



140 MIC - Lab 10

Counting of Bacteria in Milk

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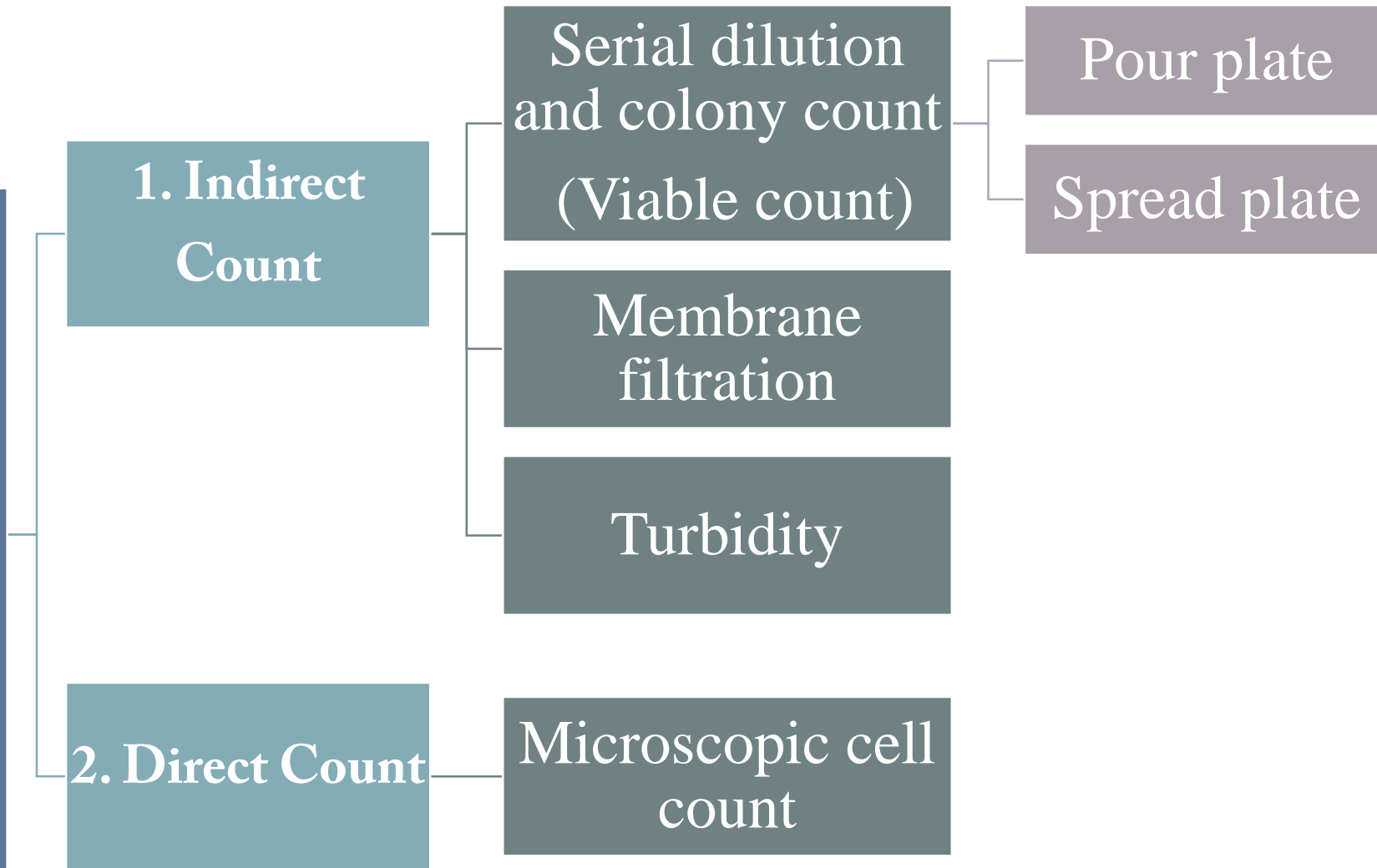
2016



Measurements of bacterial
growth

**Viable plate count by serial
Dilution Method**

Microbial Growth Measurements



- Many studies require the quantitative determination of bacterial populations.
- The two most widely used methods for determining bacterial numbers are :
 - 1. The standard plate count method.**
 - 2. Spectrophotometer (turbid metric) analysis.**

1. The Standard Plate Count Method

An indirect measurement of cell density (live bacteria).

2. Spectrophotometer (turbid metric) Analysis

Based on turbidity and indirectly measures all bacteria (cell biomass), dead and alive.

- The bacteriological tests used most often are the **Standard Plate Count (SPC)**

Dilution 1



Dilution 2



Dilution 3



Dilution 4



❖ The plate count (VIABLE COUNT)

- If the sample is serially diluted and then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies, the number of colonies can be used as a measure of the number of viable (living) cells in that known dilution.

- We are determining the number of Colony-Forming Units (**CFUs**) in that known dilution.



❖ Colony counting

- Bacteria may be clumped together.

Therefore, when doing the plate count technique, we generally say we are determining the number of Colony-Forming Units (CFUs) in that known dilution.

- The bacterial sample is diluted by factors of 10 and plated on agar.
- After incubation, the number of colonies on a dilution plate showing between 30 and 300 colonies is determined.

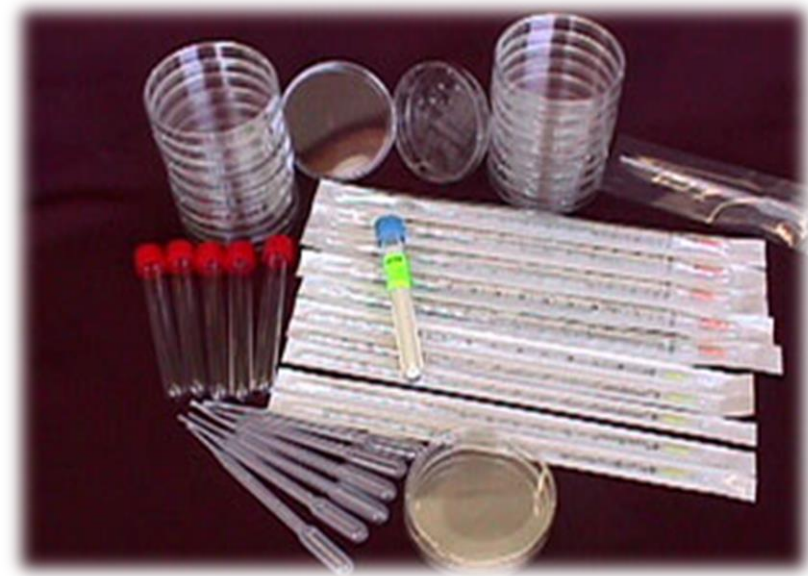
- Bacterial contamination of raw milk can generally occur from three main sources:
 1. Within the udder.
 2. Outside the udder.
 3. From the surface of equipment used for milk handling and storage.



The Experiment

❖ Material

- Test tubes.
- Pipettes (1 ml, graduated).
- Nutrient agar.
- Bent glass rod.
- Alcohol 70%.
- Raw milk



1. Take six test tubes and add 9 ml of distilled sterilized water (DSW) in each tube and label them as 1,2,3,4,5,6

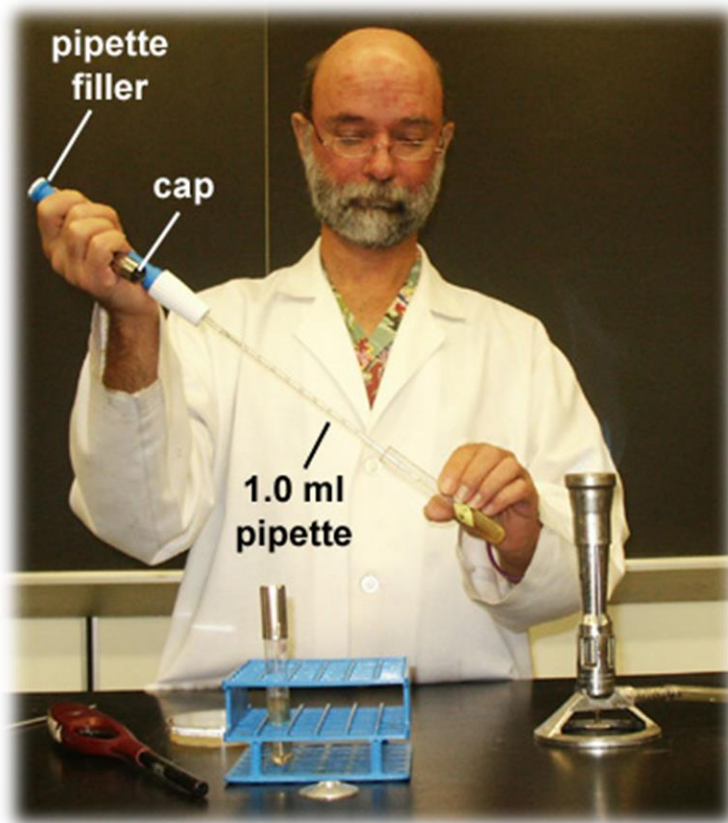
2. Transfer 1ml of the sample (unsterilized milk) to tube no.1 contained 9ml DSW and Reflame and cap the sample.

3. Mix the tube thoroughly, this will makes the first dilution.

4. Transfer 1 ml from the 1st dilution to test tube no.2. And this makes the second dilution.

5. Repeat the same pattern with other tubes as shown in the diagram.

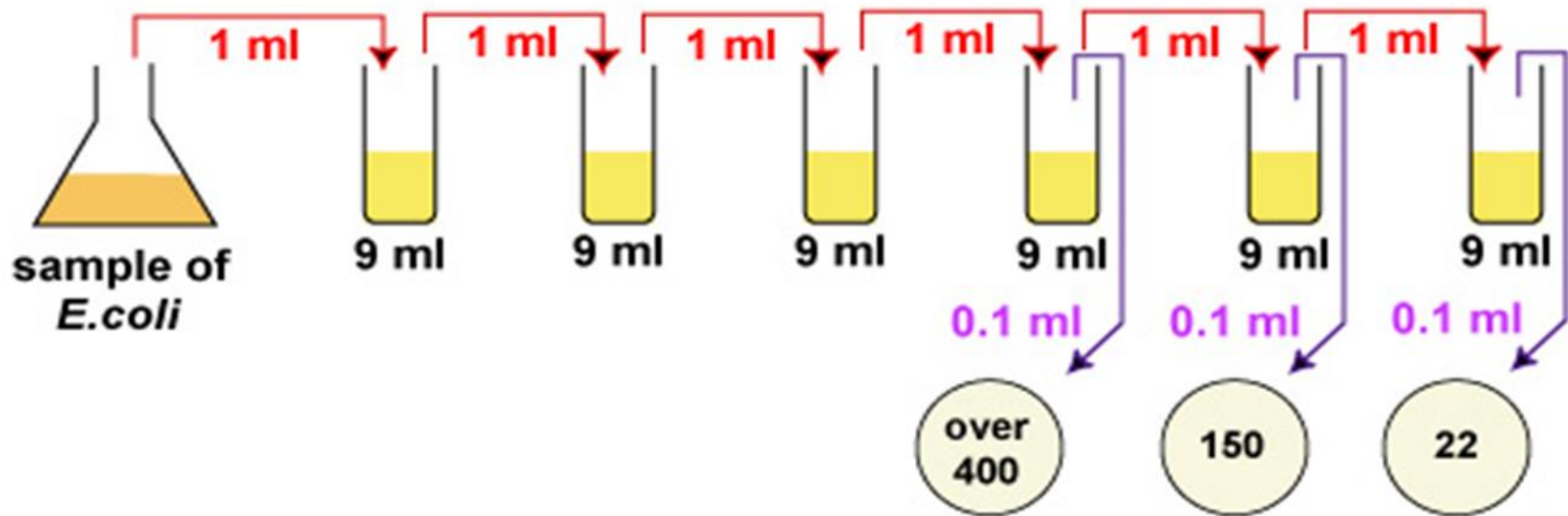
Preparation of dilutions



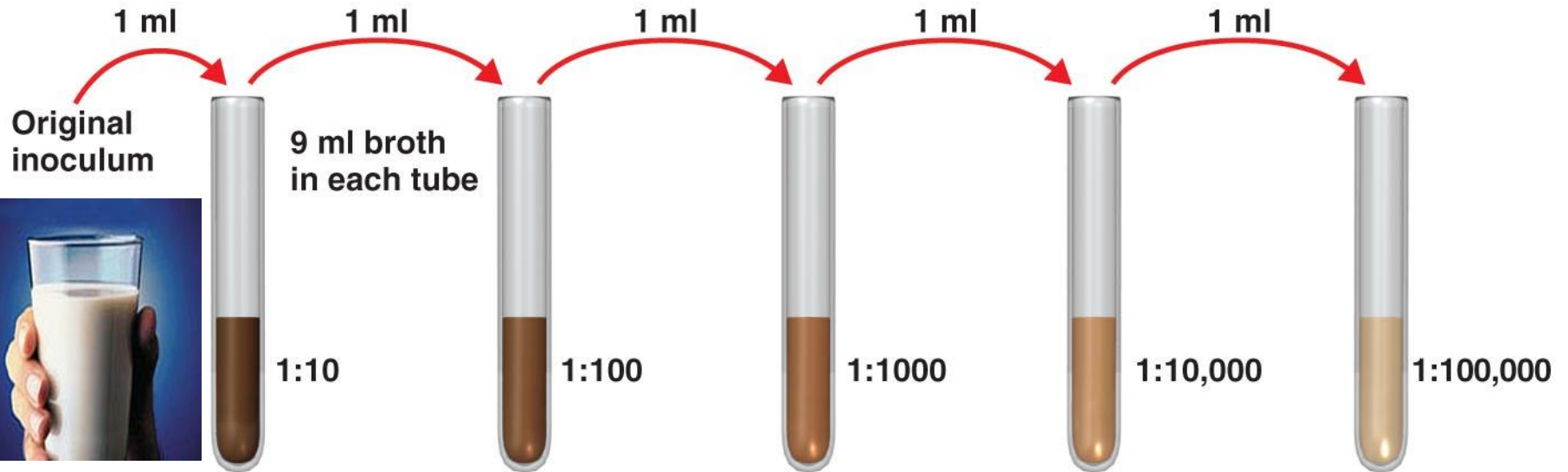
Using a Pipette to Remove
Bacteria from a Tube



Using a Vortex Mixer to Mix
Bacteria Throughout a Tube



- For a more accurate count it is advisable to plate each dilution in duplicate or triplicate and then find an average count.



6. From the last three dilutions, transfer 1ml to the nutrient agar plate.

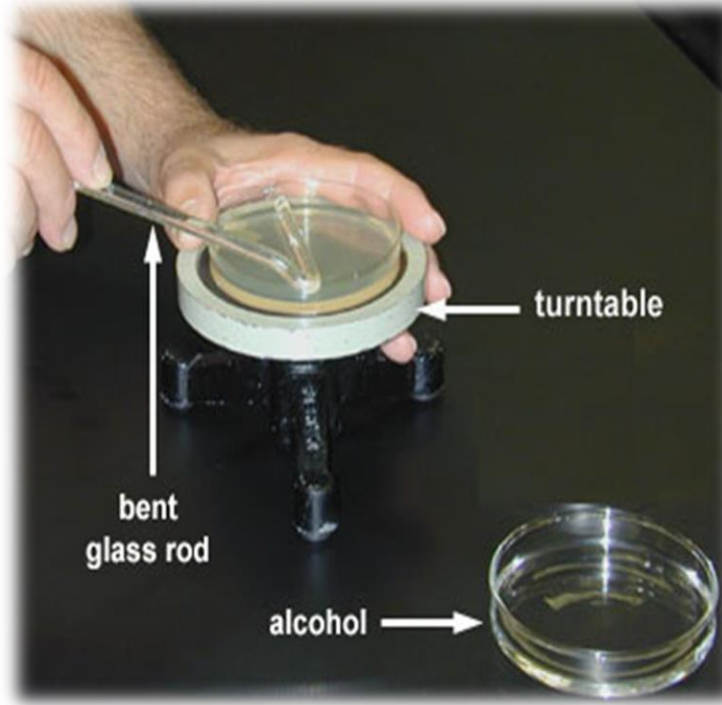
7. Using a turntable and sterile bent glass rod, immediately spread the solution over the surface of the plates.

8. Replace the lid and re-sterilize the glass rod with alcohol and flaming, and repeat for each plate.

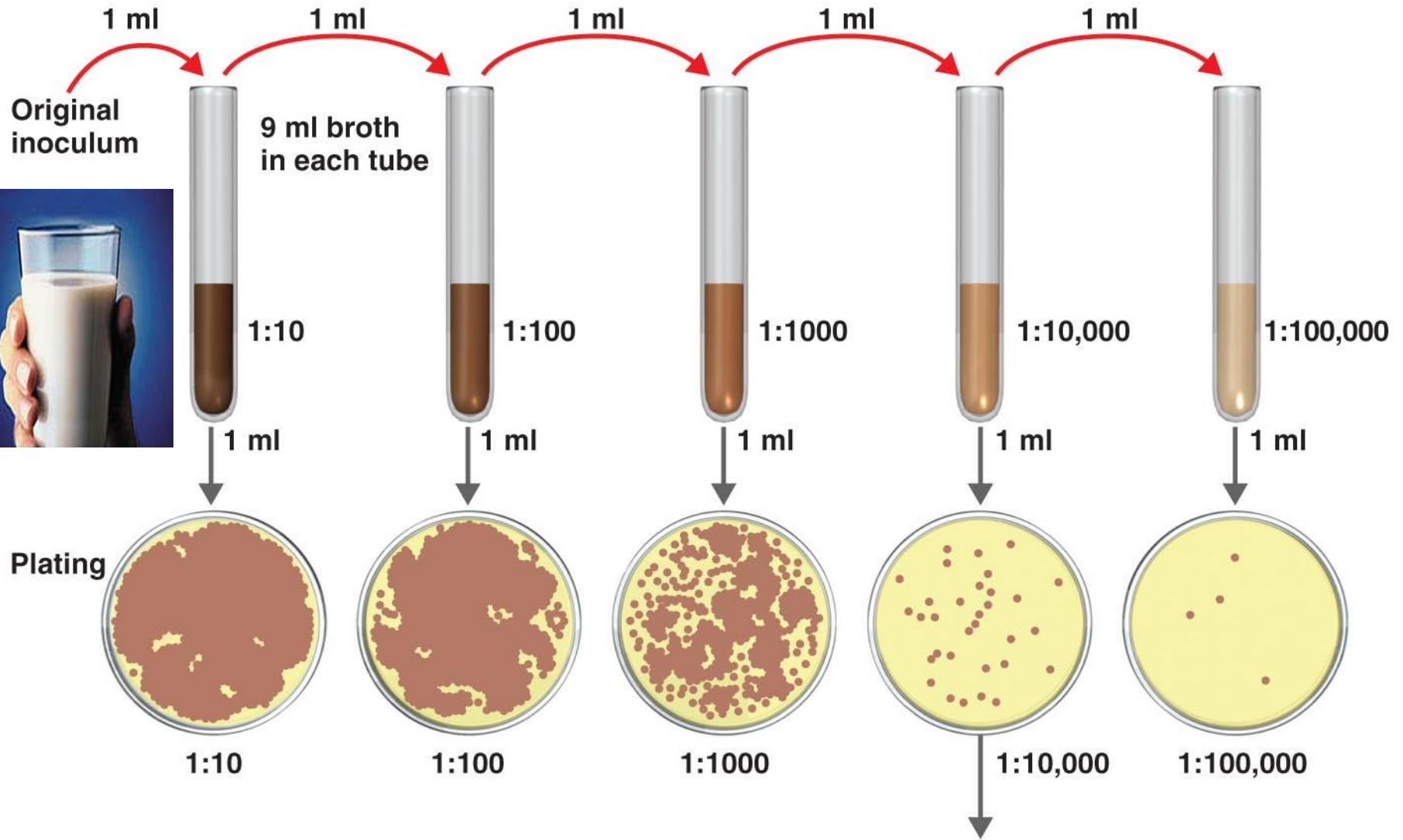
9. Incubate the plates converted for 24 hrs. at 37°C.

10. Count the colonies of bacteria after incubation.

Inoculation of Diluted Sample



- Using a Bent Glass Rod and a Turntable to Spread a Bacterial Sample.



❖ Result (Colony counting)

- Count the colonies on each plate.
- Select all of the Petri plates containing between 30 and 300 colonies.



- At the end of the incubation period, count by looking at the bottom of the plate (while keeping the Petri plate closed).
- Agar is translucent you should not have to open the plate.

- Select all of the Petri plates containing between 30 and 300 colonies. Count the colonies on each plate.



❖ Counting of bacteria in Milk (CFU)

- Calculate the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to agar plate.

- A simple calculation is given below:

$$\frac{\text{CFU}}{\text{ml}} \text{ in original sample} = \frac{\text{\# colonies counted}}{(\text{dilution factor})(\text{volume plated, in ml})}$$

$$\text{CFU} = \text{C} \div (\text{D} \times \text{V})$$

- While :
 - CFU = Number of bacteria cell in ml.
 - C = Number of colonies in plate.
 - D = dilution of sample.
 - V = volume plated in ml.

- **Example 1 :**
 - If **32** colonies in plate of **1/10.000** dilution and volume plated **0.5** then the count is :
 - $32 \div (1/10,000 \times 0.5) = 640,000$ cell /ml

- **Example 2 :**

- You count **46** colonies on your plate
- You put **1 ml** of bacterial culture into **99 ml** of saline and plated **0.1 ml**
- Dilution **1/100**

$$\text{CFU} = 46 \div (1/100 \times 0.1) = 46 \times 100 \times 10 = 46.000 \text{ CFU/ml}$$

- A plate having 30-300 colonies is chosen because this range is considered statistically significant.

This plate has between 30 and 300 colonies and is a suitable plate for counting.



- If there are less than 30 colonies on the plate, small errors in dilution technique or the presence of a few contaminants will have a drastic effect on the final count. 'too few to count (TFTC)'.

This plate has less than 30 colonies and is unsuitable for counting.



- Likewise, if there are more than 300 colonies on the plate, there will be poor isolation and colonies will have grown together. ‘too numerous to count (TNTC)’.

This plate has over 300 colonies and cannot be used for counting.



Any Questions



❖ Activity

Calculate CFU

- If you count **75** colony in plat of **1/1000** dilution and volume plated **0.5 ml**.

