to cross into the first sector during the first streak or two.



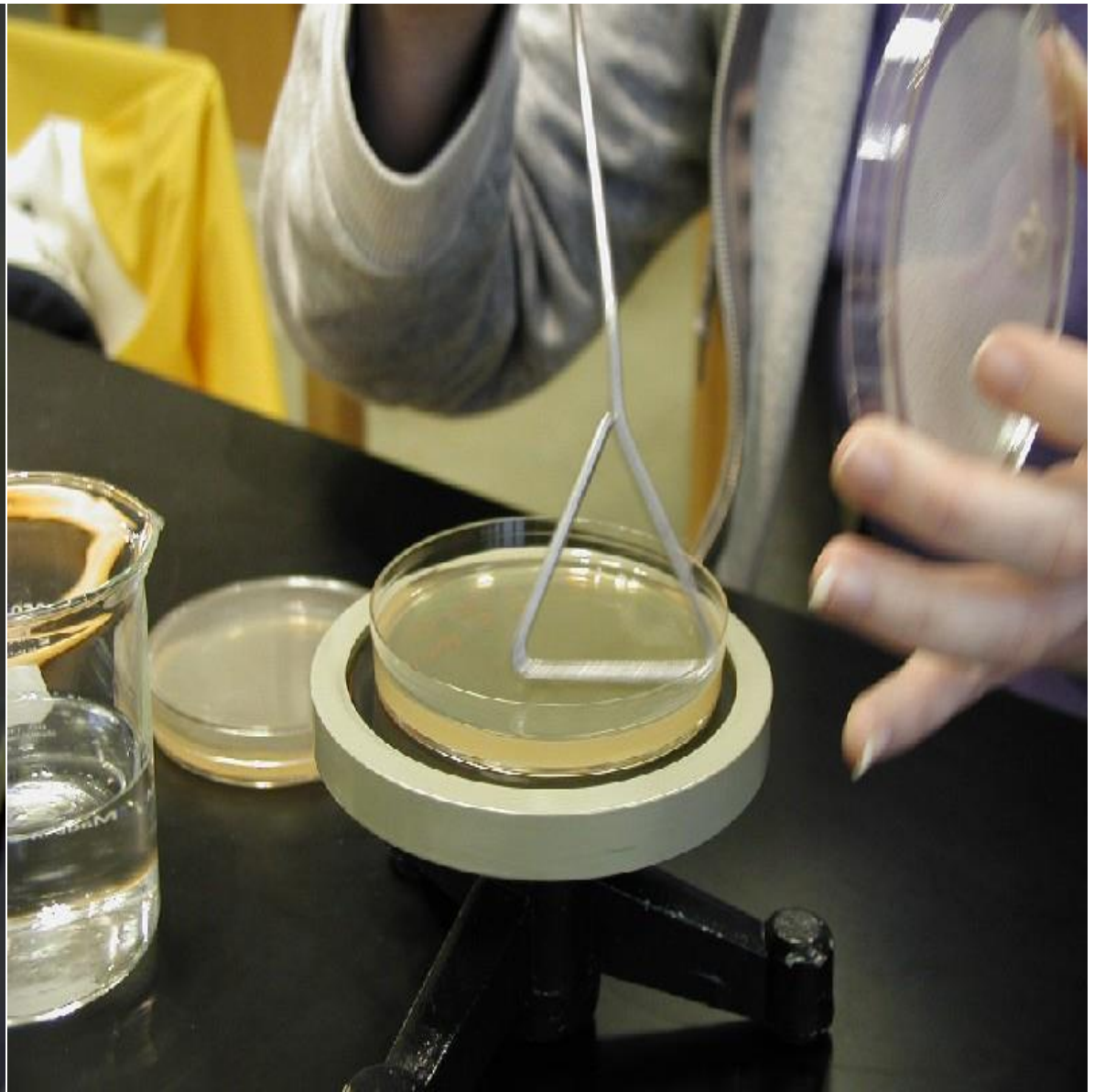
Step 3: Repeat step two, but streak across sector 3.

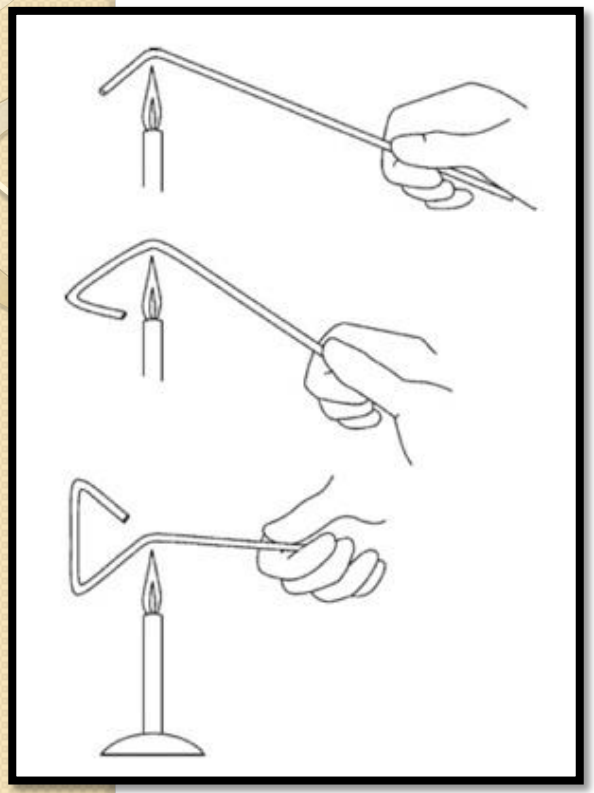
Spread Plate method

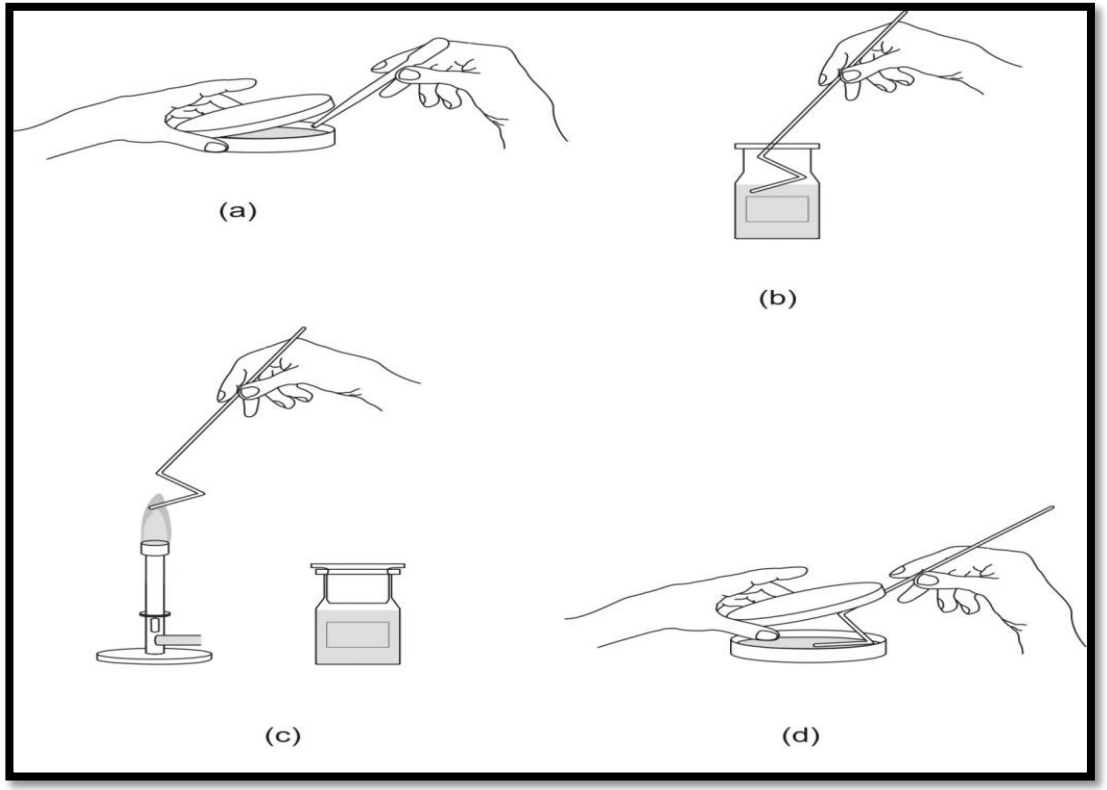
- Serially diluted specimen spread over the solidified agar media plates as a thin layer with the help of a sterile L-shape glass rod (Spreader)

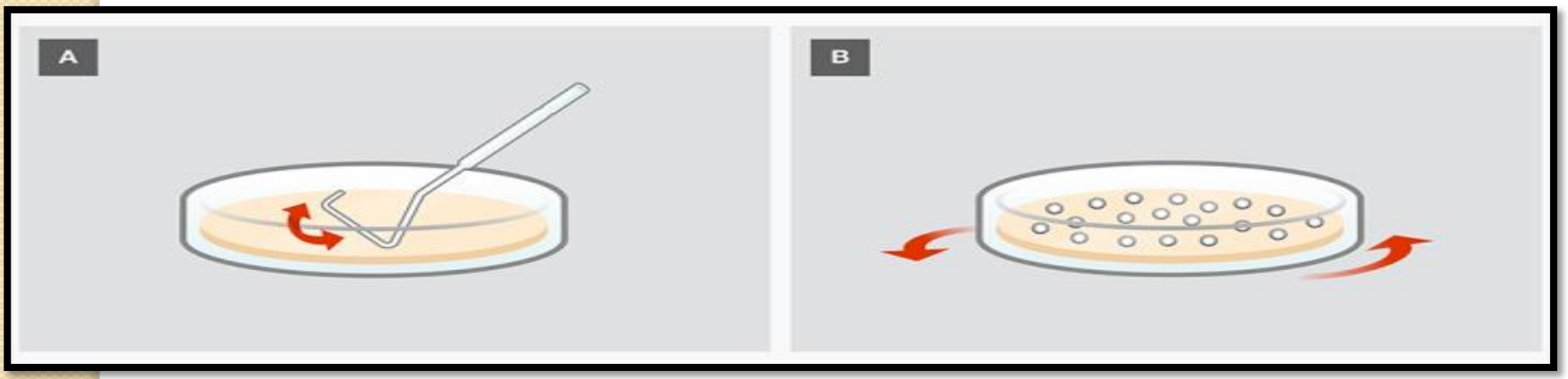


spread plate technique






(a)
(b)
(c)
(d)

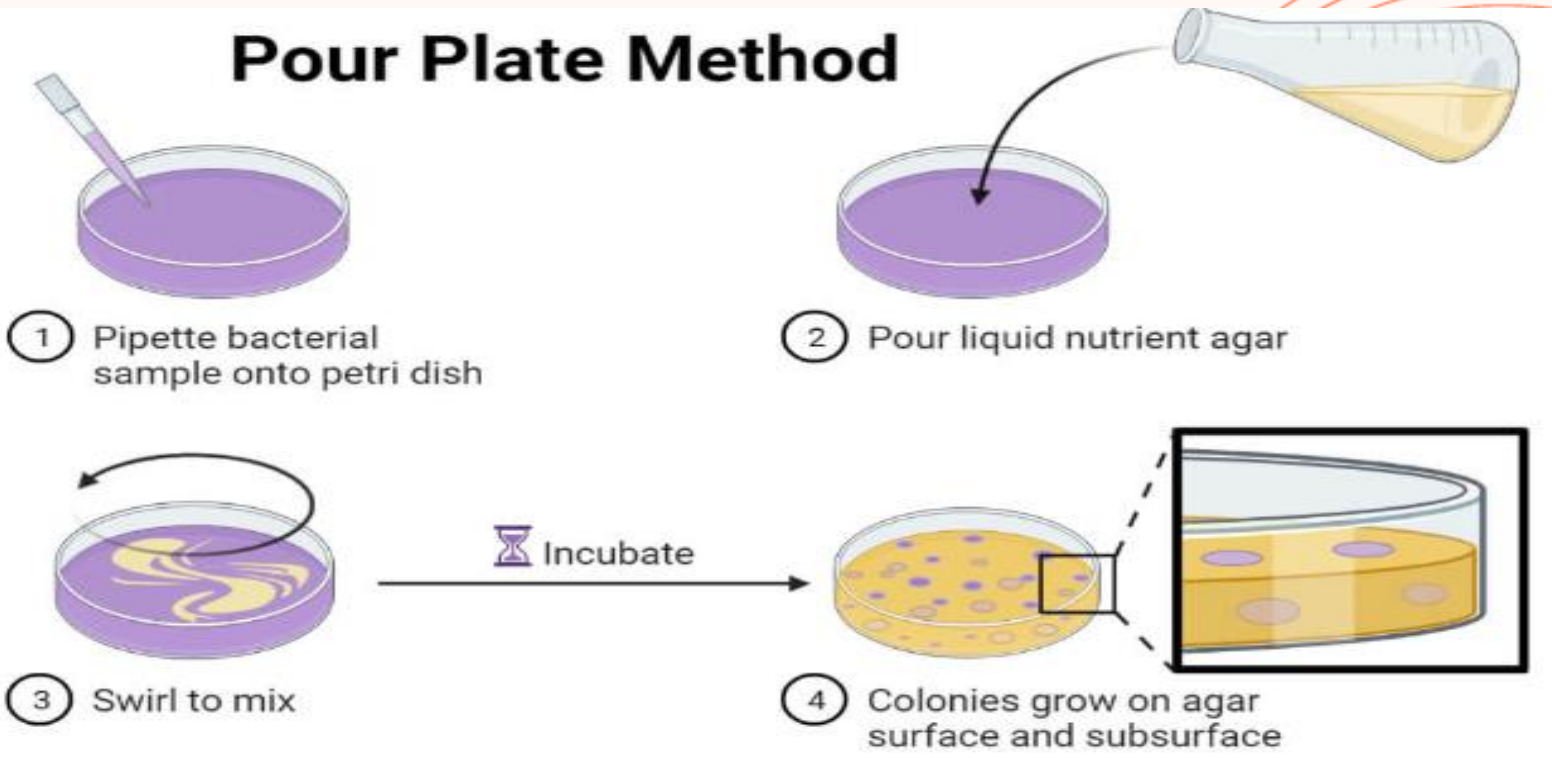

A
B

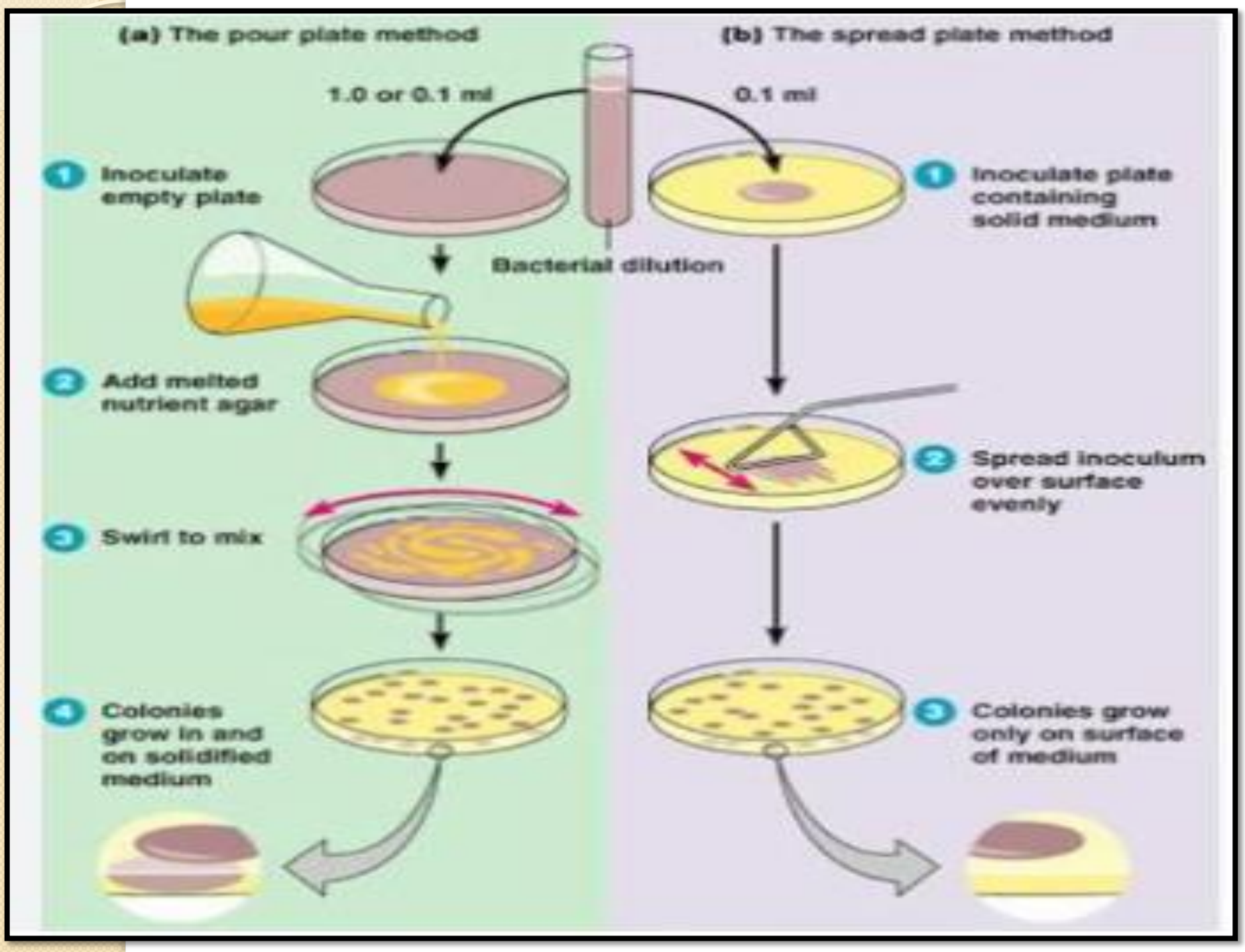
Spread Plate Method

1. Sample (0.1 mL) poured onto solid medium
2. Spread sample evenly over the surface
3. Plate incubated until bacterial colonies grow on the surface of the medium

Pour Plate Method

- Inoculum from a broth/sample placed in centre of sterile Petri dish using a sterile pipette
- Molten cooled agar then poured into the Petri dish containing the inoculum, mixed well and allowed to solidify
- After incubation, discrete bacterial colonies found growing both on the agar and in the agar




(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
Bacterial dilution
1 Inoculate plate containing solid medium
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium

- **What are dilutions in microbiology?**
- **Dilution** is **the process of making a solution weaker or less concentrated.**
- In microbiology, serial dilutions (log dilutions) are used to decrease a bacterial concentration to a required concentration for a specific test method, or to a concentration which is easier to count when plated to an agar plate

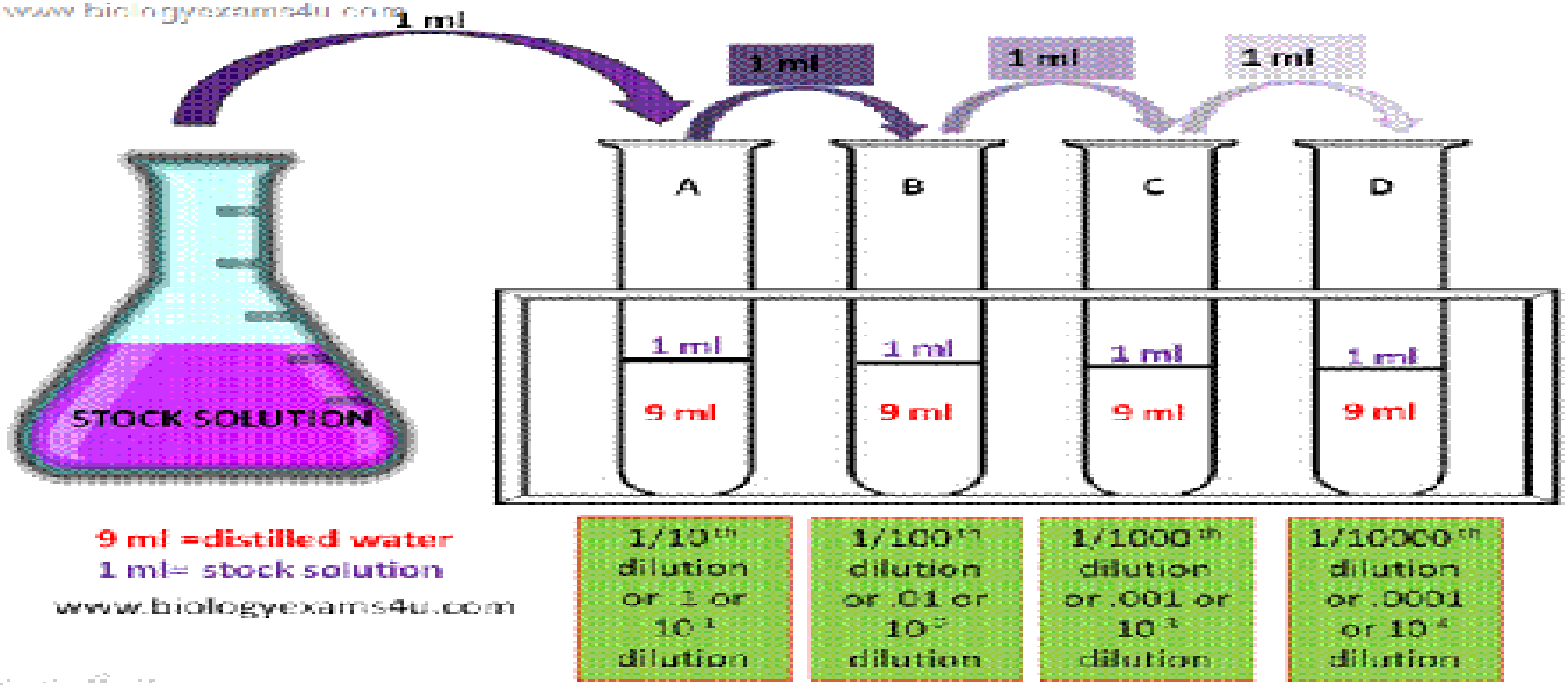
Serial Dilution Protocol

It is a method of diluting a stock solution where concentration decreases by the same quantity in each successive step.

Materials required: stock solution, test tubes, pipettes, beaker, and distilled water.

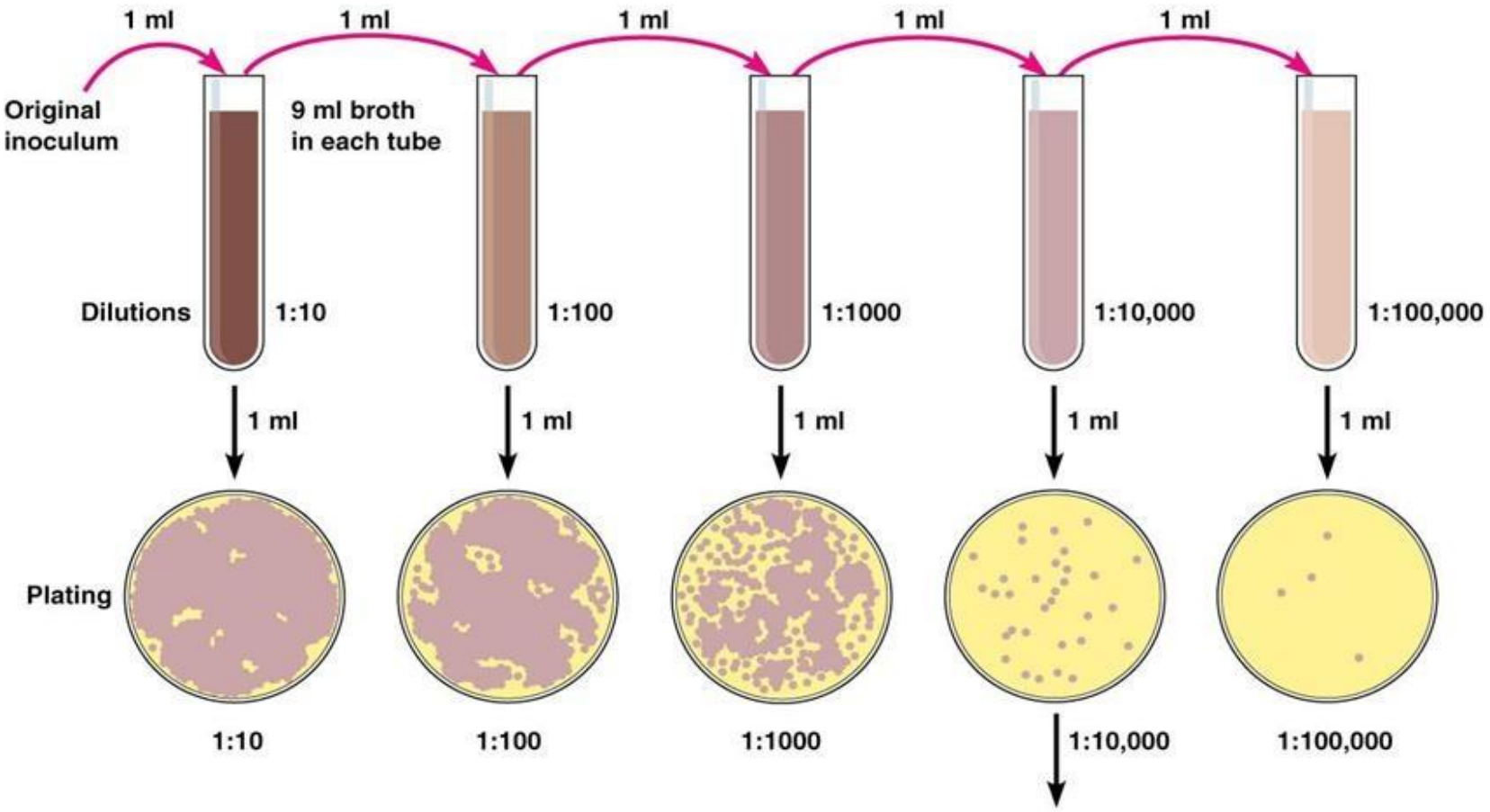
Procedure:

1. Take 4 test tubes label A, B, C and D
2. Pour 9 ml of distilled water to these test tubes
3. Transfer 1ml of stock solution to the test-tube labeled A and mix well
4. Transfer 1 ml of solution from test tube A to test tube B and mix well
5. Transfer 1 ml of solution from test tube B to test tube C and mix well
6. Transfer 1 ml of solution from test tube C to test tube D and mix well and continue up to the required dilution. Here we are making 10^{-4} dilution.



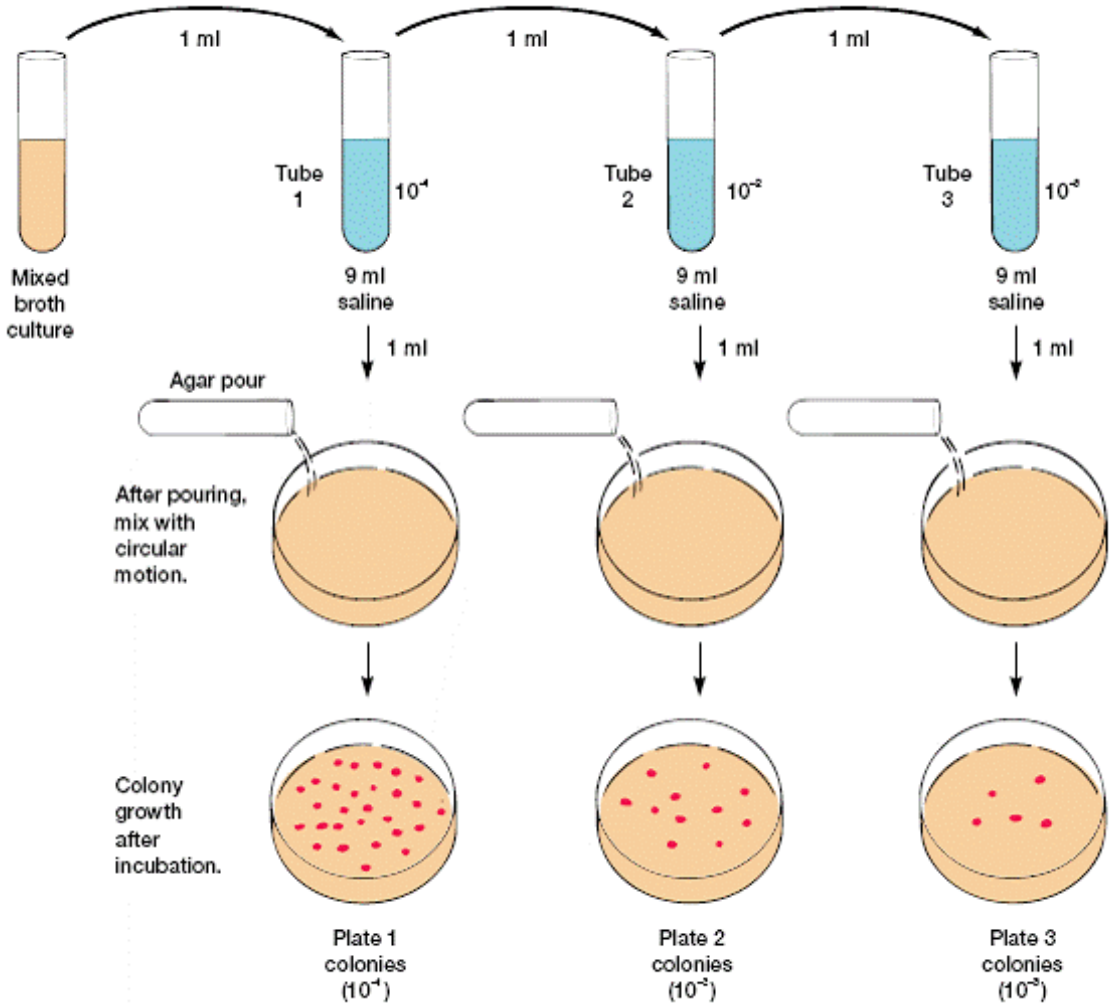
Inference:

- Test tube A has 10 times dilution or $1/10^{th}$ or 10^{-1} dilution of the stock solution.
- Test tube B has 100 times dilution or $1/100^{th}$ or 10^{-2} dilution
- Test tube A has 1000 times dilution or $1/1000^{th}$ or 10^{-3} dilution
- Test tube A has 10000 times dilution or $1/10000^{th}$ or 10^{-4} dilution.



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

pour plate technique




Thank you for
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