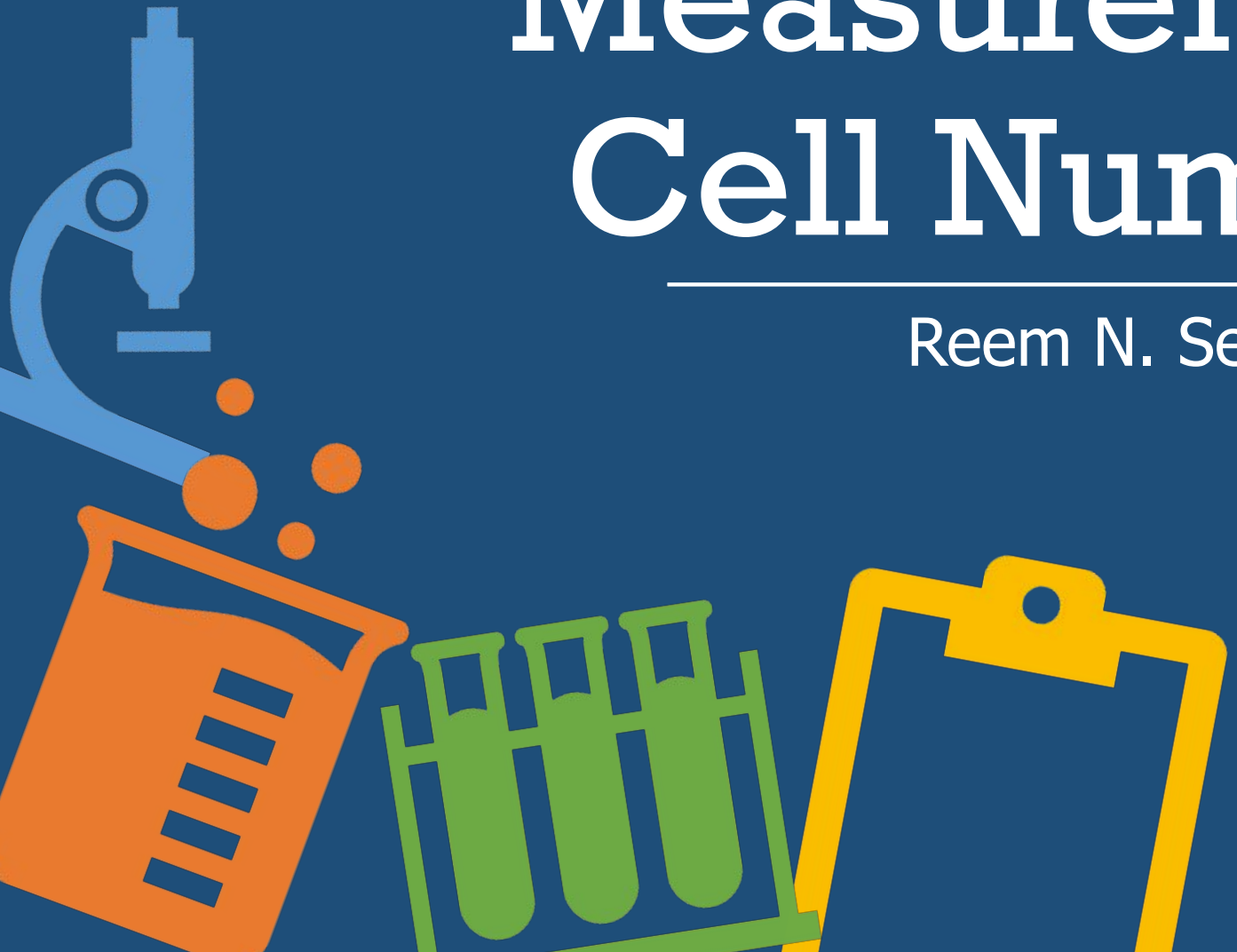
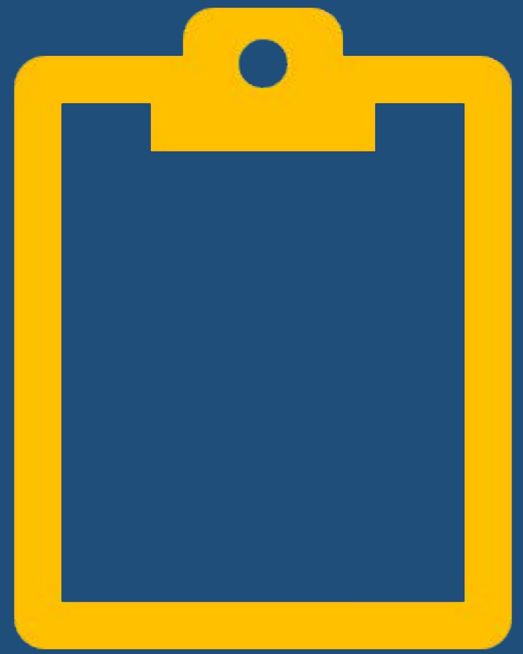
Measurement of Cell Numbers:

Reem N. Seyala



Direct Methods

A major advantage of direct counts is the fast results are obtained. However, because large numbers of bacteria in uncultured products give evidence of unsanitary conditions, no matter what type of organisms are present or whether they are viable (living) cells, this method does have value as a quality control procedure.



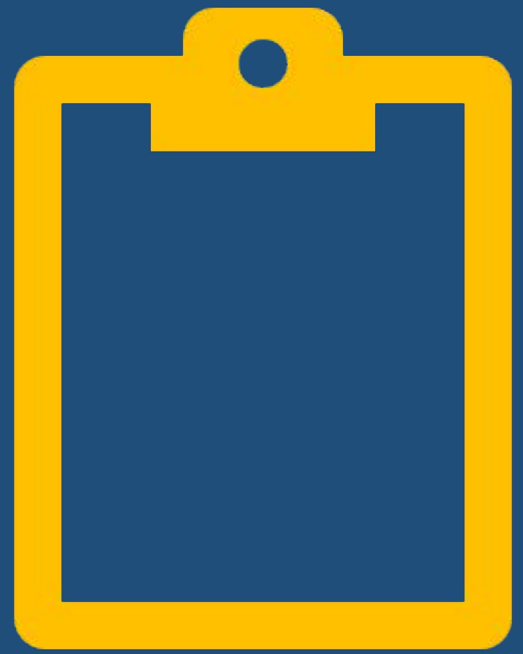
Breed's Method

Especially useful for counting bacterial cells in Dairy products.

- A known volume of microbial cell suspension (0.01 ml) is spread uniformly over a glass slide covering a specific area (1 sq. cm).
- The smear is then fixed by heating, stained and examined under oil immersion lens, and

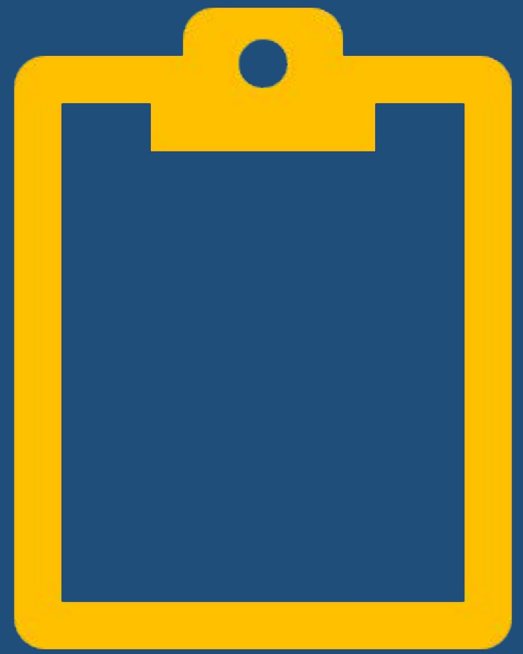
the cells are counted.

- Cells in a few microscopic fields are counted because it is not possible to scan the entire area of smear



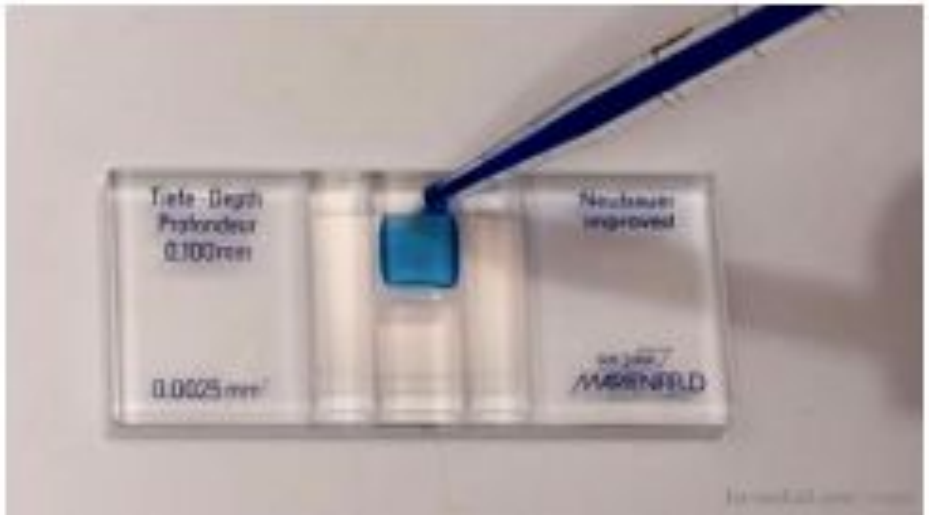
- The counting of total number of cells is determined by calculating the total number of microscopic fields per one square cm. area of the smear .The total number of cells can be counted with the help of following calculations:

- No. of cells / ml = Average no. of microbes per microscopic field x 5000 x 100



2- Hemocytometer

The hemocytometer (or haemocytometer or counting chamber) is a specimen slide which is used to determine the concentration of cells in a liquid sample. It is frequently used to determine the concentration of blood cells (hence the name “hemo-”). It’s called hemocytometer. Hemo, for blood; cyto, for cell; meter, for measuring. So altogether: measuring blood cells. The cover glass, which is placed on the sample, does not simply float on the liquid, but is held in place at a specified height (usually 0.1mm).



Procedure:

1- Wash the hemocytometer, rinse and dry.

2- Place a clean cover over the chamber area.

3- Dilute bacterial suspension such that clouding is barely visible with the naked eye.

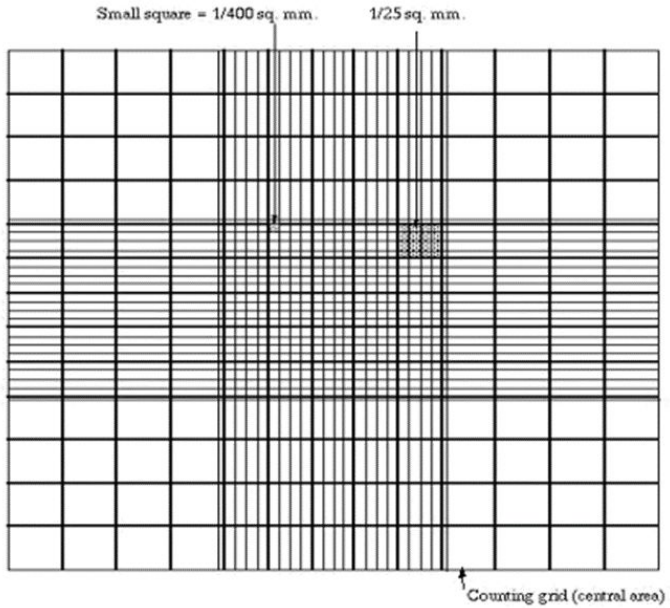
4- Combine 1 volume of cell suspension and 1 volume of trypan blue (0.4%). Mix thoroughly and allow to stand for 5 minutes. Viable cells don't take up trypan dyes, whereas dead cell

do.

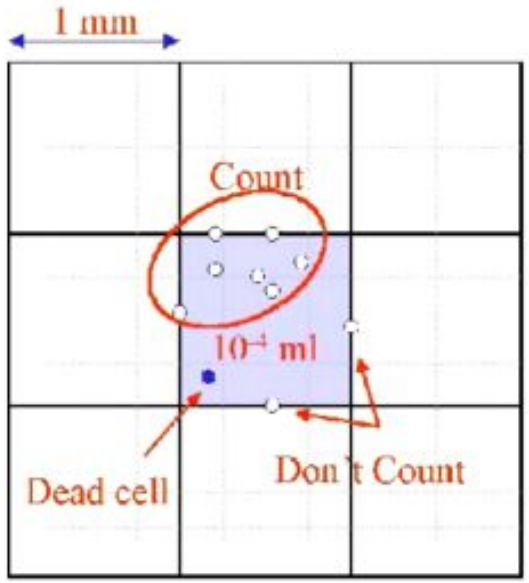
5- Using a Pasteur pipet or syringe, place the tip in the V-shaped indention near the edge of the cover.

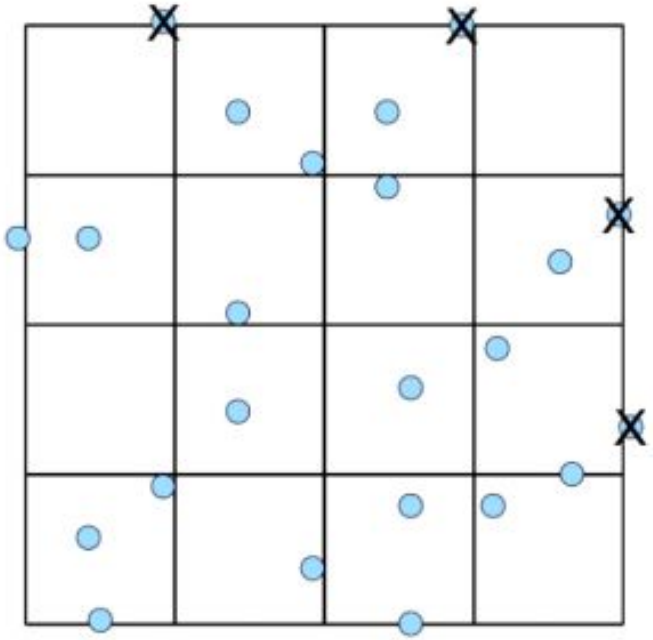
6- Slowly, let the chamber fill by capillary.

7- Place the hemocytometer on the stage and count bacterial cells in small squares. Count cells on top and left touching middle line. Do not count cells touching middle line at bottom and right



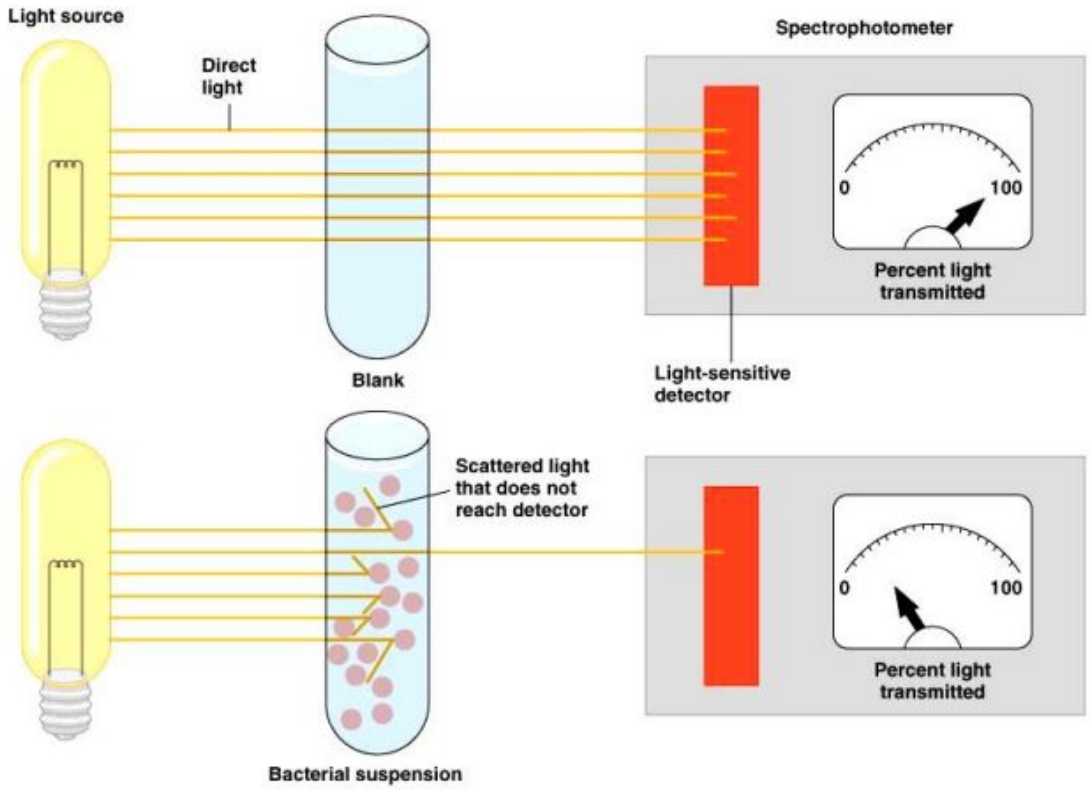
- No. cells / ml = Average no. of cells / small square x 4 x 106 x 1/dilution.





3- Turbidity (spectrophotometer).

A quick and efficient method of estimating the number of bacteria in a liquid medium is to measure the turbidity or cloudiness of a culture and translate this measurement into cell numbers. This method of enumeration is fast and is usually preferred when a large number of cultures are to be counted.

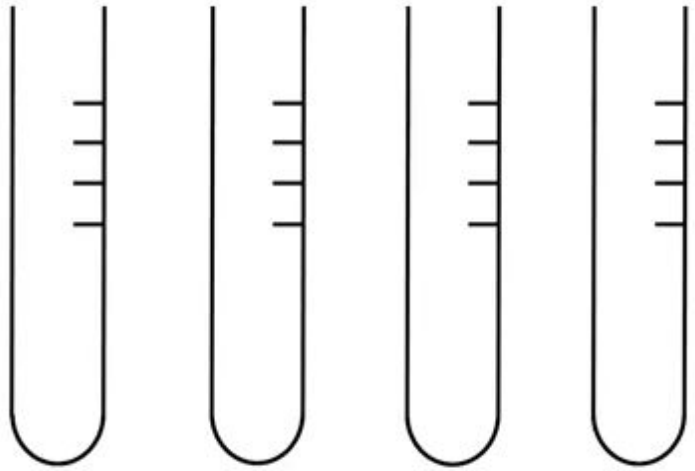


Indirect Methods:

- 1- Plate count
- 2- Dry Weight
- 3- Filter membrane

How to prepare a serial dilution

Serial Dilution
10-fold



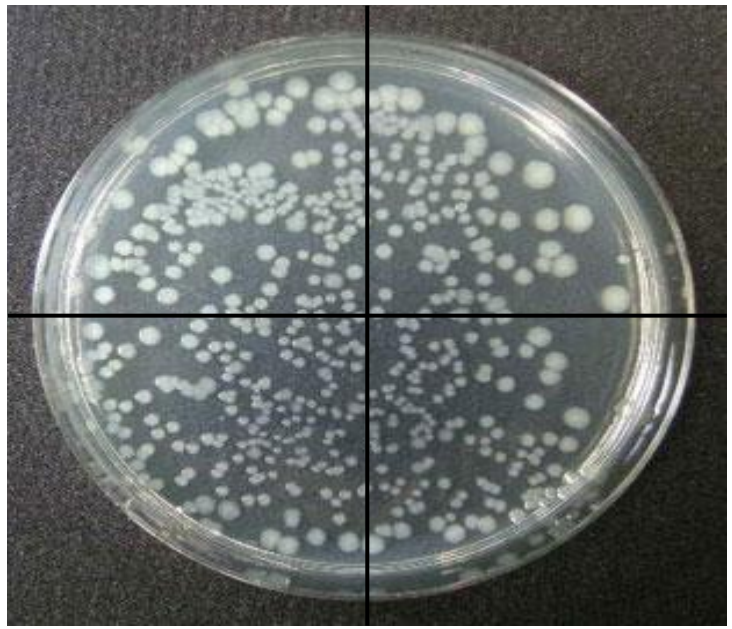
Total plate count:

- After the cells have been diluted, they are incubated on an agar medium until colonies form. It is at this time that the cells may be counted. Each colony represents a "colony forming unit" (CFU). In the methods below, ONLY viable (living cells are counted) which makes these methods more accurate than direct methods above. There are two main methods of plate counting: 1- Spread plate method . 2- Pour plate method.

CFU formula

CFU/mL or CFU/g = Number of Colonies Counted ÷(Volume Plated × Dilution Factor)

- Calculate cfu for this plat if the dilution factor is 10 -3 ?




Safety first,
science always!