FE 204 Experiment 3

Pure Culture Techniques

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Pure Culture Techniques

Definitions:

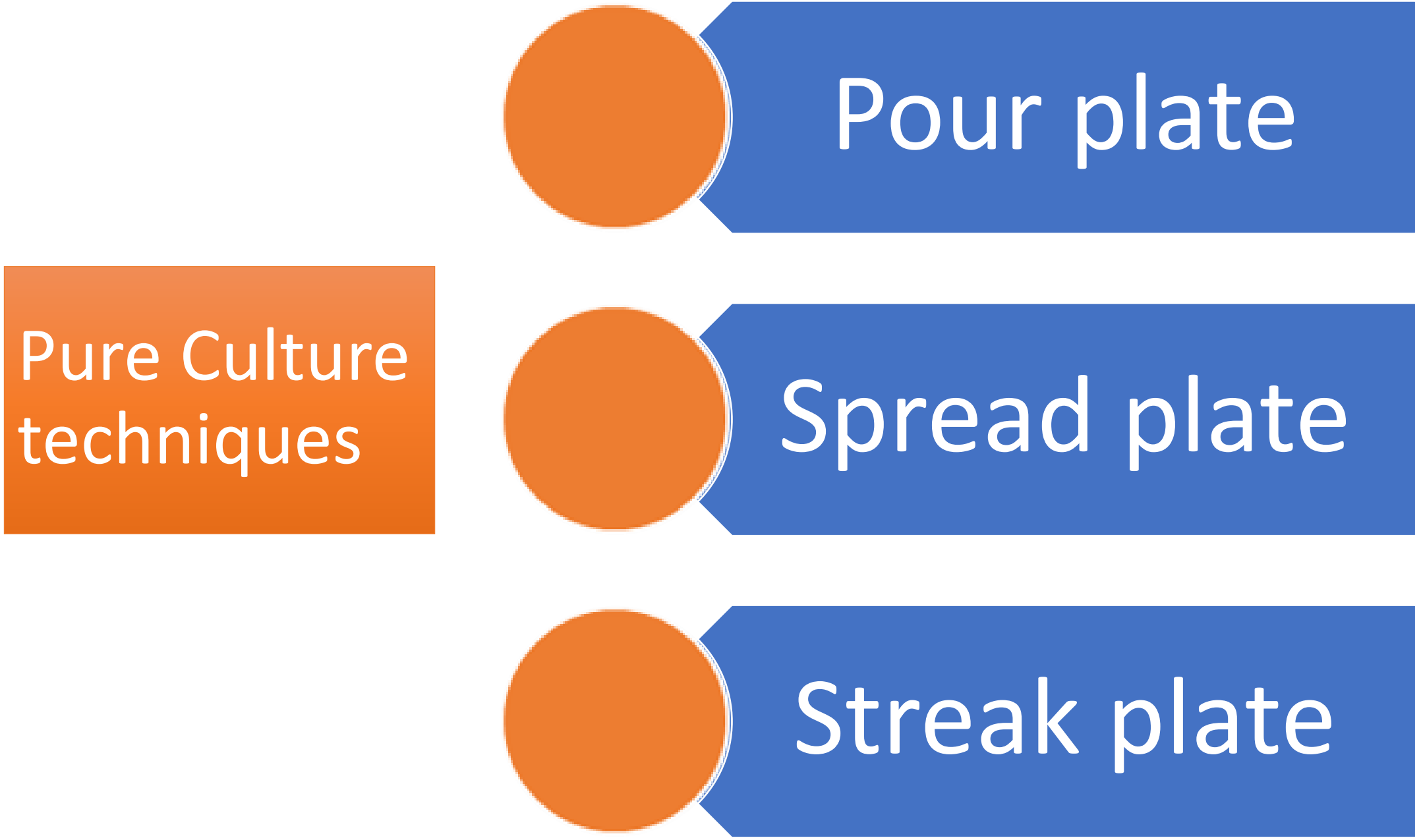
Culture media: Microorganisms on nutrient materials are called as culture media.

Inoculum: The substance used for inoculation.

Pure Culture: is a culture consisting of only one type of microorganisms.

Mixed Culture: Many different cultural types are associated together in one medium, so this is called as mixed culture.

Colony: Microbial accumulation which can be seen by naked eye on an agar surface.


Pure Culture techniques
Pour plate
Spread plate
Streak plate

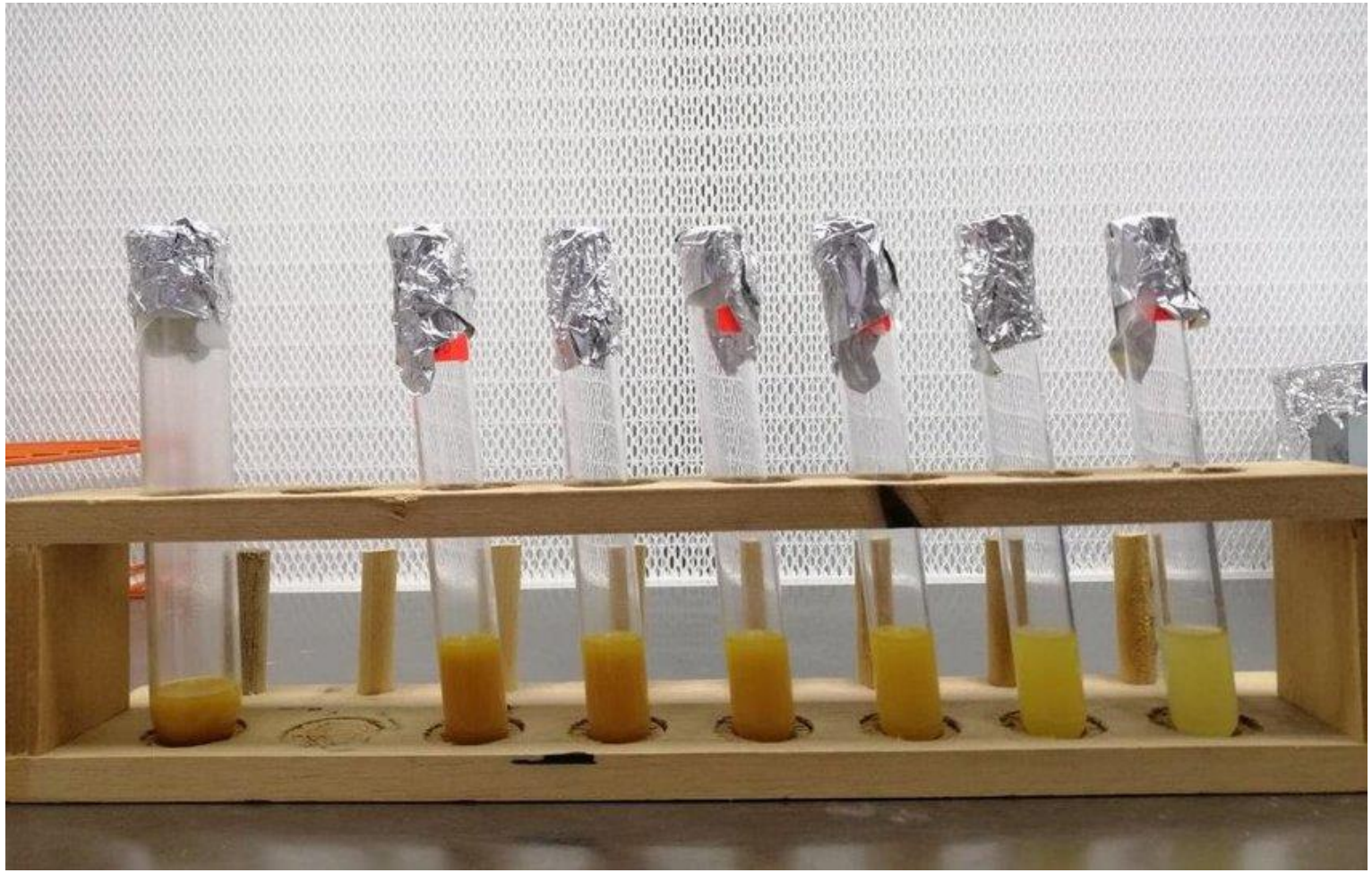
Pure Culture Techniques

Pure Culture Technique	Aim and purpose	Type of microorganisms grown
a) Pour Plate	To count formed colonies and calculate # of microorganisms / ml (g) of sample	Mostly for anaerobic microorganisms. Inoculum is placed at the bottom of media
b) Spread Plate	To count formed colonies and calculate # of microorganisms / ml (g) of sample	Mostly for aerobic microorganisms groth is on the surface media.
c) Streak Plate	To isolate colonies of microroganisms on a petri plate	Mostly aerobic microorganisms form colonies on the surface of agar.

Materials used in LAB

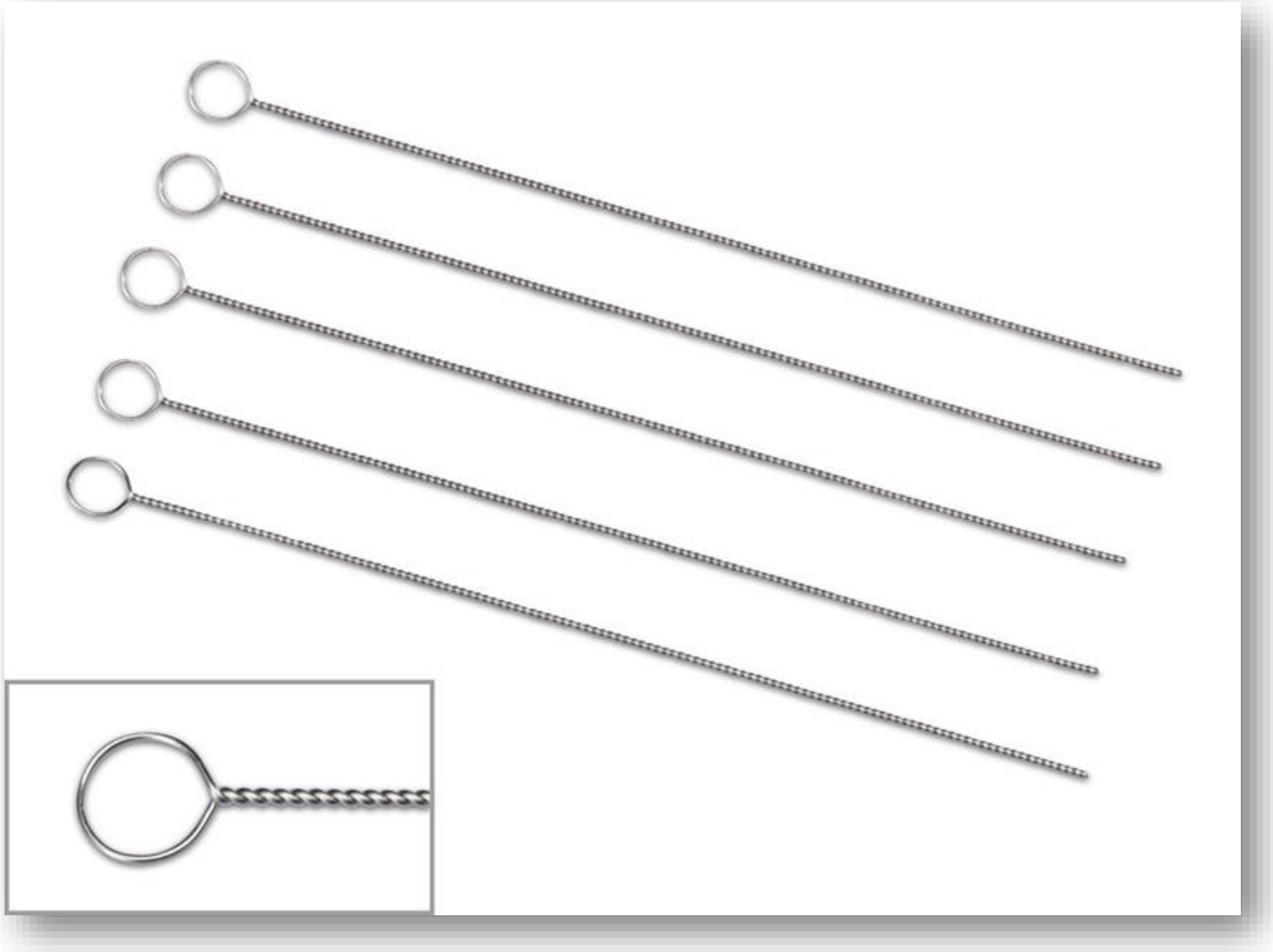
- Plate Count Agar petri plates
- Sterile dilution water (9 ml)
  test tubes
- 1 ml pipettes
- Inoculating loop
- Bunsen burner
- *E. coli* broth culture

Sterile Dilution Water (9 ml) tubes



Laboratory pipettes

Inoculating Loop

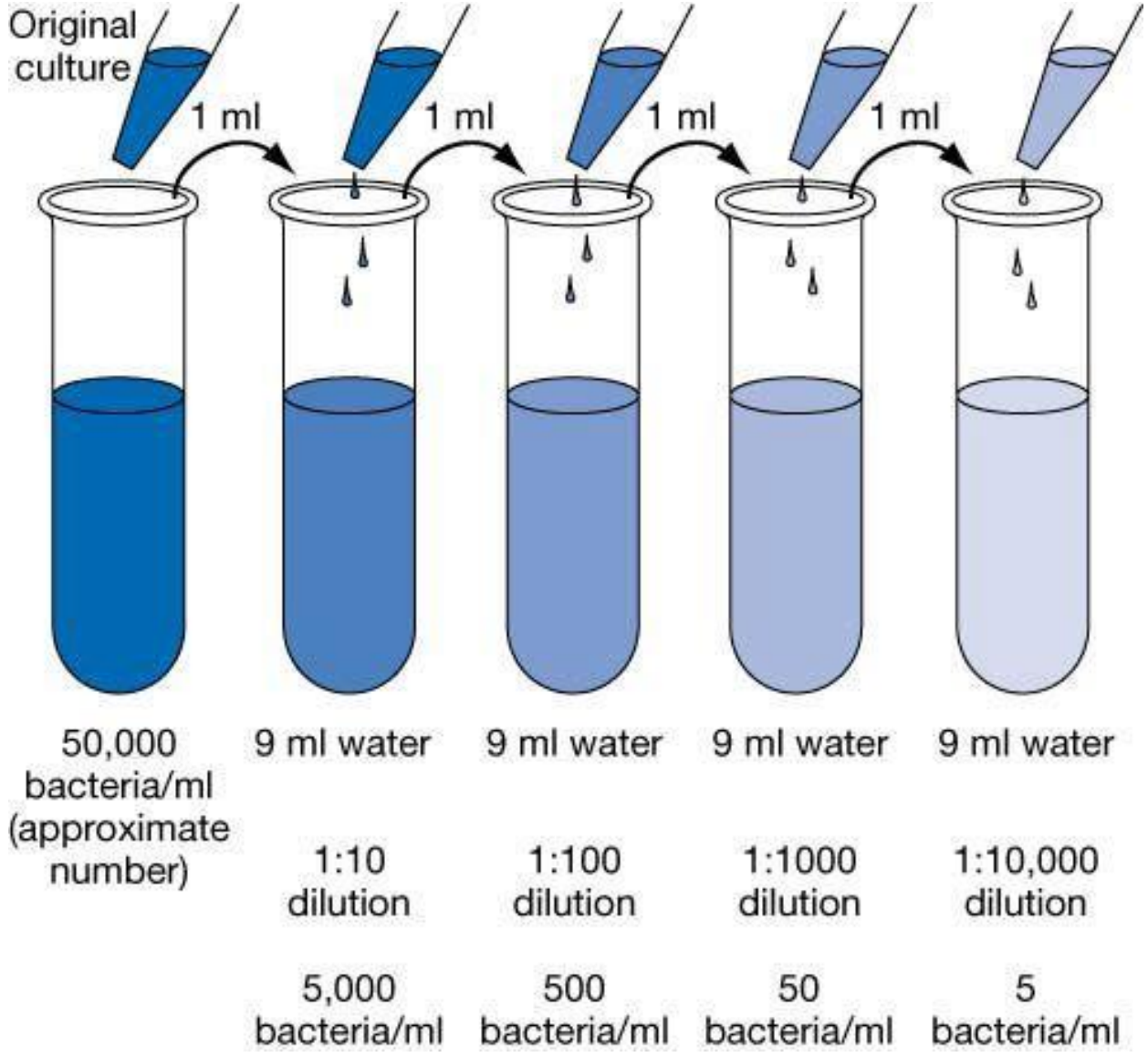


Procedure Steps

- Preparation of Serial Dilution
- Inoculation in Plate Count Agars
- Pour plate technique
- Spread plate technique
- Streak plate technique

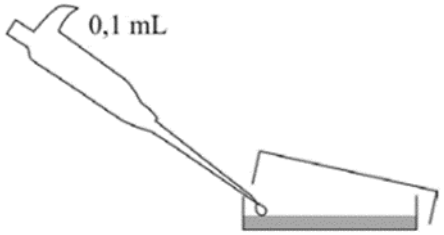
Preparation of Serial Dilution

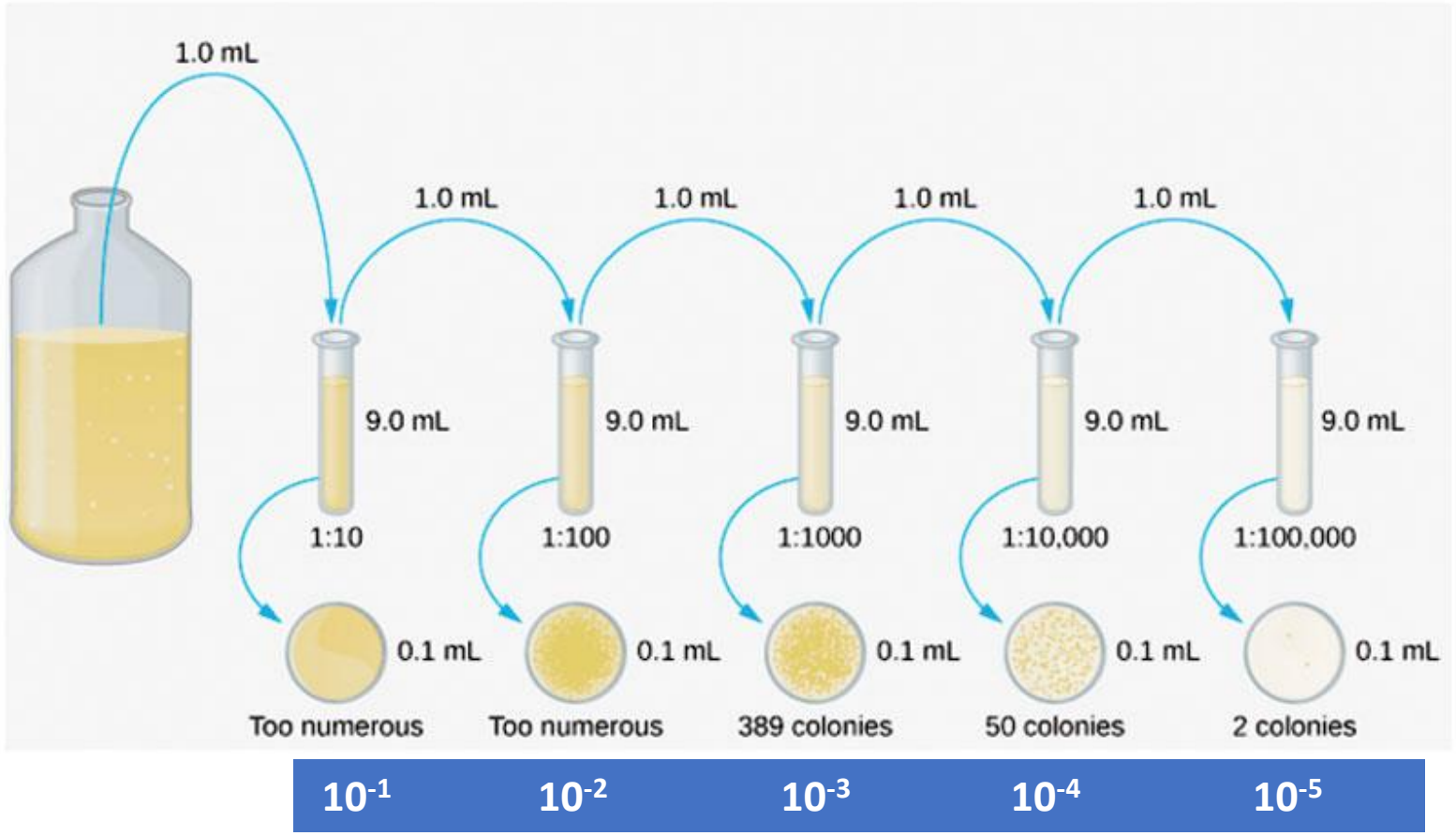
- In order to make a successful counting method we need serial dilution of samples.
- 9 ml of Sterile 0.1 % peptone water tubes are used for dilution.
- 1 ml sample was taken from non diluted sample and added into 9 ml of dilution water in order to obtain 10^{-1} dilution.



Procedure of Spread Plate Technique

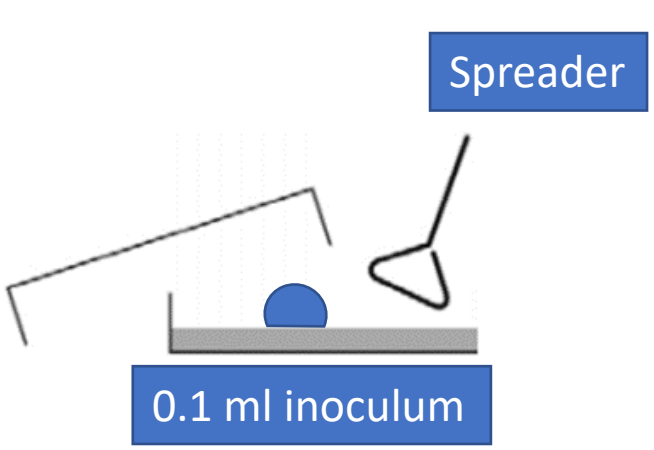
- Take 0.1 ml of sample from each dilution by using sterile pipette at Aseptic Conditions.
- Place the sample on PCA petri plate near flame.

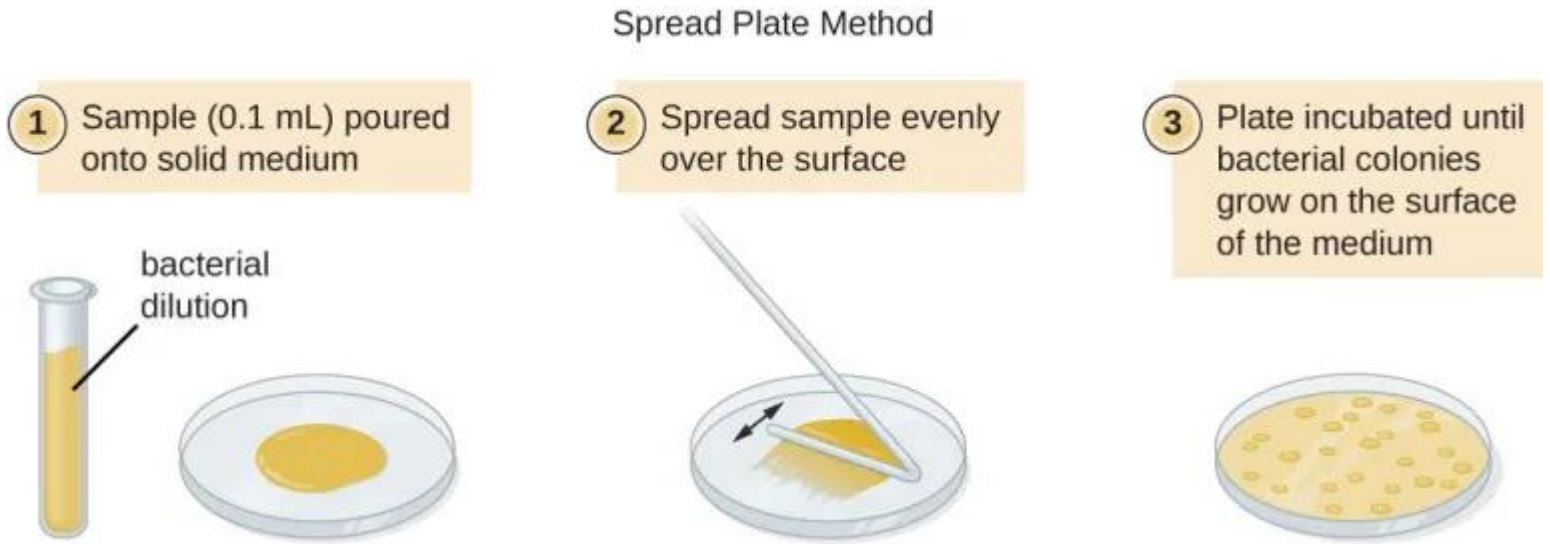




Procedure of Spread Plate Technique

- Sterilize spreader with dipping in alcohol and passing through flame.
- Spread the sample on petri plate by using spreader.

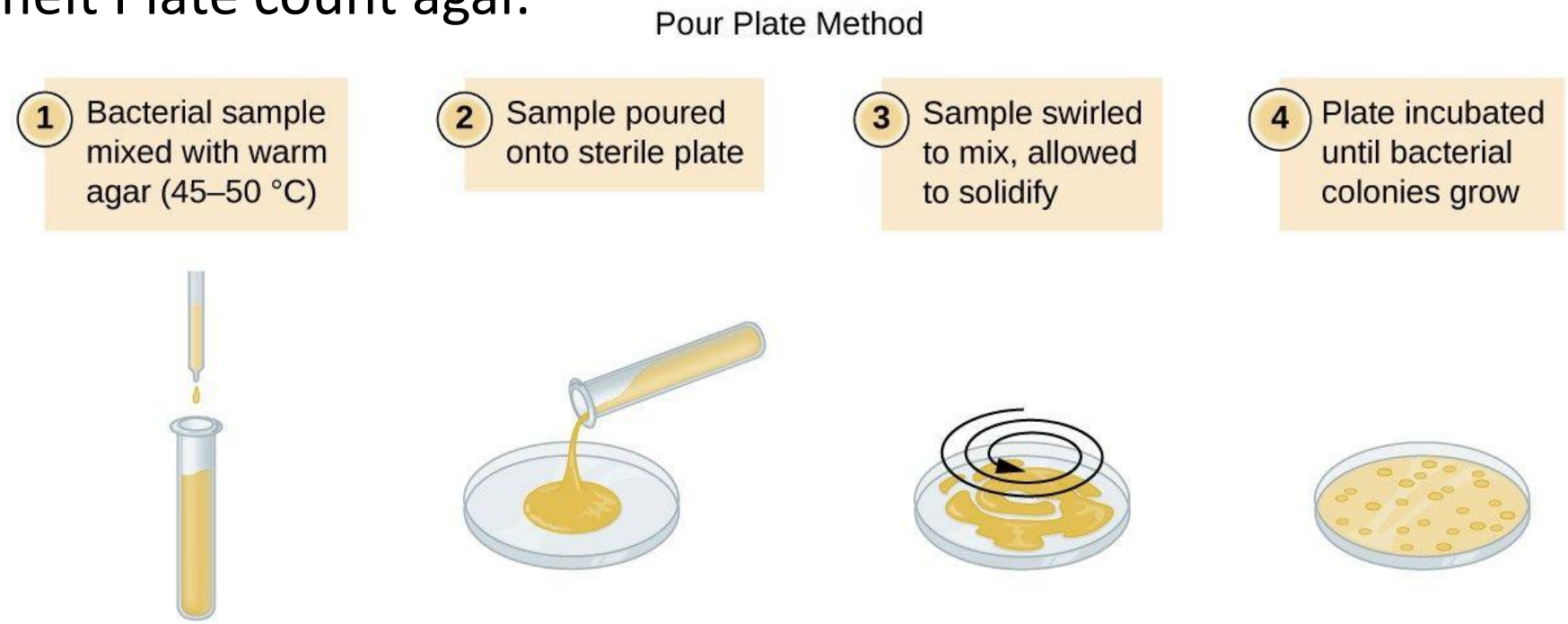




- Incubate inoculated petri dishes at 37°C Oven for 24-48h. Count formed colonies and calculate # of microorganisms / ml(g) of sample.

Procedure of Pour Plate technique

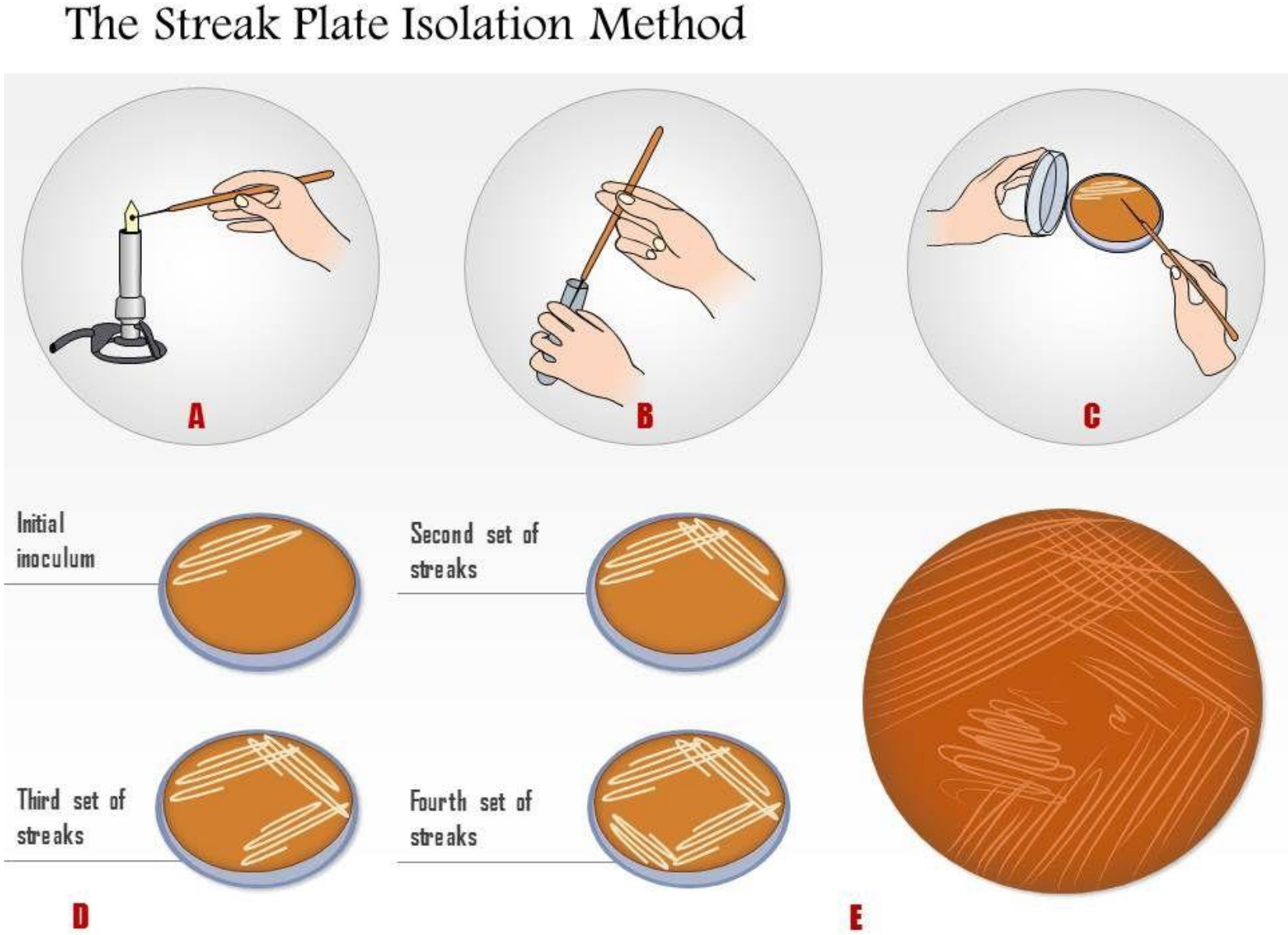
- From each dilution tube take 1 ml or 0.1 ml sample by using sterile pipette at aseptic conditions.
- Place the inoculum in a sterile empty petri plate or place the inoculum inside warm melt Plate count agar.



- Incubate inoculated petri dishes at 37°C Oven for 24-48h. Count formed colonies and calculate # of microorganisms / ml(g) of sample.

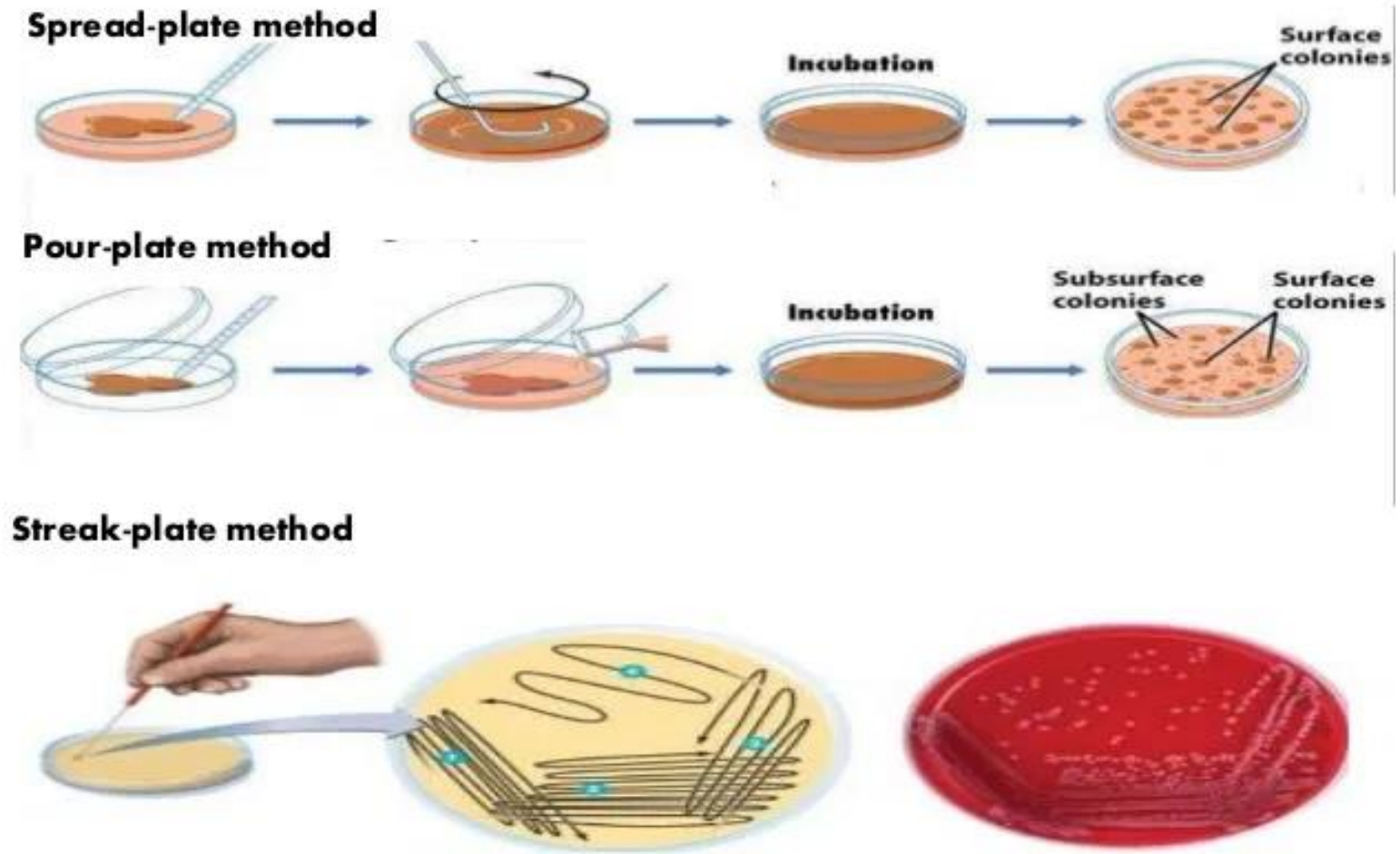
Procedure of Streak Plate technique

- Sterilize the inoculating loops by holding on flame of bunsen burner.
- Take 1-2 loops of culture sample from Positive tubes
- Inoculate sample by using streak plate method and incubate.



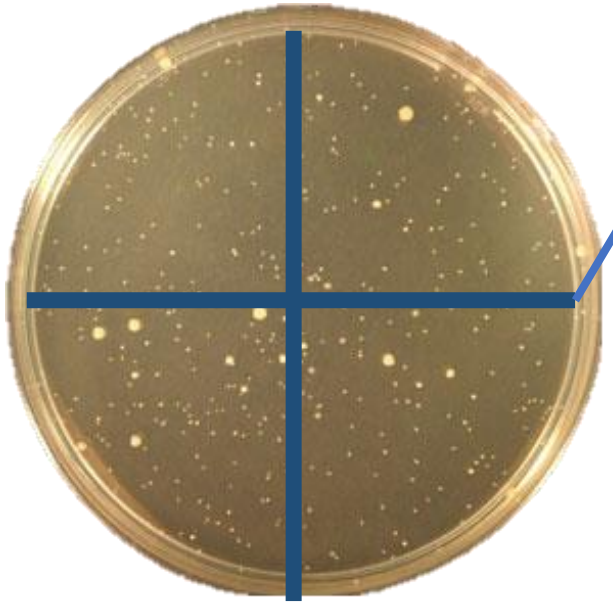
Summary of Pure Culture Techniques

Method of isolating pure cultures



Results of Spread and Pour Plate

- Count formed colonies on every petri plate for each dilution and record results on the given table below.



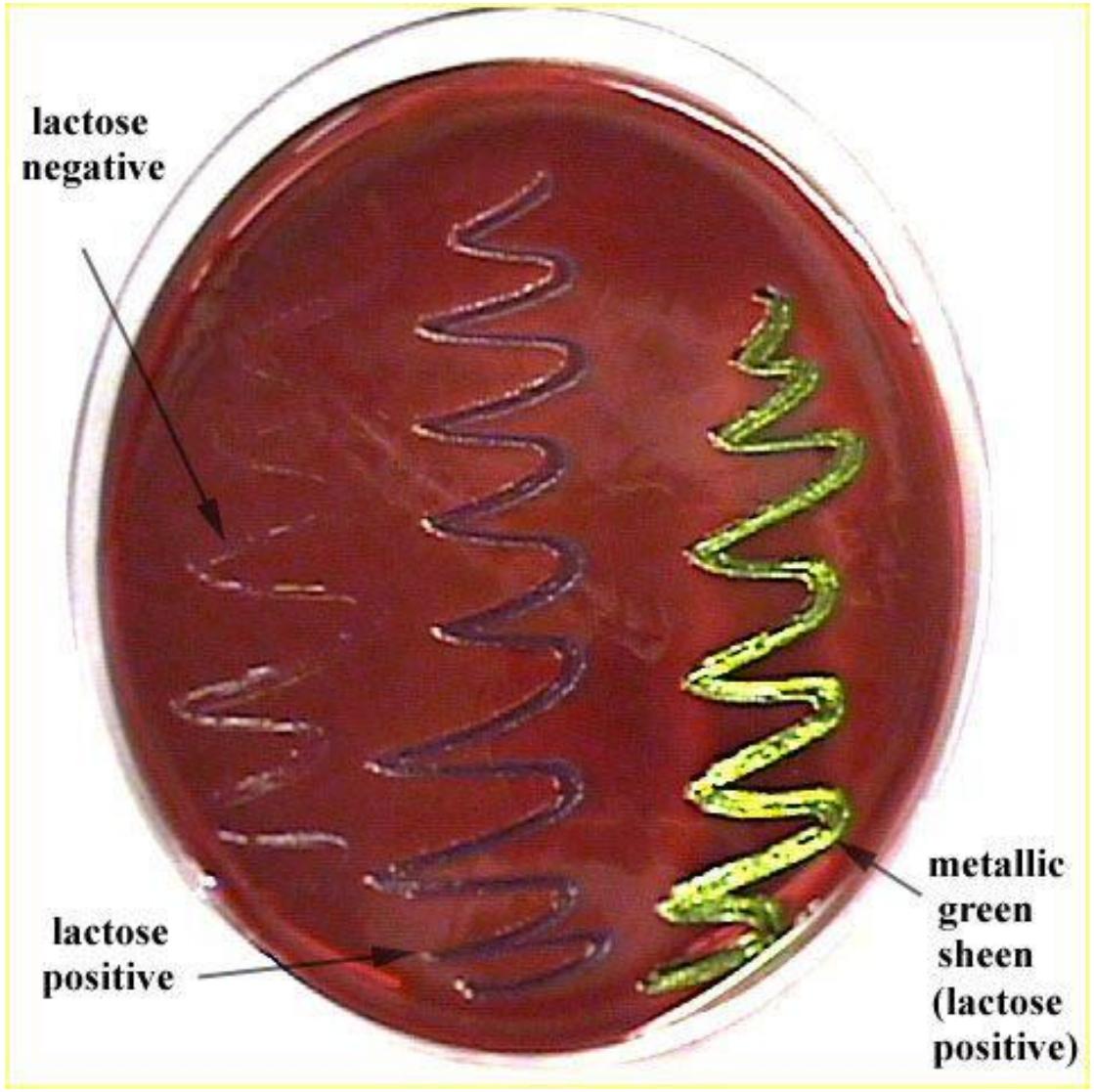
- You can divide petri into equal parts to count easily.
- Ex: E. coli broth
  - Non → Too number to count → TNTC
  - -1 → 546
  - -2 → 186
  - -3 → 34
  - -4 → 0

Sample names	Dilutions					
	non	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
E. coli broth	TNTC	546	186	34	0	0

Calculation

- # of microorganisms / g or ml of sample= $\frac{Count\ of\ microorganisms\ in\ one\ petri}{inoculum\ amount\ in\ one\ petri}\ X\ Dilution\ factor$
- Take only counts between 30<x<300 colonies into calculation.
  - Non: TNTC
  - -1: 546 → No calculation above 300
  - -2: 186 → $\frac{186}{0.1\ ml} \times \frac{1}{10^{-2}}$
  - -3: 34 → $\frac{34}{0.1\ ml} \times \frac{1}{10^{-3}}$
  - -4: 0 → No calculation below 30
- Take average of these two results
- # of microorganisms / g or ml of sample= $\frac{186000+340000}{2} = 263000$ microorganism / ml of sample

Result of Streak Plate



Like *Enterobacter*

Like *E. coli*

Results Evaluation

Pure Culture Technique	Evaluation	Type of microorganisms grown
a) Pour Plate	Calculate # of microorganisms / ml (g) of sample	Record results as # of microorganism / ml of sample
b) Spread Plate	Calculate # of microorganisms / ml (g) of sample	Record results as # of microorganism / ml of sample
c) Streak Plate	Isolate colonies of microroganisms on a petri plate	Draw results or take photography to record formed colonies on surface.