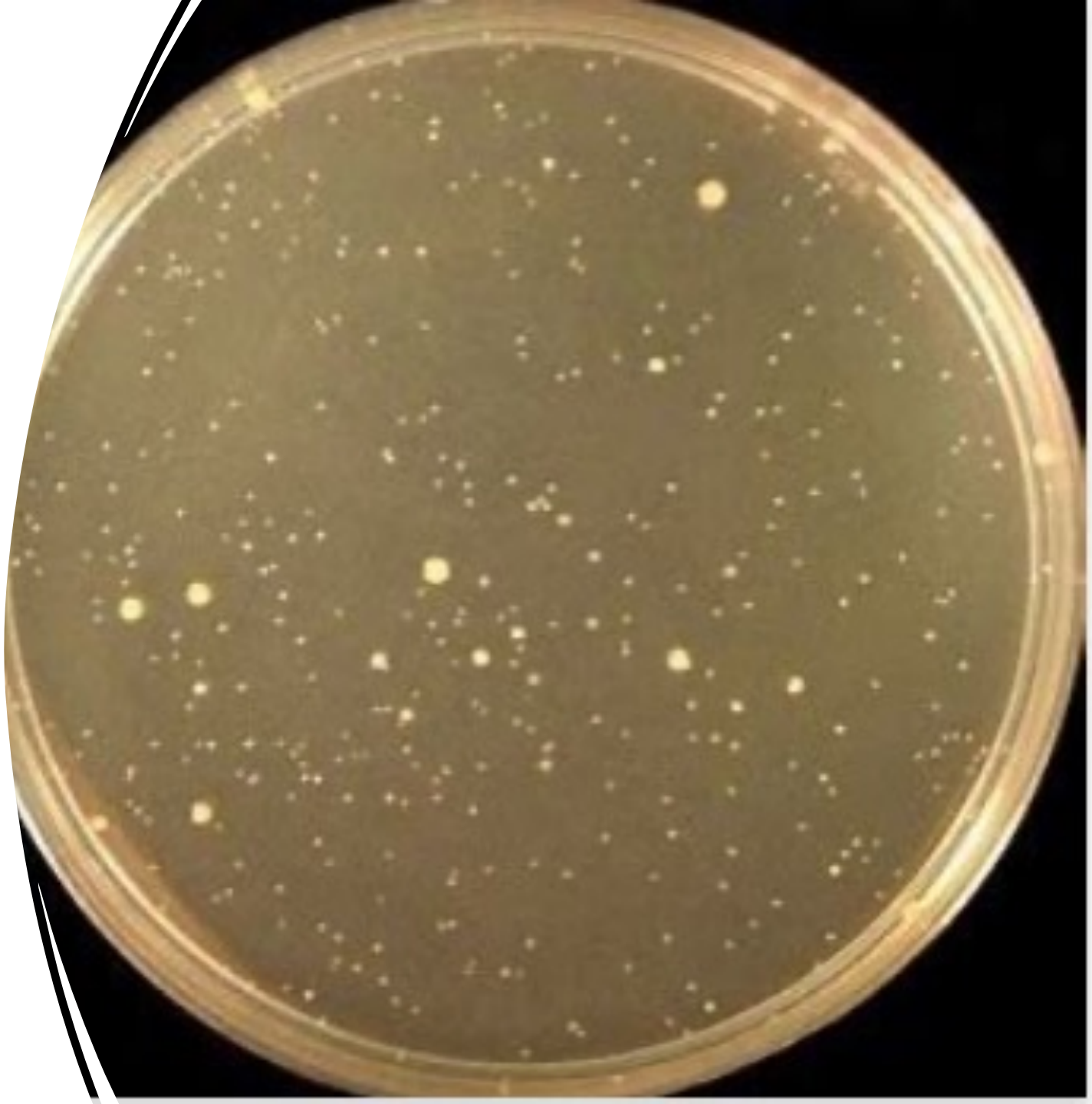
Heterotrophic CFU counts from environmental samples

- **Spread Plate Method: microbiological laboratory technique for isolating and counting the viable microorganisms in a sample by spreading a certain volume of the sample over a solidified culture media**

- One of the widely used culture techniques in microbiology laboratories due to its ease and simplicity.

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



Objectives of Spread Plate Technique

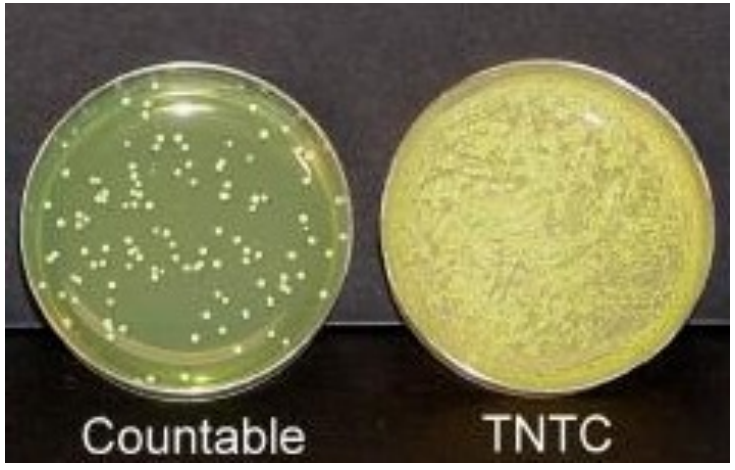
1. To isolate the microorganisms from a sample
2. To calculate viable microbial load by counting colony formation unit (CFU) per mL of sample (in our case environmental samples)
3. To isolate microorganisms in discrete colonies to study their colony characters and diversity
4. To isolate the pure culture of microorganisms from a mixed population
    - To obtain sufficient growth for characterisation of the microorganisms: e.g. antimicrobial sensitivity and production testing, genomic characterisation, and biochemical studies

Principle of Spread Plate Method

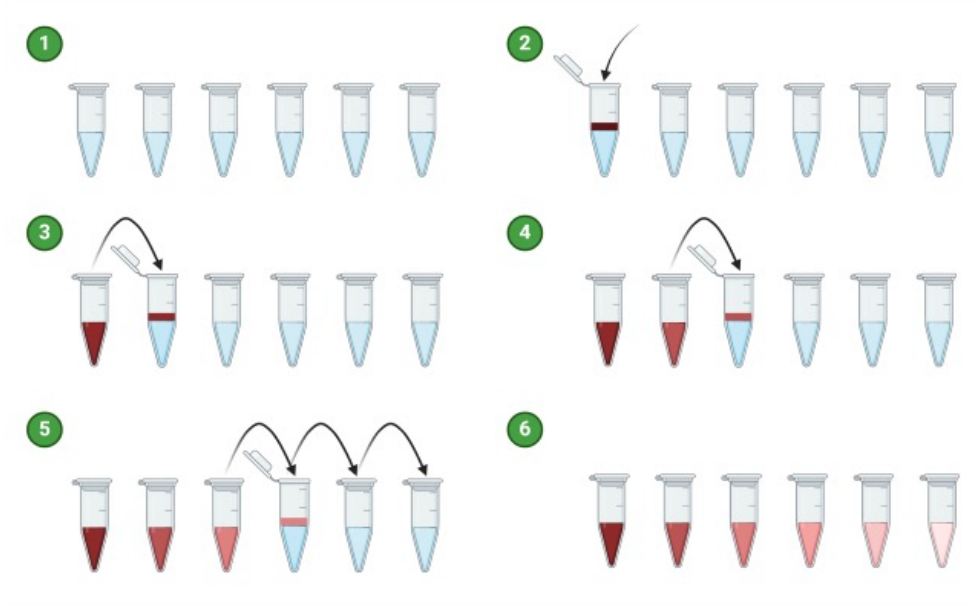
- When a diluted specimen containing microorganisms is spread over a solid agar media, each of the viable microorganisms will multiply forming a separate colony.
- These colonies can be counted and expressed in terms of the CFU/mL which can be used to calculate the microbial load in the sample.
- The diluted sample is dispensed and spread over the surface of a solid medium.
- Following the incubation, the viable microorganisms in the sample will grow into discrete visible colonies on the surface of the medium.
- The visible colonies can be counted and CFU/mL can be calculated using the following formula;

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

- The sample in the spread plate method must be liquid (stream water) or in suspension (soil in a buffer).
- The objective is to count the CFU/mL (colony forming units). Each CFU corresponds to a cell in the original samples.
- Therefore, before plating, the samples are serially diluted, otherwise there will be too much growth on the plate.
  - Sample is diluted to make the microbial load in the sample between 20 – 300 CFU/mL.
  - If we get too many colonies (>300) it becomes hard to count accurately and there could be suppression of growth of some cells
    - TNTC: too numerous to count
  - If we get too few colonies (<20) we are subject to bias as the plate may not statistically representant the sample
    - TFTC: too few to count
  - Since we do not know the concentration of cells in our samples, we plate different dilutions of the samples
- 0.1 mL of the sample (100 ul) is pipetted in the middle of a sterile solidified agar medium and evenly spread over the surface of the medium using a bent glass rod.
- Depending on the temperature of incubation, colonies are counted 1 day- 1 month later
- Based on the dilution factor and the number of colonies on the plate we can calculate and estimate the concentration of microbial cells in the environment.



Serial dilutions



Spread Plate Method