PHARMACY INDIA

MICROBIOLOGY

UNIT-1 I PART-5

Cultivation of Anaerobes, Quantitative Measurement of Bacterial Growth

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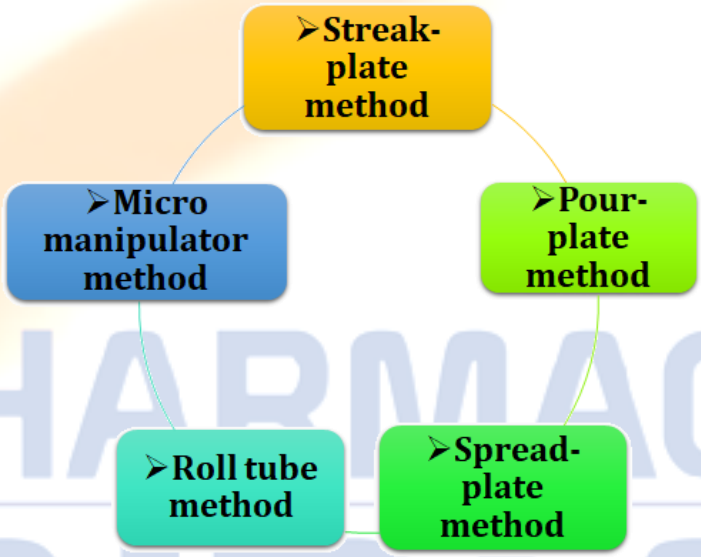
B. PHARMA 5TH SEM ONE SHOT NOTES

UNIT-1 | PART-5

ISOLATION AND PRESERVATION METHODS FOR PURE CULTURE

- Several methods of obtaining pure cultures are in use. The three most commonly used methods are:



STREAK-PLATE METHOD

The streak plate method is a microbiological laboratory technique of isolating pure cultures, and/or getting well-isolated colonies of bacteria from a mixed population.

It is mostly used to get pure cultures of bacteria; however, yeasts can also be isolated by this method.

It is one of the most commonly used aseptic techniques in microbiology to isolate and propagate bacteria.

It is a mechanical isolation technique used in microbiology, commonly known as the "streaking method".

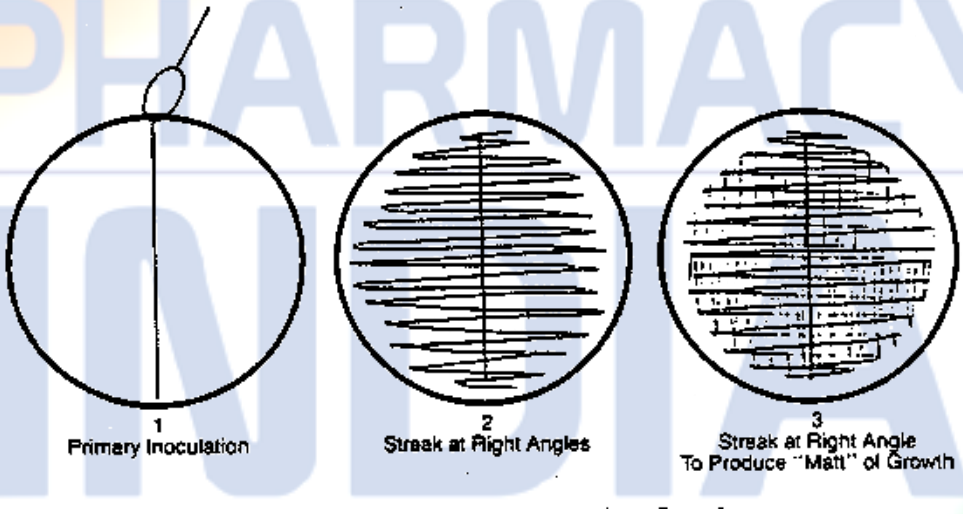
- **Principle:**
  - ✓ The **streak plate method is based on dilution during** the process of mechanical spreading of inoculum over the surface of solidified culture

media in order to obtain well-isolated colonies of the sample at the terminal streaks.

- ✓ **Sample can be either colony on solid media** or suspension in broth. The sample is picked by using different tools, mostly using a sterile inoculating loop or swab.
- ✓ **The sample is placed over a surface of sterile** solid media at one edge of the petri dish and a smear is prepared.
- ✓ Using the tool, the **smear is successively streaked over the agar medium on different patterns.**

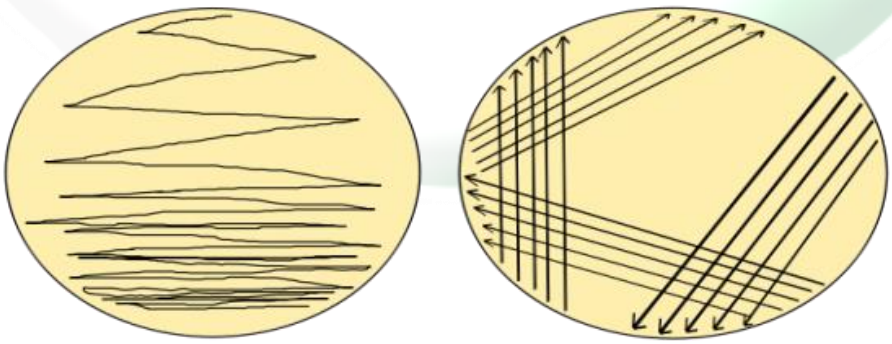
➢ **The two most common methods of streaking are:**

1. **Continuous streaking method:**
   - ➢ This method of streaking is continuous in the sense that there is no overlapping of streaking **zones and the loop is sterilized only once in the beginning and at the end.**



2. Discontinuous streaking method:

- In this method streaking is discontinuous and the loop is sterilized between streaking.



POUR PLATE METHOD

The pour Plate Method technique was established in the laboratory of Robert Koch and is still being used widely since his period.

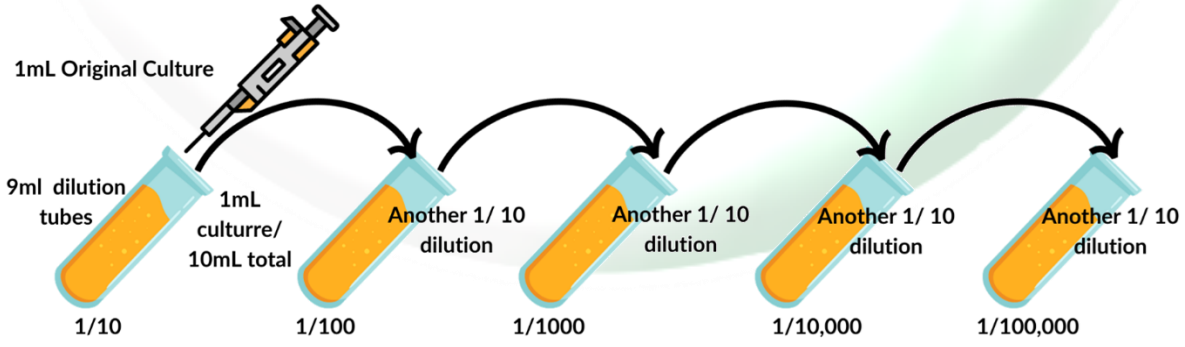
This method is suitable for facultative, Microaerophilic, and anaerobic microorganisms.

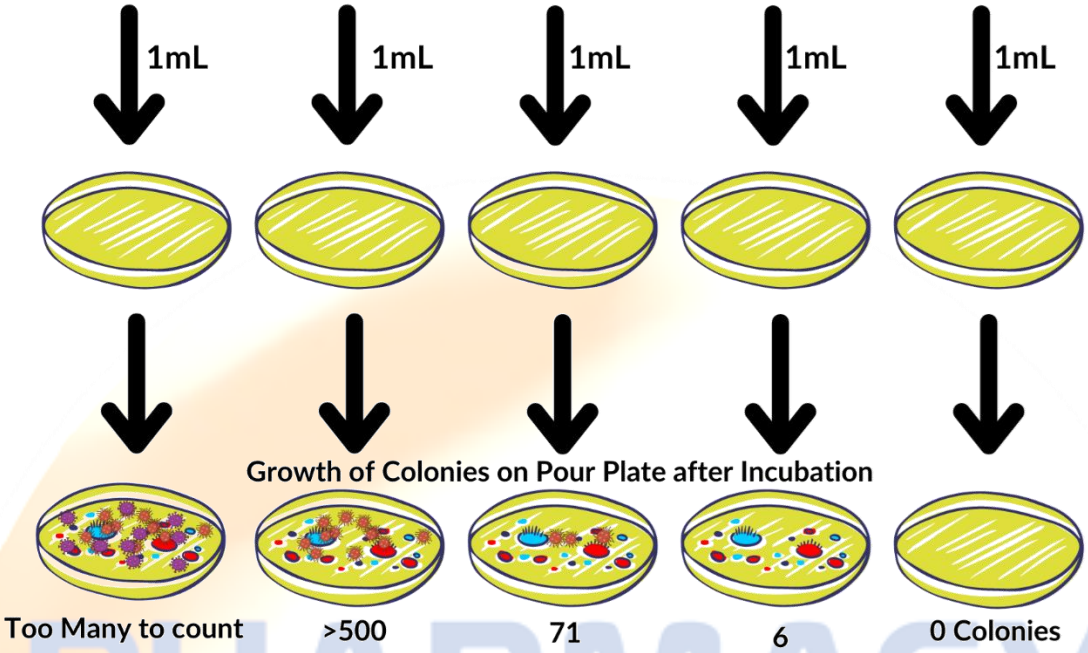
It is simple, less resource-consuming, easy, and economical; however, it requires the sample to be in liquid or suspension.

Principle –

- The pour Plate Method is based on the fact that when an agar medium mixed with microorganisms is **incubated, each of the viable microorganisms will multiply forming a separate colony.**
- In this **method, a certain volume, usually 1 mL, of the serially diluted liquid sample is mixed properly with approximately 15 mL of specific** molten agar medium of about **40 – 45°C (less than 50°C) in a Petri plate**.
- The medium is allowed to solidify and is incubated, usually at 37°C for 24 – 48 hours.
- Following the incubation, the viable microorganisms in the sample will grow into visible **colonies on the surface of and within the medium.**
- **The visible colonies can be counted** and CFU/mL can be calculated using the following formula;

$$CFU/mL = \frac{Total\ number\ of\ colonies\ obtained \times dilution\ factor}{volume\ of\ specimen\ used\ (aliquot)}$$





Procedure

1. Arrange all the requirements, **put on the PPE, sterilize the work surface, and set up the laboratory types of equipment.**
2. **Sample preparation:**
   - If the sample is in liquid form, serially dilute it to make the **microbial load to the range of 20 – 300 CFU/mL.** *(Prior pilot test may give exact value. You can prepare serial dilution up* ***to 10^{-10} and use different dilutions.*****)**
   - If the sample is in solid or semisolid form, dissolve it in sterile distilled water or sterile broth, or any other solvent. ***(Generally, 1 gm sample*** *is mixed with 9 ml of solvent to get the concentration of 10^{-1} gm/mL.)*

3. Media preparation:

- Suitable media (general-purpose media like Nutrient Agar and Plate Count Agar for bacteria, and Potato Dextrose Agar or Sabouraud Dextrose Agar for fungi) are prepared and autoclaved. The media is allowed to cool to about 40 – 45°C (maximum up to 55°C), but don't let it solidify.
- If the **media is prepared already and solidified,** melt it by placing it over a water bath or other heat source.
- If you want to mix the sample in media prior to pouring it into the Petri plate, you can either add approximately 15 mL of media in one test tube or beaker and autoclave it. Alternatively, a fixed volume of media can be prepared in a large beaker or bottle and a sample can be added later by calculating the volume which will be equivalent to 1 mL sample per about 15 mL of media.

4. Arrange sterile Petri plates. Label at the edge of the bottom of the plate with the dilution factor, date, name, sample ID, and other required information.

5. Inoculation:

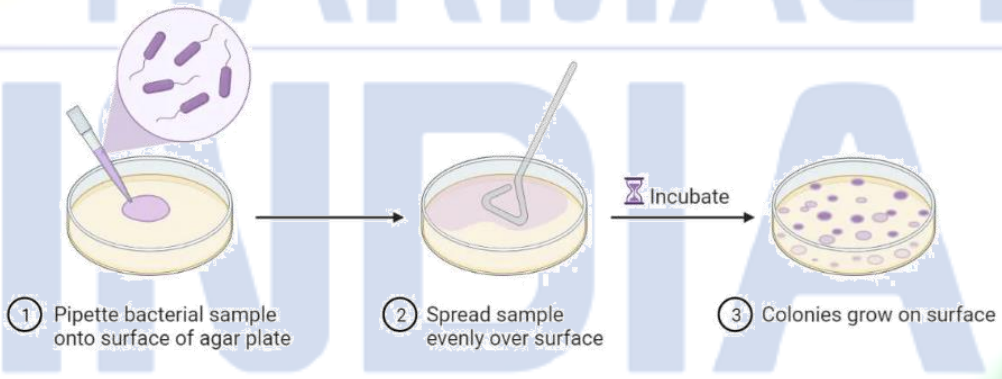
Dispense 1 ml of diluted sample in the center of the Petri plate using a sterile micropipette or calibrated pipette.

Open the lid of the bottle and flame its mouth. Pour about 15 mL of sterilized molten media at the appropriate temperature above the sample.

Close the lid of the plate then mix the sample in the media properly by gently swirling the plate. The plate is generally swirled in an "S" or "8" shape.

SPREAD PLATE METHOD

The spread plate method is a microbiological laboratory technique for isolating and counting the viable microorganisms present in a liquid sample by spreading a certain volume of the sample over an appropriate solidified culture media.



Procedure

i. Take three Nutrient Agar plates and label them with the name of the organisms to be inoculated.
ii. Aseptically inoculate the plates with a loopful of the given organisms.
iii. Place plate 1 on the turn table (revolving).
iv. Sterilize the spreader by putting it first in ethanol (95%) in a beaker, then on the flame of Bunsen burner and cool the rod for 30 seconds.
v. Remove the lid of plate and spin the turn table.
vi. Touch the spreader gently on the surface of agar and move it forth and back to spread bacterial cells on the agar surface when the turn table is spinning.
vii. Sterilize the spreader again and repeat the same process for the other two plates.
viii. Incubate all the plates at 37 °C for 24 hours.

ROLL TUBE METHOD

- This method is used for isolation of obligate anaerobes.
- A stoppered anaerobic culture tube coated with a pre-reduced agar medium containing oxygen free nitrogen is used for isolation.
- When the stopper is removed, the tube is kept anaerobic continuously flushing it with oxygen free carbon dioxide from a gas cannula.
- Inoculation is done with transfer loop held against the agar surface as the tube is being rotated by a motor.
- Inoculation starts from the bottom and draws the loop gradually upward.
- After inoculation, the tube is re-stoppered and incubated anaerobically to get isolated colonies.

MICROMANIPULATOR METHOD

Micromanipulator are devices that can pick up single microbial cell (from hanging drop preparation) from a colony of mixed culture and are used in conjunction with microscopes.

The single microbial cell is gently sucked into a micropipette and transferred a large drop of sterile medium on another cover slip.

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