FE 305 Experiment 2

Bacteriological Examination of Water

Contents

- Total Aerobic Mesophilic Bacteria Count
- Coliform Count
  - Presumptive Test
  - Confirmed Test
  - Completed Test

Experiment No from Book: **4-15-46**

Samples needed for this experiment

- **River Water directly obtained**
- **Pool water**
- **Tap water**
- **Bottled Drinking water**

Water types

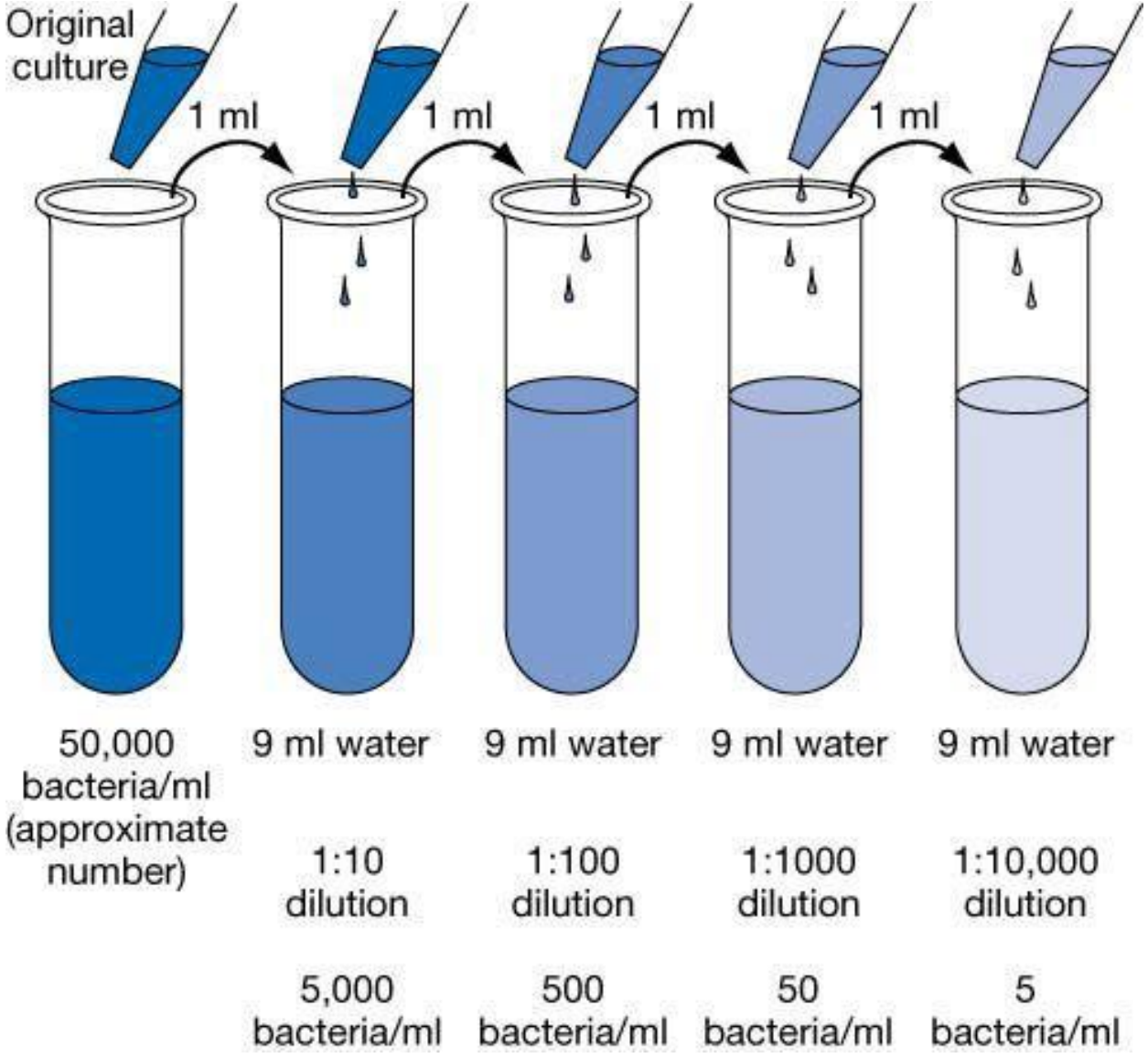
- Spring or well water
- Specifically prepared drinking water
- Purified water
- Fluoridated water
- Mineral water

Importance of examination of water

- Main food material
- Can be consumed directly or added to food materials
- In order to keep product safe added water should be safe

Preperation of Serial Dilution

- In order to make a succesfull 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.

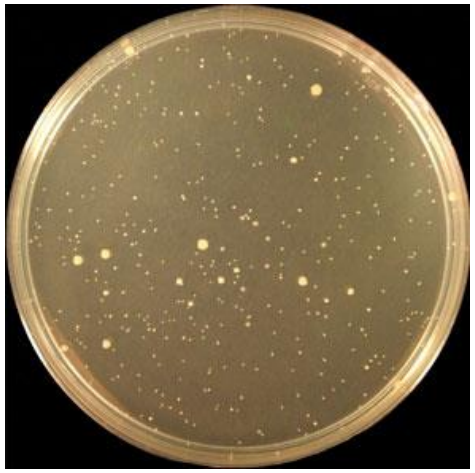


Total Aerobic Mesophilic Bacteria Count

- Media: Plate Count Agar

- Incubation conditions: **37 C for 24-48 hours**

- Spread Plate Technique
- Count formed colonies

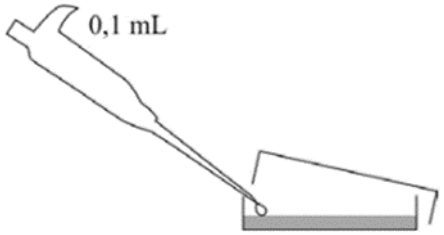


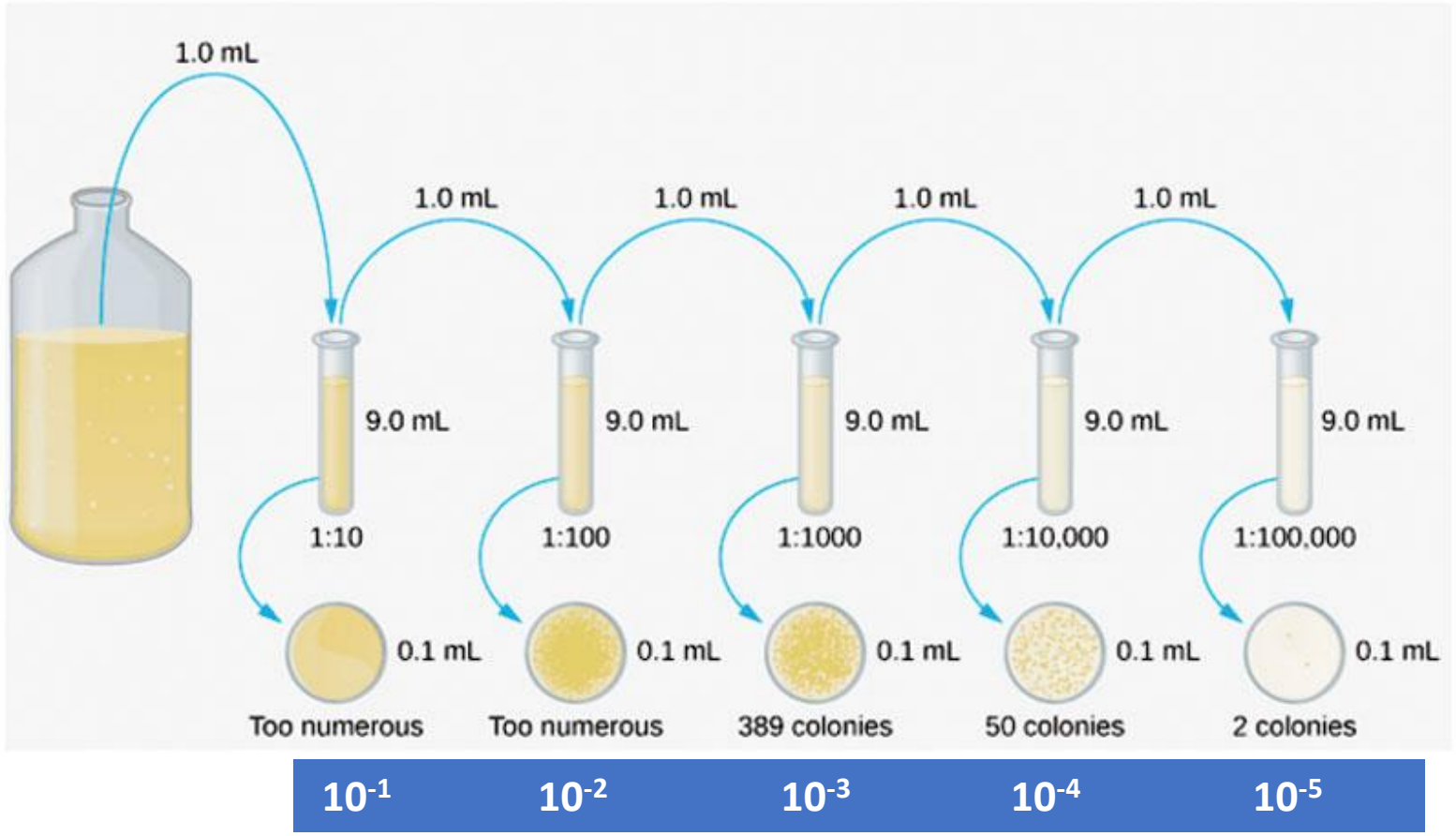
Material and Methods

- Sterile PCA petri plates
- Sterile Serial Dilution water tubes (9 ml)
- Spreader
- Alcohol
- Pipette (5 and 1 ml)
- Bunsen burner
- Incubator 37 C

Procedure of Total Count

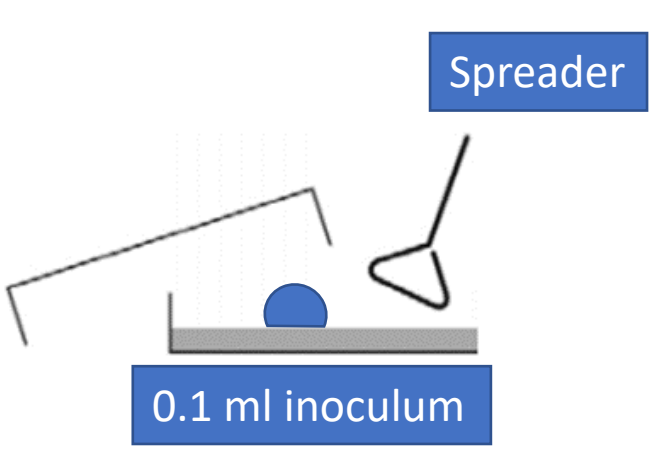
- Take 0.1 ml of sample from each dilution by using sterile pipette at Aseptic Conditions.
- Place the sample on PCA petri plate near flame.

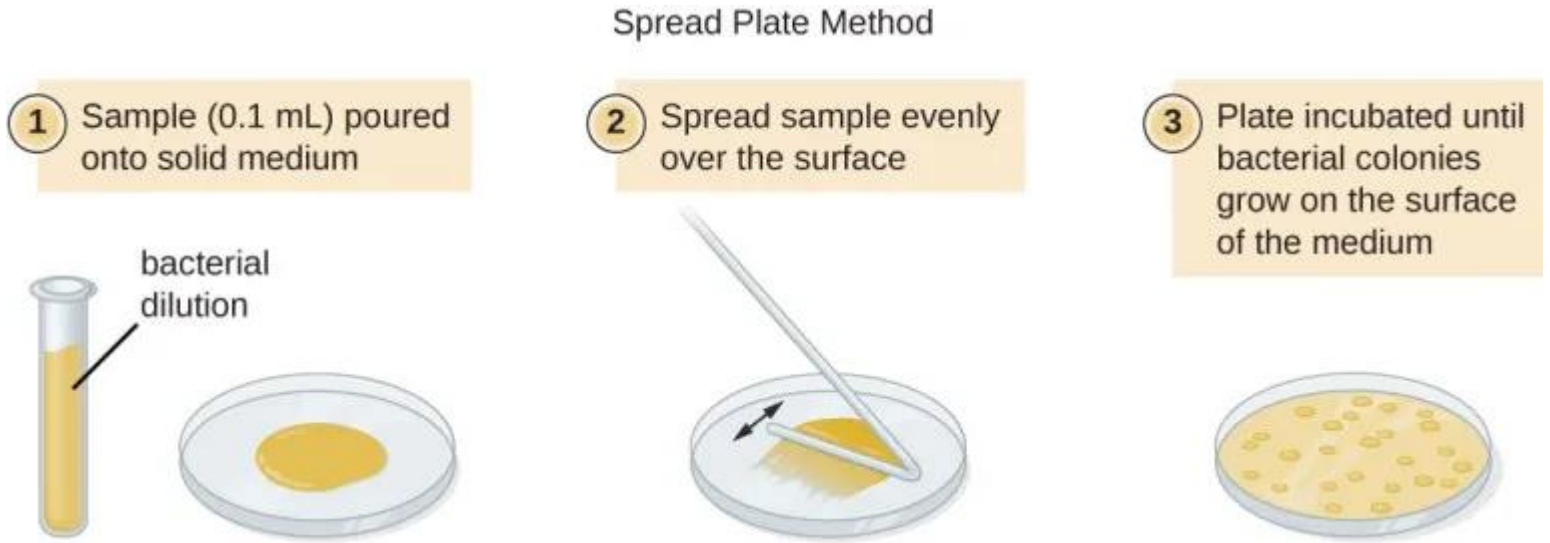




Procedure of Total Count Spread Plate

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

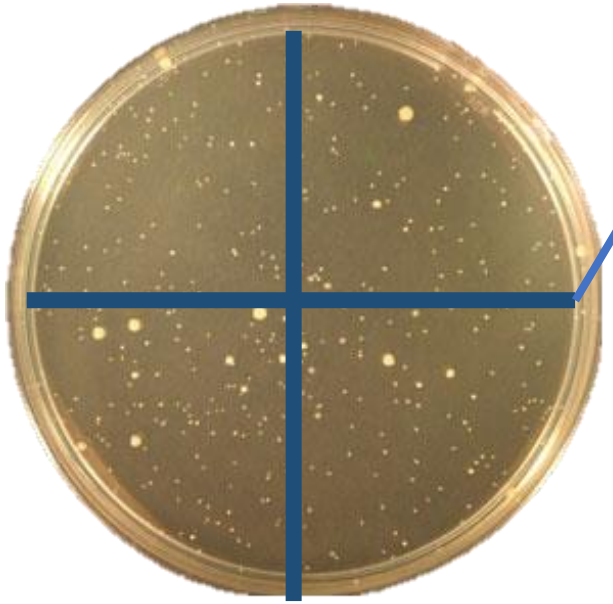




- Incubate inoculated petri dishes at 37°C Oven for 24-48h. Count formed colonies

Results of Total Count

- 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: For river water
  - Non → Too nuber 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}
River water	TNTC	546	186	34	0	0
Pool water						
Tap water						
Drinking water						

Calculation

- # of microorganisms / g or ml of water= $\frac{Count\ of\ microorganisms\ in\ one\ petri}{inoculum\ amount\ in\ one\ petri}\ X\ Dilution\ factor$
- Ex: For river water 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 water= $\frac{186000+340000}{2} = 263000$ microorganism / ml of water

Coliform Count

- Presumptive Test
  - Media: Brillant Green Bile Broth
  - Incubation: 37 C for 48 hours
  - Most Probable Number method 3 tubes
  - Decide Positive tubes and calculate # of coliforms / ml water
- Confirmed Test
  - Media: Eosine Methylene Blue Agar
  - From Positive Presumptive Test Tubes
  - Streak Plate Technique
  - Observe isolated colonies on petri
- Completed Test
  - Samples from colonies formed on petri plates
  - Observation under microscope 40x and 100x with Gram Staining

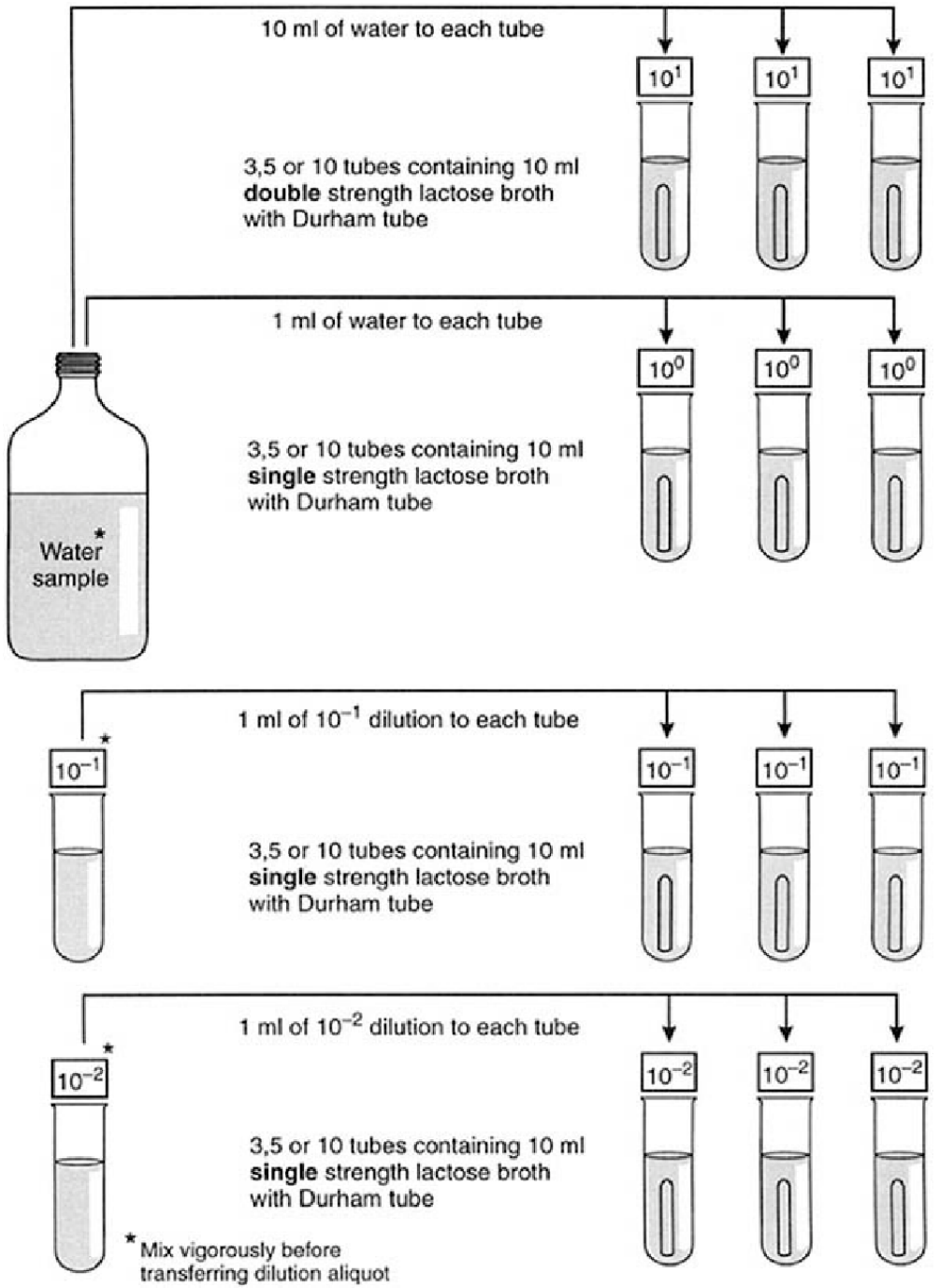
Presumptive Test
MPN method materials

- Water sample
- Serial Dilution Water Test tubes
- BGBB with durham tubes
- Sterile pipette (10 and 1 ml)
- Bunsen burner
- Tube rack

MPN method procedure

- Take 1ml of sample from each dilution by sterile pipette and inoculate 1ml in each of three seperate BGBB broth tubes at aseptic conditions.
- By this, you will have 3 BGBB tubes for each dilution sample inoculated.

- Place the tubes in a tube rack in order from non diluted to diluted.
- Incubate at 37°C for 48 h. Observe results and decide positive test tubes.



Results of MPN method

Gas formation in the durham tubes

Turbidity at the broth

- For each dilution count positive test tubes from 3 test tubes.
- Record results for each dilution.
- Calculate # of coliforms / ml of water from MPN table

Sample	non	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
River water	3	3	2	1	0	0

Positive tubes

2 1 0
from MPN Table

Calculation

Positive tubes	MPN/g ou ml	Confidence limits (95%) Low	High
0-0-0	<3.0	–	9.5
0-0-1	3.0	0.15	9.6
0-1-0	3.0	0.15	11
0-1-1	6.1	1.2	18
0-2-0	6.2	1.2	18
0-3-0	9.4	3.6	38
1-0-0	3.6	0.17	18
1-0-1	7.2	1.3	18
1-0-2	11	3.6	38
1-1-0	7.4	1.3	20
1-1-1	11	3.6	38
1-2-0	11	3.6	42
1-2-1	15	4.5	42
1-3-0	16	4.5	42
2-0-0	9.2	1.4	38
2-0-1	14	3.6	42
2-0-2	20	4.5	42
2-1-0 →	15 (MPN number)	3.7	42
2-1-1	20	4.5	42
2-1-2	27	8.7	94

$$\#\ \text{of coliforms / ml of water} =$$

$$\frac{MPN\ number\ from\ table}{100} \times Dilution\ Factor\ of\ middle\ tube$$

- For 2 1 0 → MPN number from table = 15

$$\frac{15}{100}\ X\ Dilution\ factor\ of\ middle\ tube$$

2 **1** 0

- Look at the records at dilution table and find for dilution of middle tube which is **10^{-3}** → Dilution Factor 10^3

$$\frac{15}{100}\ X\ 103$$

$$= 150$$

150 coliforms / ml of water

Confirmed test

- Made from positive presumptive test tubes
- To confirm culture growing in tubes as coliforms

Materials

- Sterile EMBA plates
- Positive presumptive test tubes
- Inoculating loops
- Bunsen burner

Method

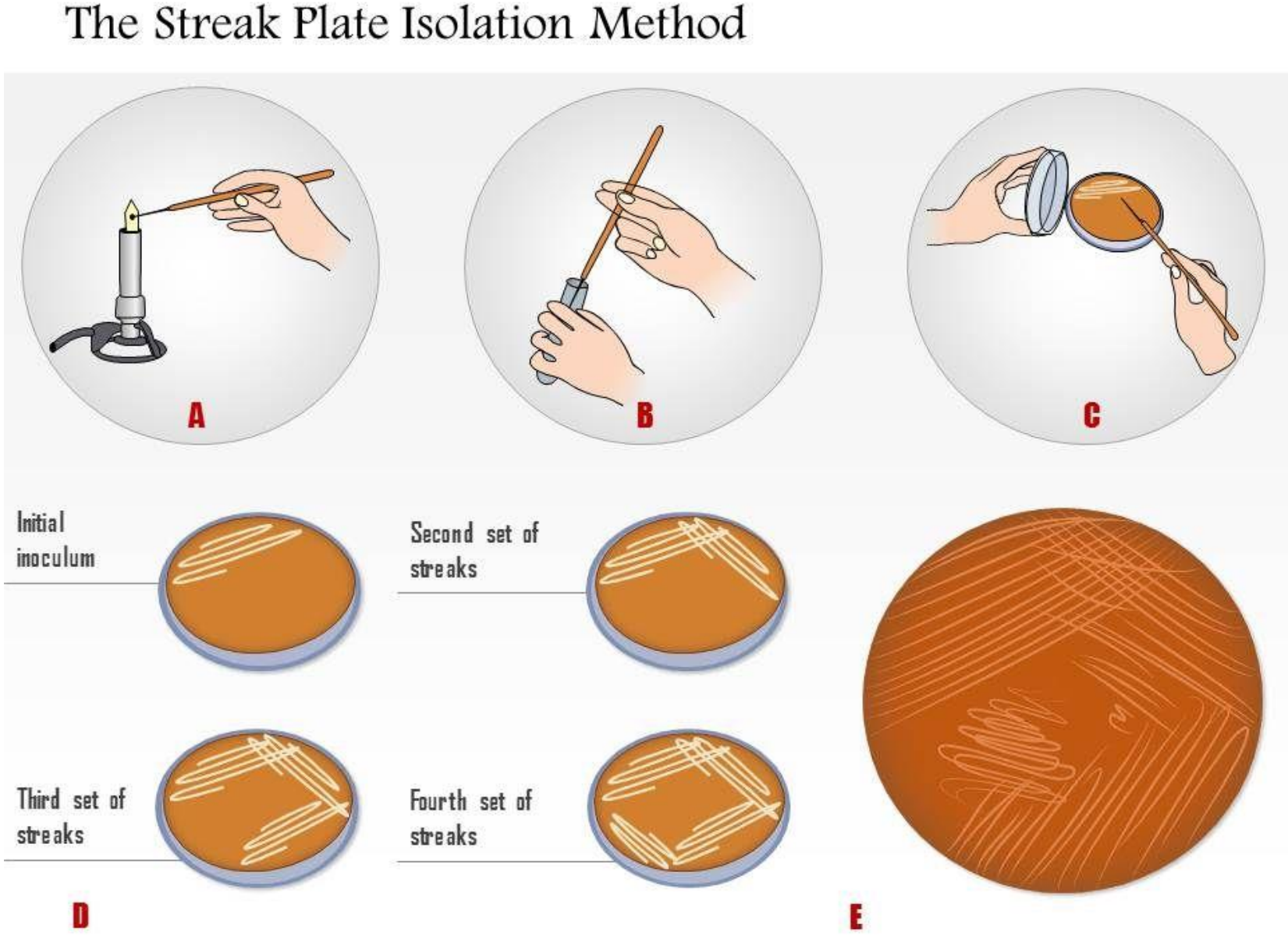
- Streak Plate Method

Incubation

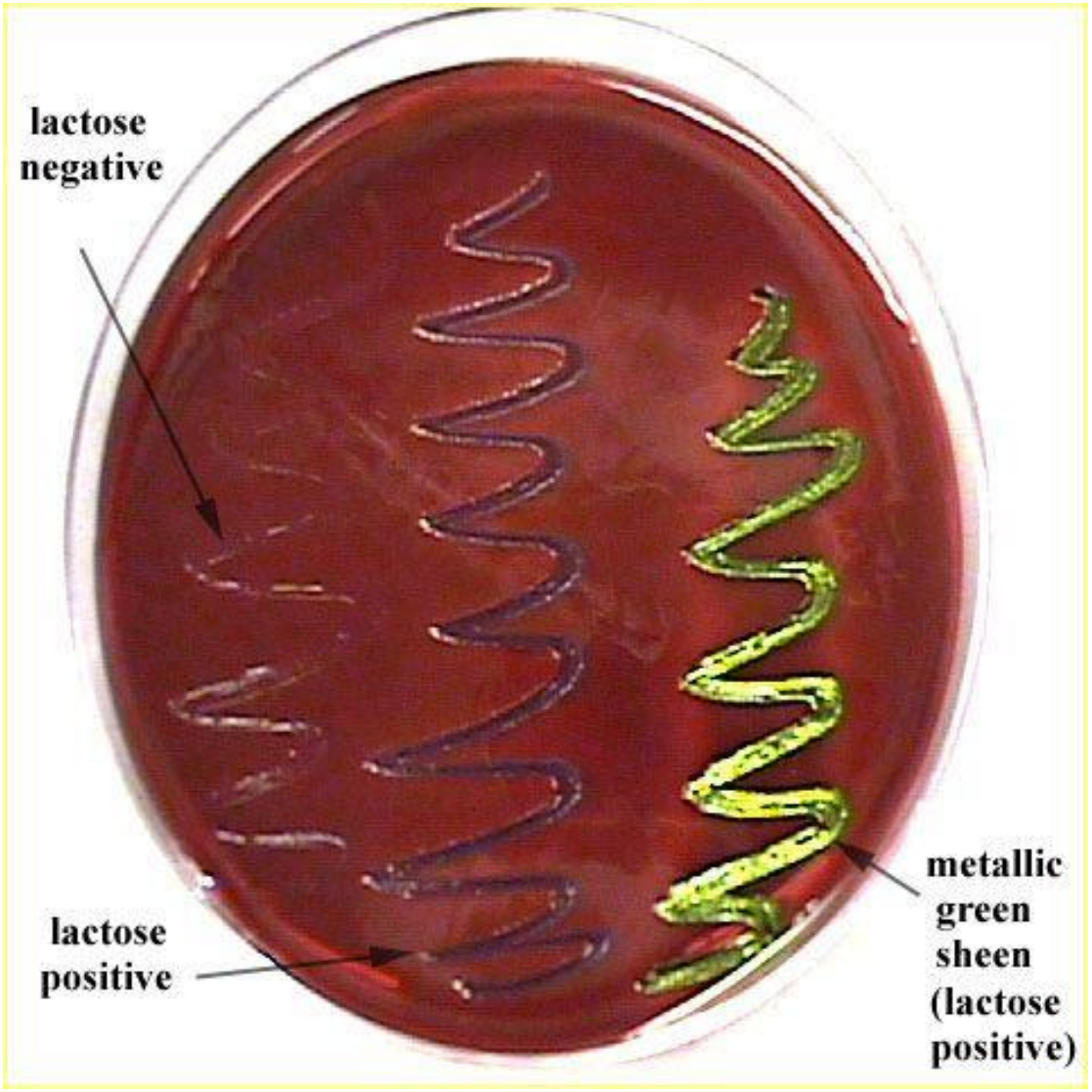
- Incubate inoculated petri plates at 37°C for 24 – 48 hours.

Procedure

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



Results



Like *Enterobacter*

Like *E. coli*

Completed test

- Morphological examination aand Gram staining of growing cultures on EMBA.
- Most of the lactose fermenters seems as Gram negative (-)

Materials

- Light Microscope with high power and oil immersion objectives
- Gram staining kit (Crystal Violet, Safranine, Iodine, Alcohol solutions)
- Pasteur pipette
- Glass slide
- Forceps
- Inoculating loop

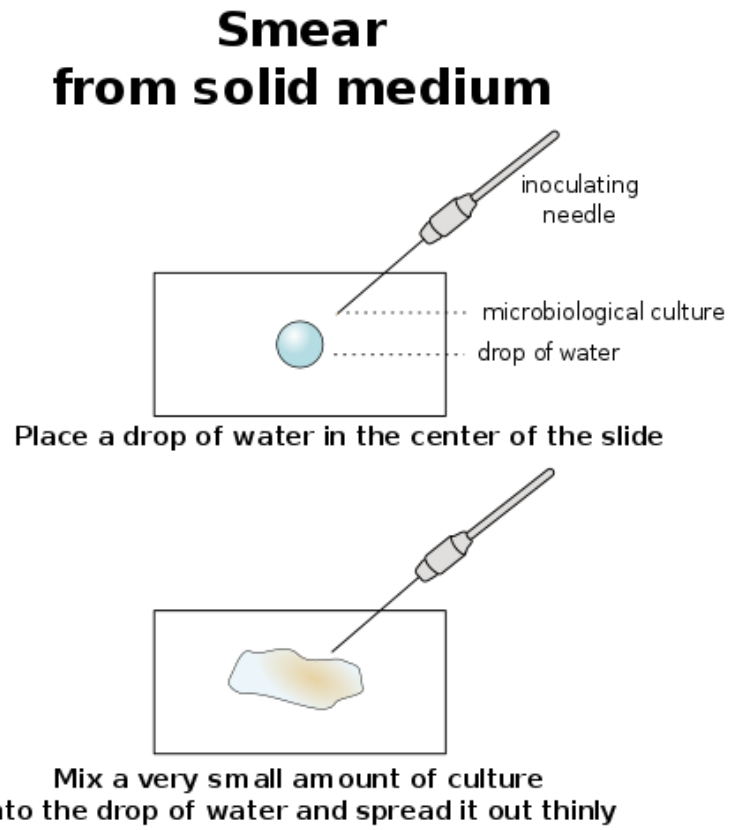
Method

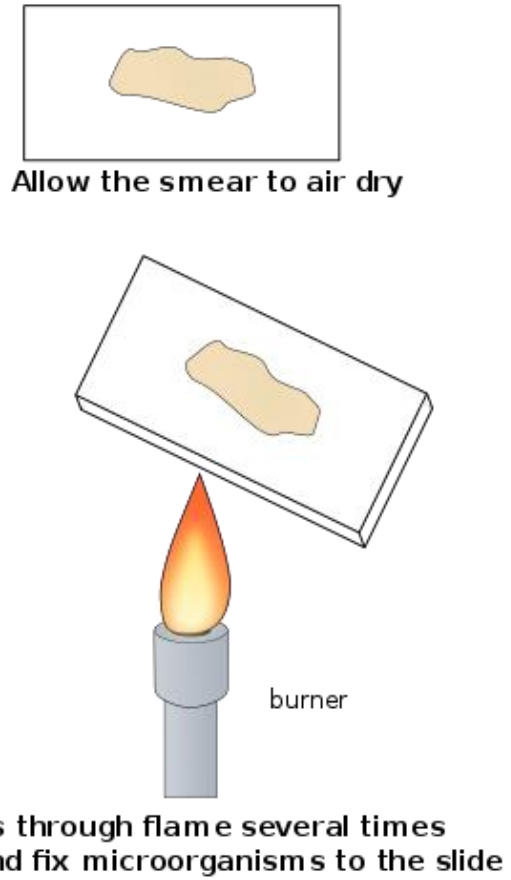
- Gram Staining

Procedure

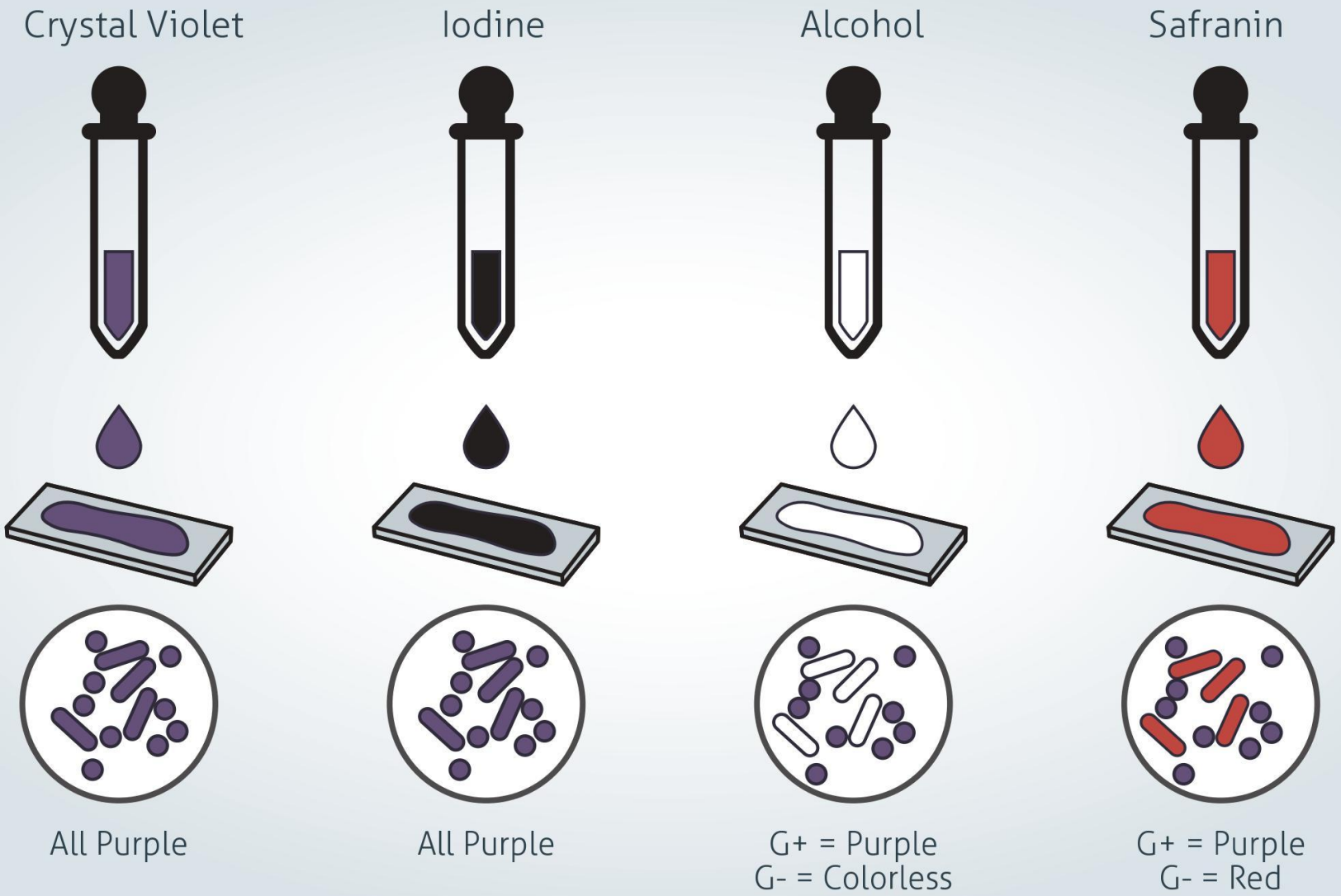
- Take samples from EMBA plate by using sterile loop and place on a sterile glass slide.

Smear Preparation



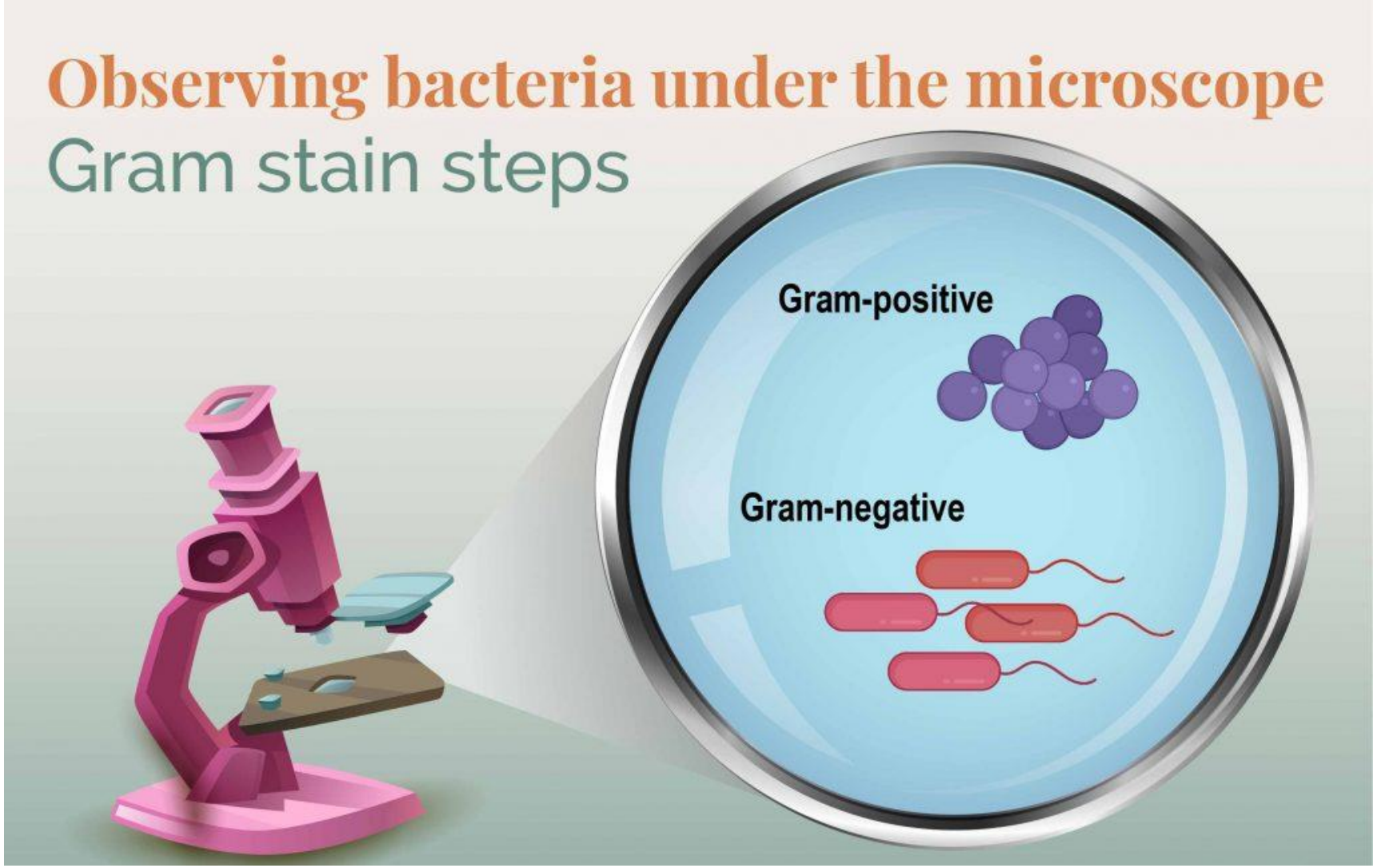


Staining Smear

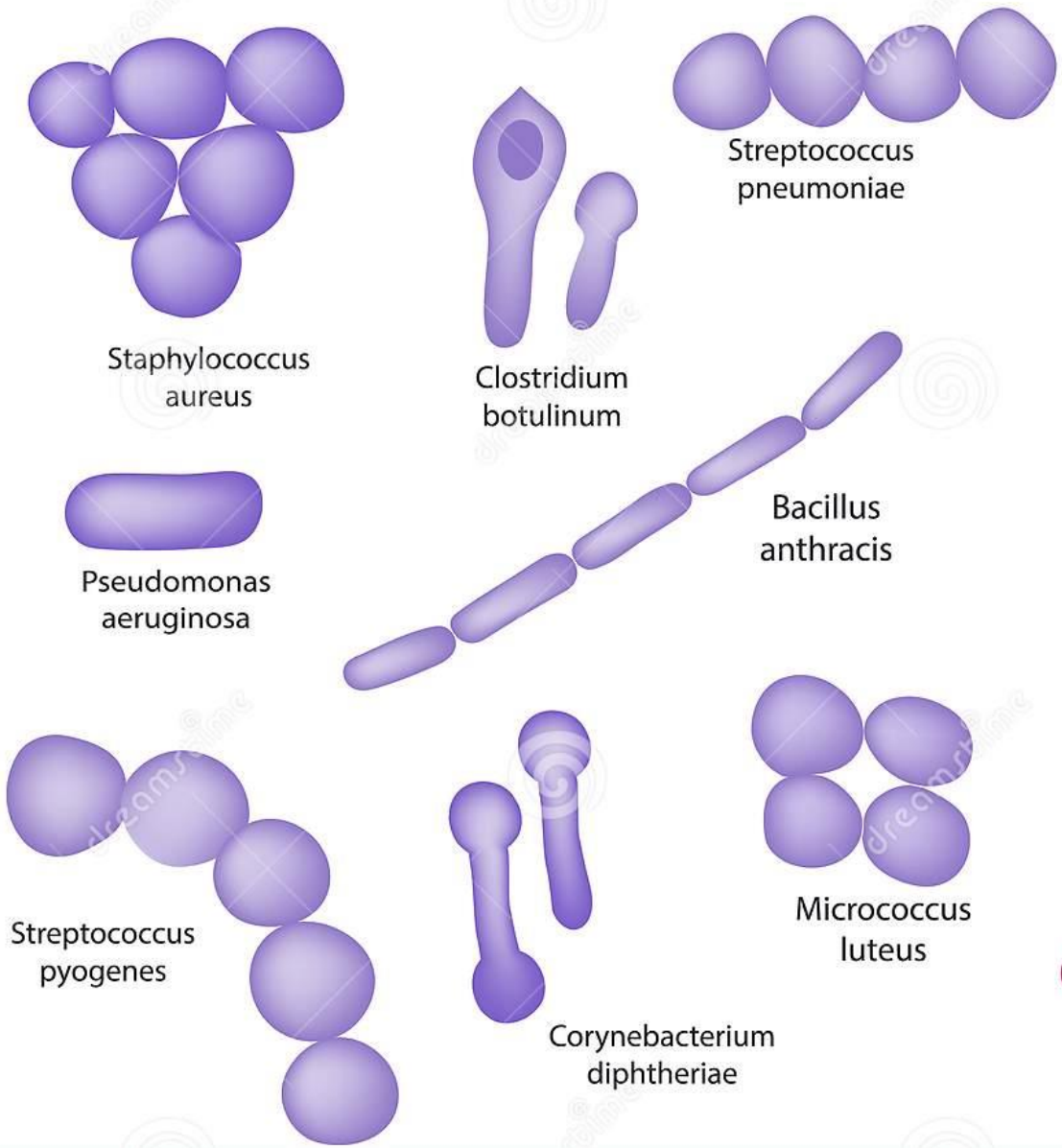


Observation Under Microscope

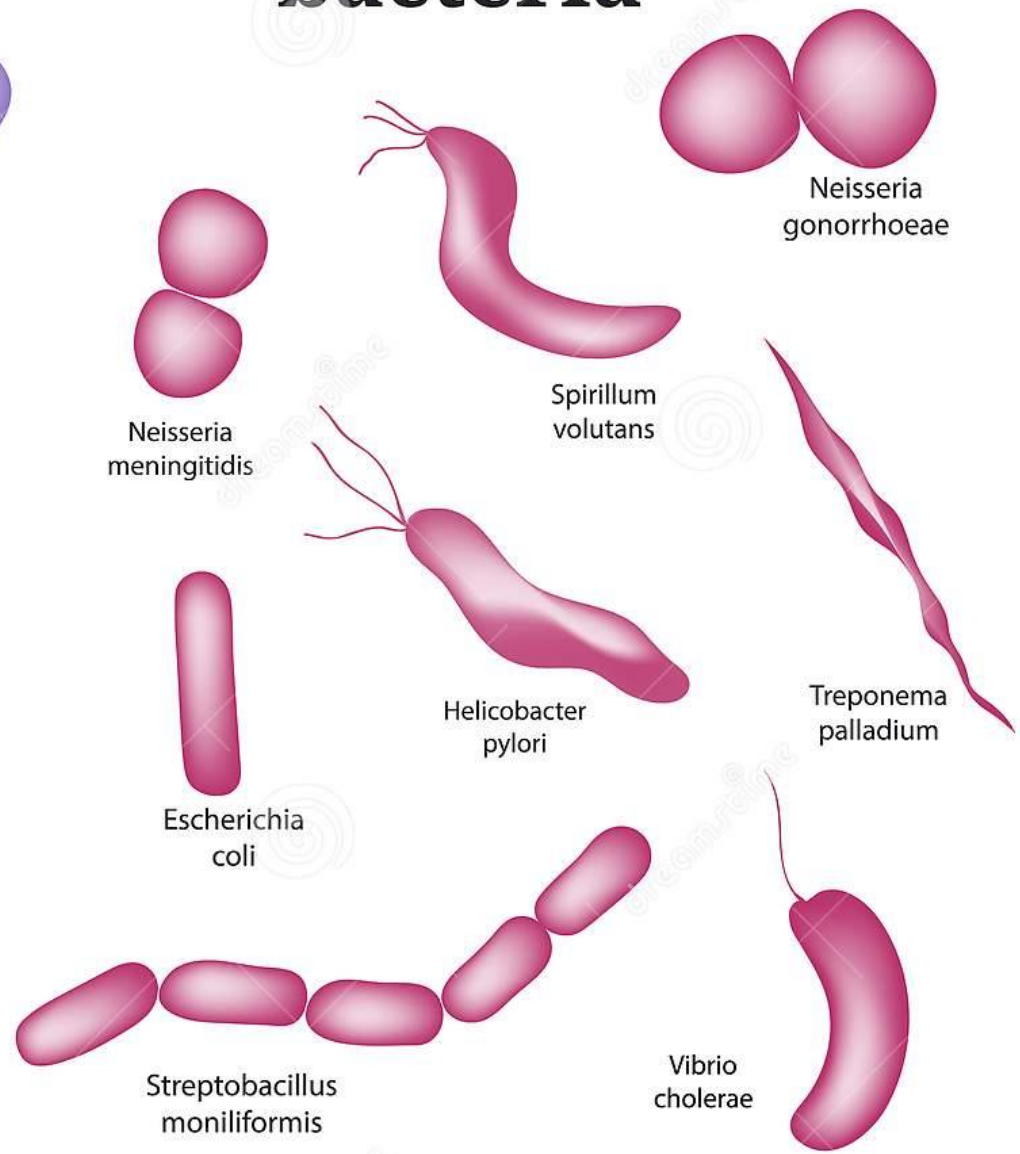
- After staining place glass slides on Microscope stage and put a drop of immersion oil. Observe under 100x objective and decide G + or G – according to colors.



Gram positive bacteria



Gram negative bacteria



Evaluation of Results

- **Total count** → Calculate and record # of microorganisms / ml of water
- **Coliform count**
  - Presumptive test MPN method
    - Calculate and record # of coliforms / ml of water (It should be zero for drinking water
  - Confirmed test
    - Record EMBA growth results of positive presumptive test tubes.
  - Completed test
    - Record Gram stain results of colonies isolated on EMBA plate

Presence of coliforms in drinking water indicates contamination from sewage because coliforms are a group of microorganisms fermenting sugars and producing carbondioxide. These microroganisms normally present human intestine and should not be present in dirnking water.