بسم الله الرحمن الرحيم

Lab 10 :

Counting of bacteria in Milk

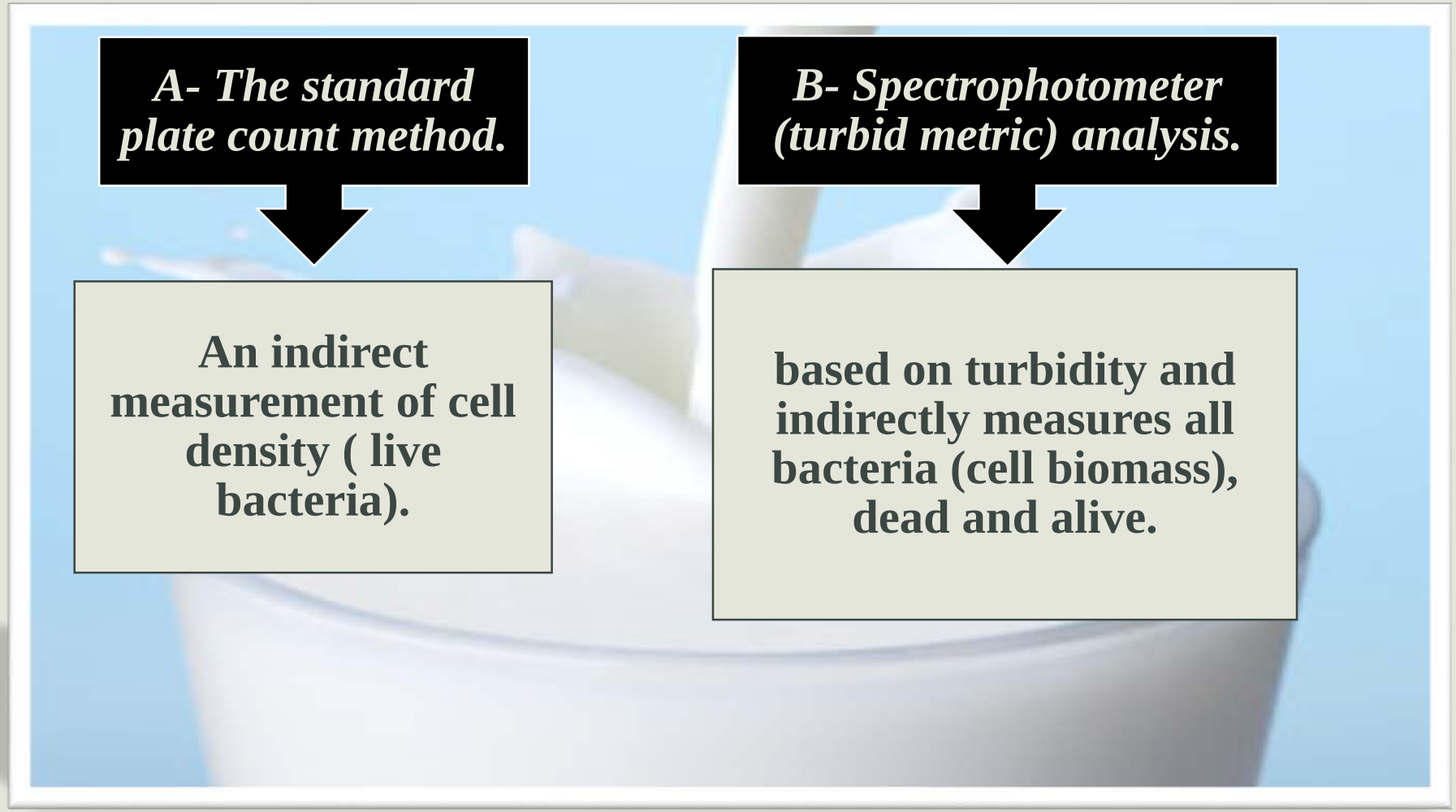
Subtitle

طرق قياس النمو البكتيري
Measurements of bacterial growth

العد الحيوي للخلايا البكتيرية بطريقة التخفيف المتسلسل على الأطباق

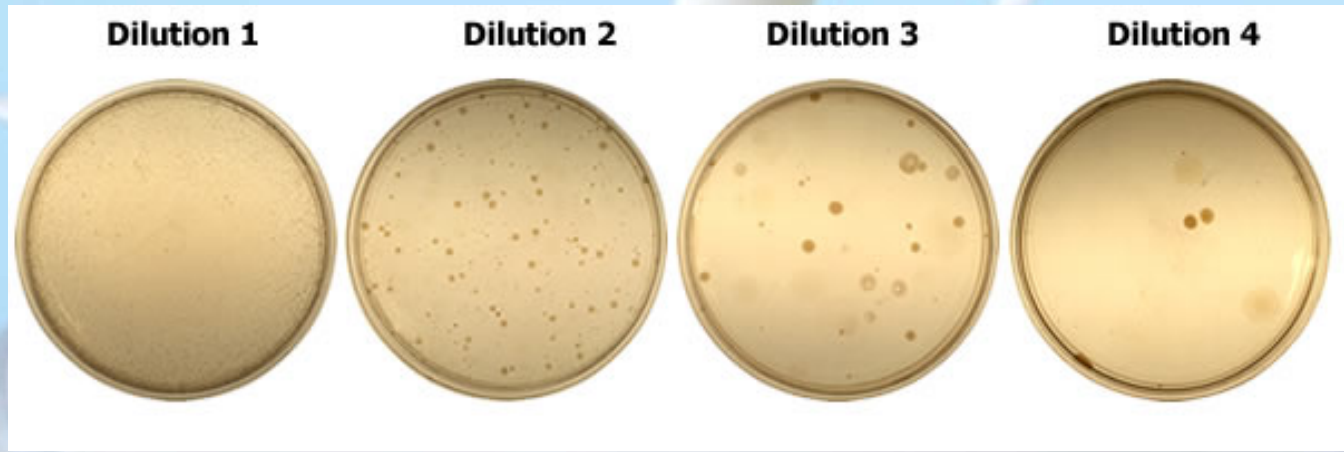
Viable plate count by serial Dilution Method

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



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.

Materials

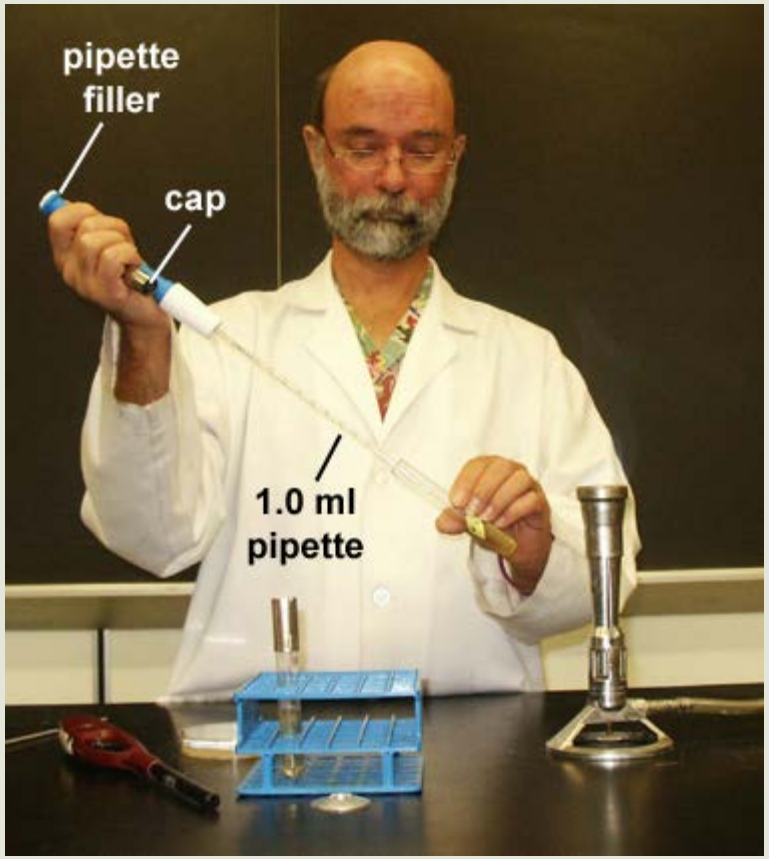
- Test tubes
- Pipettes (1 ml, graduated).
- Petri plates
- Nutrient milk agar
- Bent glass rod.
- Alcohol 70%.

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.

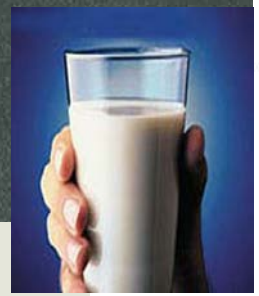
Preparation of dilutions :

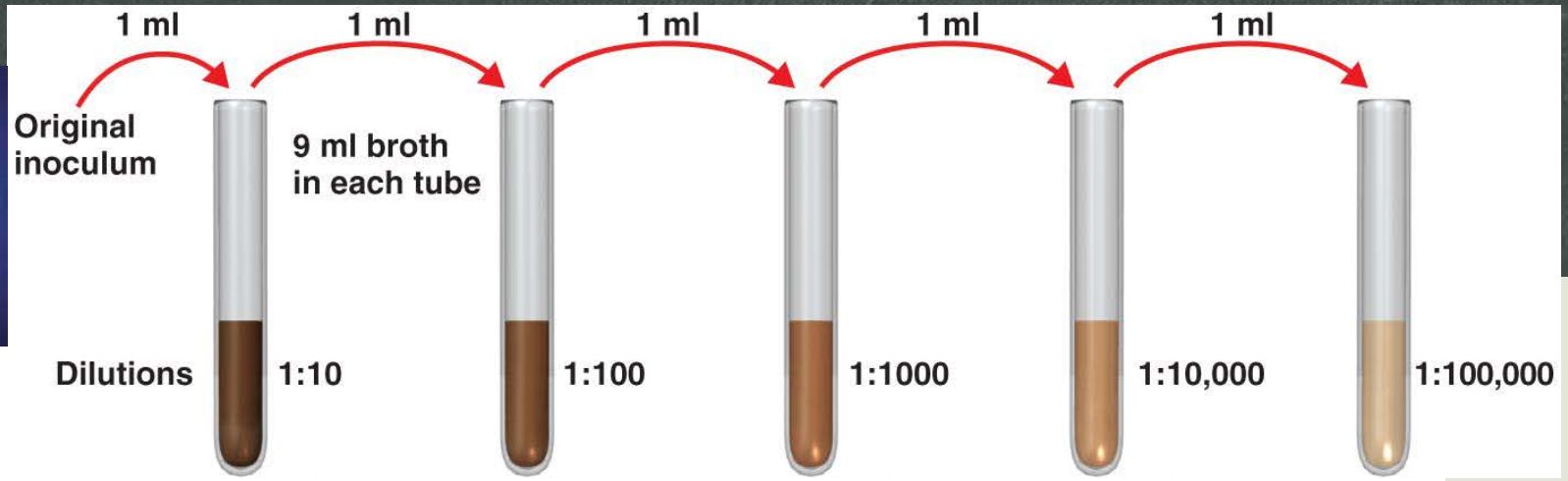
1. Take six test tubes and add 9 ml of distilled sterilized water (DDW) in each tube and label them as 1,2,3,4,5,6
2. Transfer 1ml of the sample (unsterilized milk) to tube no.1 contained 9ml DDW and re-flame and cap the sample.
3. Mix the tube thoroughly. And this makes the first dilution.
4. Transfer1 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.

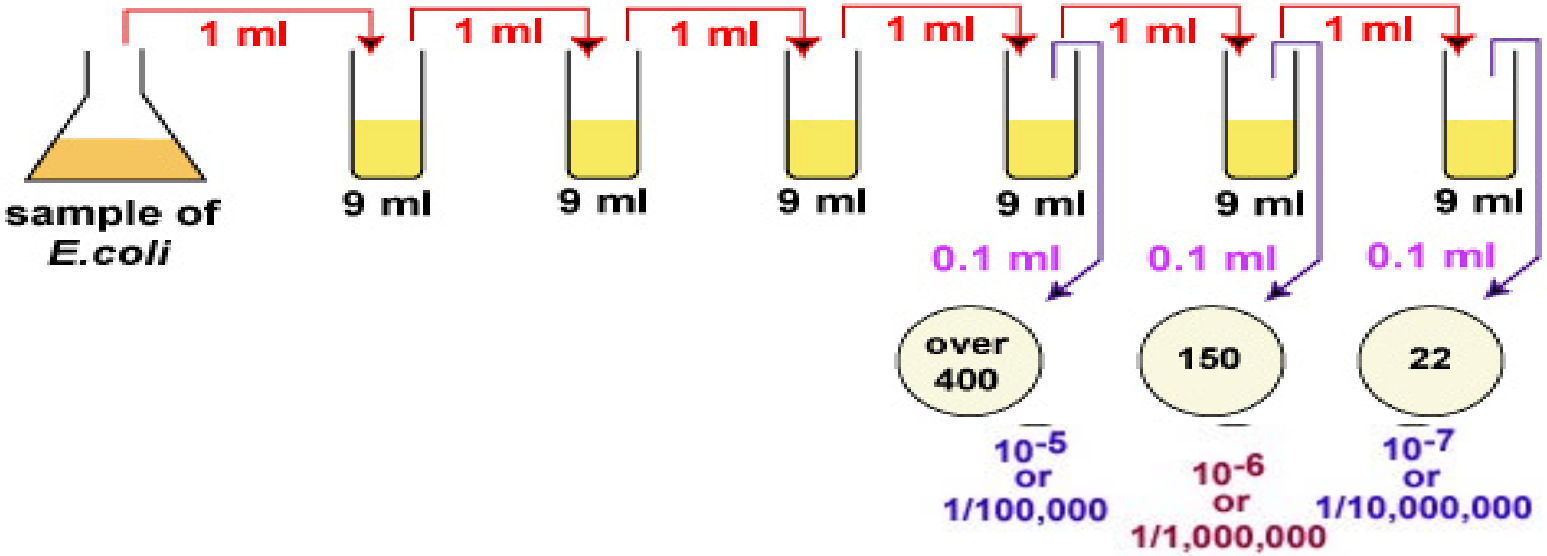


Using a Pipette to Remove Bacteria from a Tube

Using a Vortex Mixer to Mix Bacteria Throughout a Tube

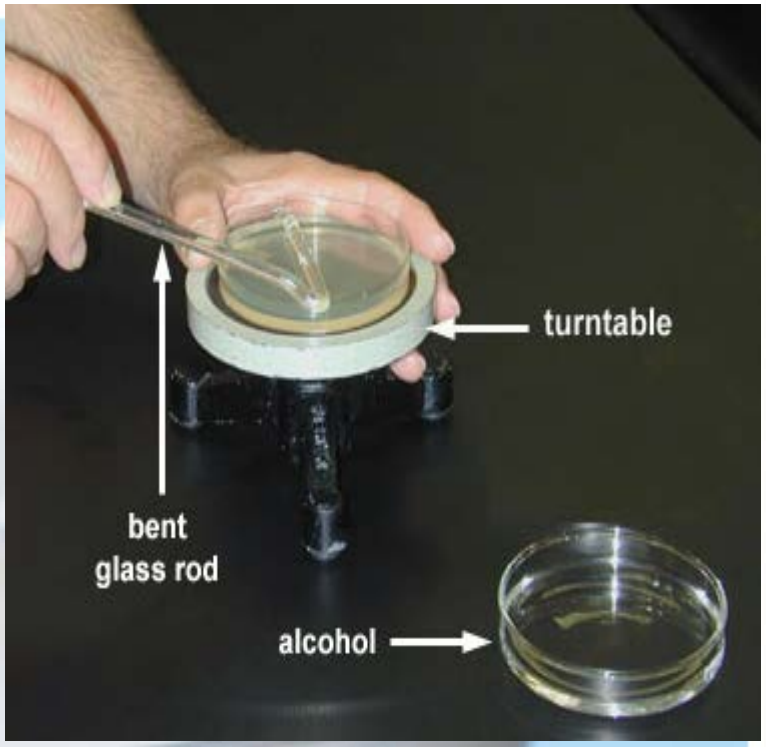




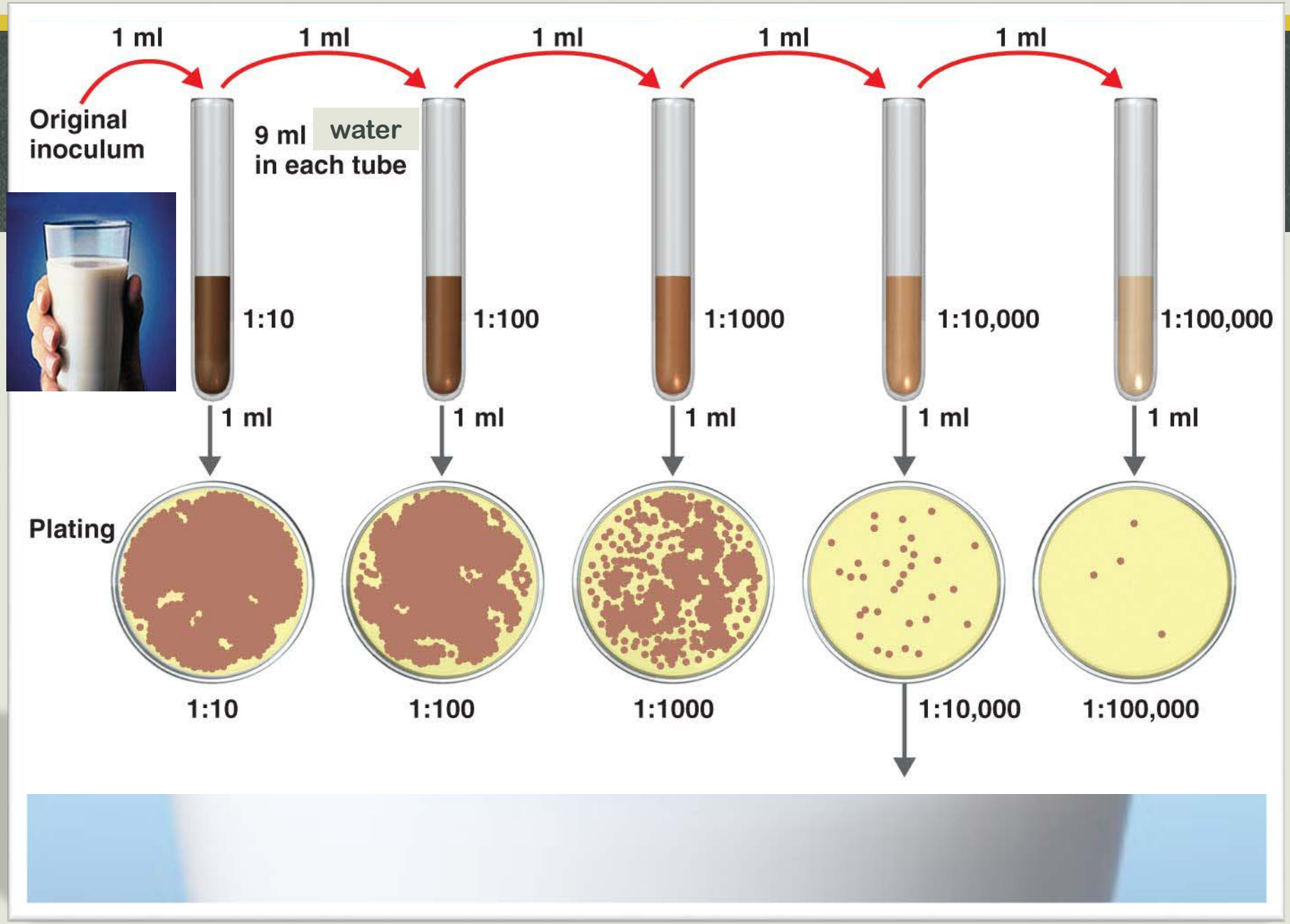


- For a more accurate count it is advisable to plate each dilution in duplicate or triplicate and then find an average count.

6. From the last three dilutions, transfer **1ml** to 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


1 ml
1 ml
1 ml
1 ml
1 ml
Original inoculum
9 ml water in each tube
1:10
1:100
1:1000
1:10,000
1:100,000
1 ml
1 ml
1 ml
1 ml
1 ml
Plating
1:10
1:100
1:1000
1:10,000
1:100,000

After incubation (The Results)

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 ÷ (dilution of sample x 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 :

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

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.

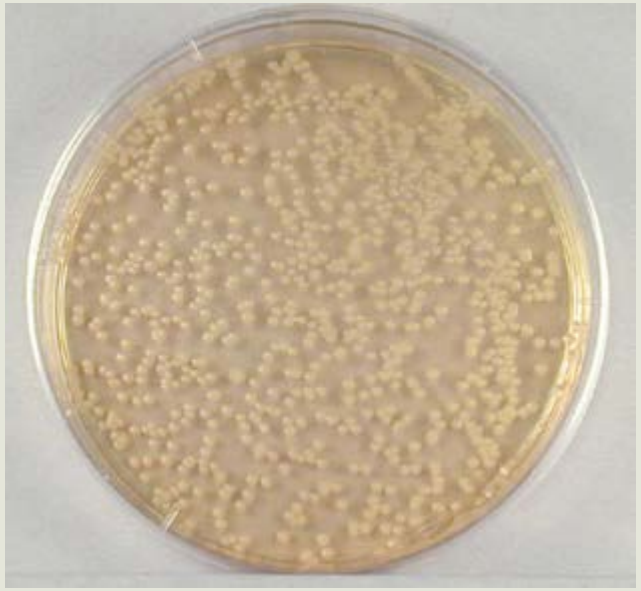
Colony counting

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)’.

This plate less than 30 colonies and is unsuitable plate 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.




Good luck
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