

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

140 micro

Lab 8 : Counting of bacteria in Milk



طرق قياس النمو البكتيري

Measurements of bacterial growth

**العد الحيوي للخلايا البكتيرية بطريقة التخفيف
المتسلسل على الأطباق**

Viable plate count by serial Dilution Method

- Many studies require the quantitative determination of bacterial populations. The two most widely used methods for determining bacterial numbers are:

*A- The standard
plate count method.*

**An indirect
measurement of cell
density (live
bacteria).**

*B- 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)**



The plate count (VIABLE COUNT)

- However, 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.

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.



A close-up photograph of a glass filled with milk. A stream of milk is being poured from above into the center of the glass, creating a large splash and ripples on the surface. The background is a solid light blue color.

((The experiment))

Materials

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



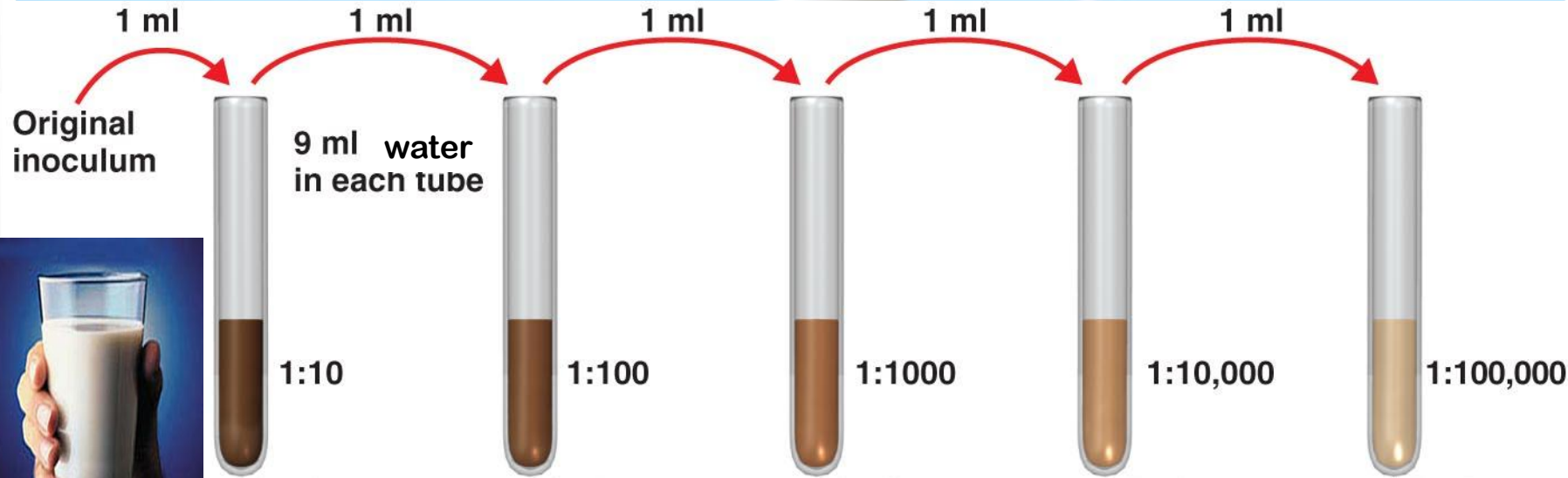
The background of the slide is a photograph of several petri dishes. Each dish contains a yellow, semi-transparent agar medium. The dishes are arranged in a slightly overlapping pattern, and the lighting creates soft reflections on the surfaces of the agar and the glass of the dishes. The overall tone of the image is light and clinical.

First, Preparation of milk agar media

- **Prepare 200ml nutrient agar media**
- **Add 1ml of sterilized milk in the prepared sterilized media**
- **Mix it thoroughly**
- **Pour the media in petri plates and let them solidify**

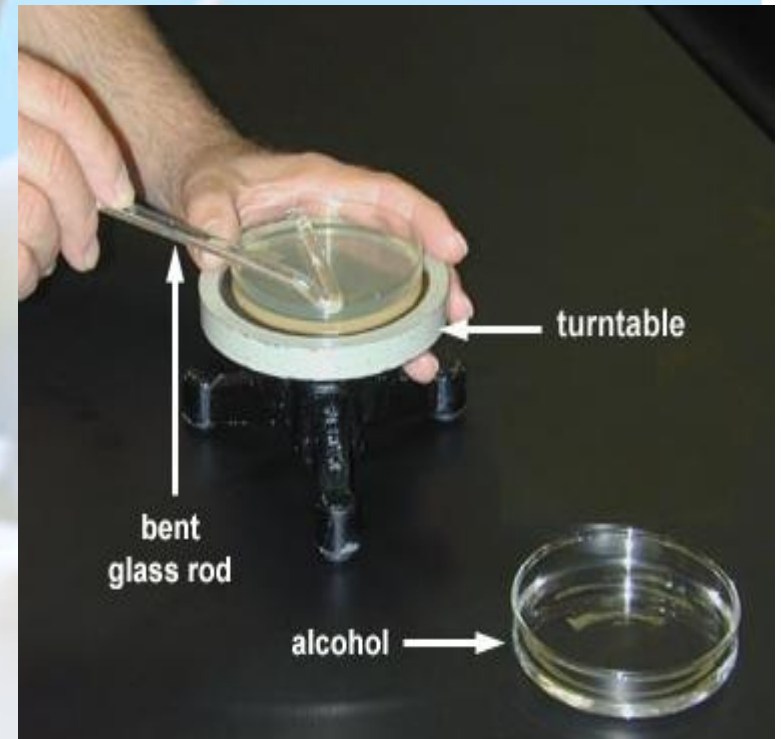
Preparation of dilutions :

1. Take six test tubes and add **9 ml** of distilled sterilized water (DDW) 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** DDW and Reflame and cap the sample.
3. Mix the tube throughlty. And this 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.

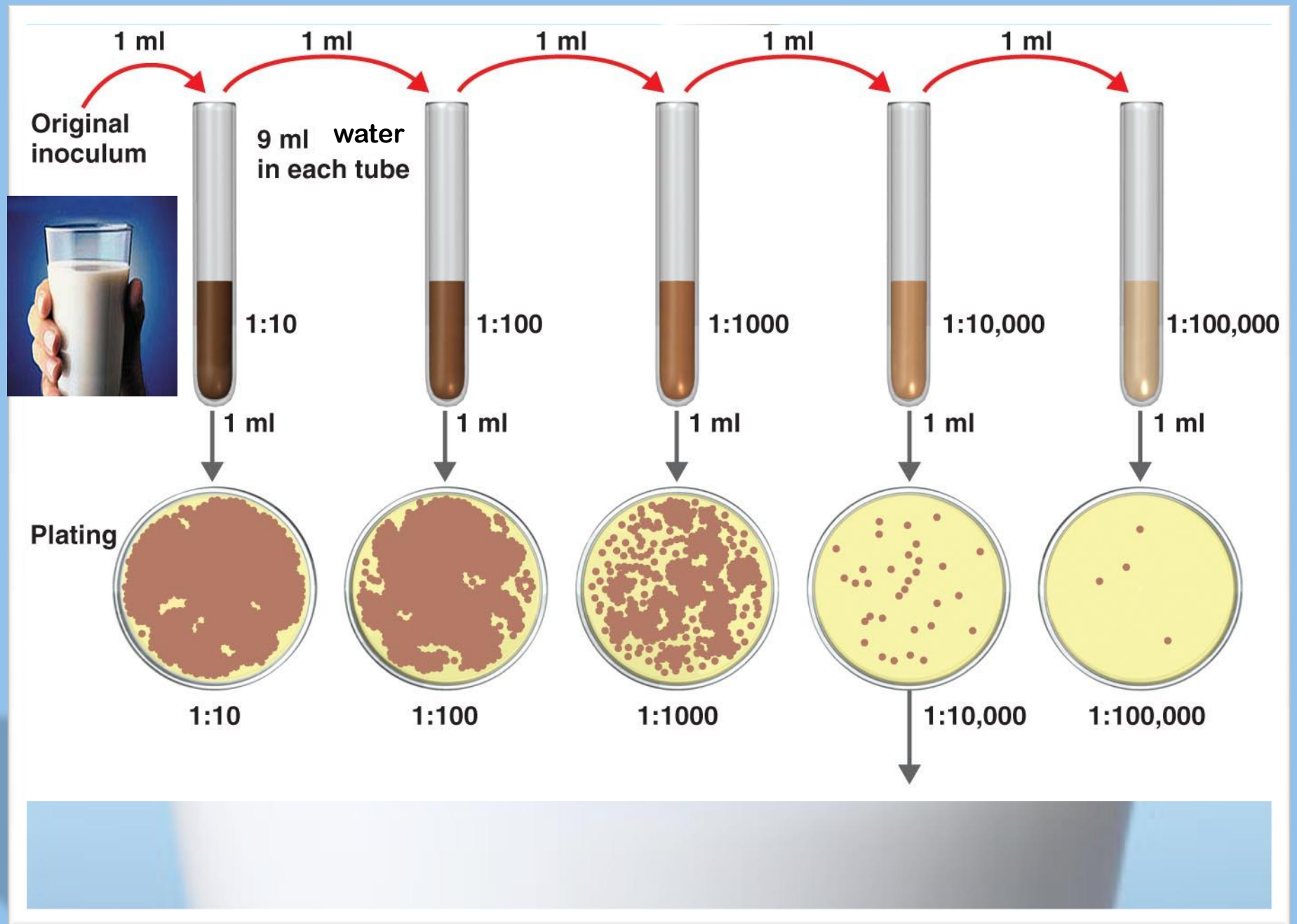


Inoculation of Diluted Sample

6. From the last three dilutions, transfer **1ml** to prepared milk 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.
9. Repeat for each plate.
10. **Incubate** the plates converted for 24 hrs at 37°C.
11. Count the colonies of bacteria after incubation.



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



After incubation



Group Cyan

Colony counting

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



Counting of bacteria in Milk (CFU)

Calculation :

Count of cell =

Number of colonies in plate \div (dilution of sample \times volume
plated in ml)

= Number of bacteria /ml.

for example; if 32 colonies in plate of 1/10,000
dilution and volume plated 0,5 then the count is :

$$32 \div (1/10,000 \times 0.5) = 640,000 \text{ bacteria /ml}$$

Colony counting

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.



Colony counting

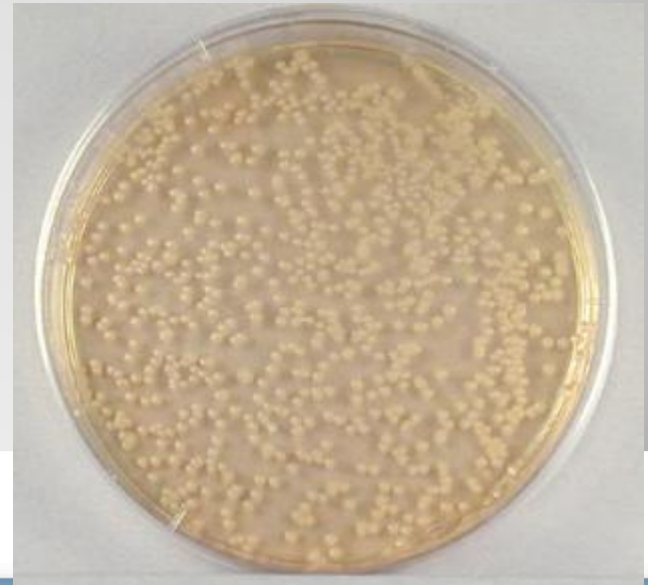
If there are less than 30 colonies on the plate, small errors in dilution technique or the presence of a few contaminants will have a drastic effect on the final count. 'too few to count (TFTC)'.

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 less than 30 colonies and is unsuitable plate for counting.



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





Good luck

أشروق الشهباني

أأمل الغامدي