Enumeration of Bacteria

Bacterial enumeration is the measurement of the number of bacterial cells per milliliter or gram (the units depends on the nature of the sample).

There are numerous reasons why researchers have to calculate the number of bacteria or compare the growing number of these microbes under certain specific conditions. It's mainly essential and a part of routine work in food, water, and dairy microbiology labs.

The knowledge of the numbers of these microorganisms in our food, including milk, buttermilk, and water, help the labs to determine if the prepared food or available water is hygienic or not for consumption.

There are a number of methods that can be used to determine the number of cells/unit and these methods can be divided up into different categories.

* **Viable counts** involve counting cells that can be cultured and/or are metabolically active.
* **Total counts** involve counting all cells including dead or inactive cells.
* **Direct methods** of enumeration involve counting actual cells or colonies.
* **Indirect methods** involve estimating the number of cells based on cell mass.

The table below gives examples of different methods.

Method of Enumeration	Category	Description
Staining and microscopy	Direct and total	Stain the cells with dyes, which make them visible. Count the number of cells using a microscope.
Standard plate count	Direct and viable	Dilute a sample in saline, spread on solid media, and count colonies. Calculate number of cells in original sample from counts and dilutions.
(Most Probable Number) MPN	Indirect and viable	Estimates numbers of cells by their patterns of growth in liquid culture media.
Spectroscopy (Optical Density)	Indirect and total	Estimate the number of cells/ml based on amount of light that passes through culture.

The two most widely used methods are the standard plate count and spectrophotometric (turbidimetric) analysis.

Direct or Slide method for bacterial counting

Direct counting methods include microscopic counts using a hemocytometer or a counting chamber. Counting chambers serve to determine the number of particles (e.g., leucocytes, erythrocytes, bacteria, fungus spores).

It is a total count method where every cell (dead or alive) is counted and it works by creating a volumetric grid divided into differently sized cubes for accurately counting the number of particles in a cube and calculating the concentration of the entire sample.

Advantage of Direct Microscopic count

* Rapid, Simple and easy method requiring minimum equipment .
* Morphology of the bacteria can be observed as they counted.
* Very dense suspensions can be counted if they are diluted appropriately.

Limitations of Direct Microscopic count
1. Dead cells are not distinguished from living cells.
2. Small cells are difficult to see under the microscope, and some cells are probably missed.
3. Precision is difficult to achieve
4. A phase contrast microscope is required when the sample is not stained.
5. The method is not usually suitable for cell suspensions of low density i.e. $< 10^7$ Cells per ml, but samples can be concentrated by centrifugation or filtration to increase sensitivity.

Calculating the cell density
The following numbers are needed:
1. **Number of cells counted in a squares,**
2. **Area of the square,**
3. **Height of the sample,**
4. **Dilution factor.**

The objective is to find the number of cells in 1ml of original solution.

The formula used for the direct microscopic count is:

Step 1: Counting:
Count all of the cells in a large square. For the purpose of this example, we use an average cell count of **25** bacterial cells in one corner square.

Step 2: Computing the volume:
It is necessary to determine the volume represented by the square. The **width** and **height** of the must be **multiplied** by the **height** of the sample (often printed on the hemocytometer,

Volume of Large Corner Square $=$ Length $\times$ Width $\times$ Height
$= 1\ mm \times 1\ mm \times 0.1\ mm$
$= 0.1\ cm \times 0.1\ cm \times 0.01\ cm$
$= 0.0001\ cm^3$
$= 10^{-4}\ cm^3$ or $10^{-4}\ ml$

Step 3: Calculating the number of cells in 4 squares and then convert the volume to 1 ml:
If there are 25 cells (**4 squares * 25 bacterial cells=100 bacterial cells/ 10^{-4} ml**), then how many cells are there in 1ml (=1000 μl)?

100 bacterial cells	10^{-4} ml
x	1 ml

We do simple direct proportion:

$x = 100$ bacterial cells $/ 10^{-4}$ ml
$= 100$ bacterial cells$/ 0.0001$ ml
$= 1.000.000$ bacterial cells$/ 1$ ml of sample
$= 10^6$ bacterial cells$/ 1$ ml of sample

Step 4: Correcting for dilution:

If the sample was diluted before counting, then this must be taking into consideration as well. We assume that the sample was **diluted 1:10**. The final result is therefore

$$x = 10^6 \text{ bacterial cells/ 1 ml of sample (before dilution)}$$

So after dilution (1:10)

$$x = 10^6 \text{ bacterial cells} \times 10$$

$$= 10^7 \text{ bacterial cells/ 1 ml of sample}$$

Procedure

1. Ensure the cover slip and hemocytometer are clean (use alcohol to clean).
2. Mix equal volumes of **0.4% methylene blue stain** and a well-mixed bacterial cell suspension, e.g. **mix 10 ml methylene blue stain** with **10 ml bacterial cell** suspension for **10 minutes**.
3. Pipette **methylene blue/cell mix** (approximately **10 μl**) at the edge of the cover-slip and allow running under the cover slip.
4. Visualize the hemocytometer grid under the microscope; please note:
   A. Methylene blue is a "vital stain"; it is excluded from live cells.
   B. Bacterial cells appear purple under phase contrast.
5. **Count bacterial cells** in all **four large corner squares** which used for **WBC count** and record cell counts.
6. It is advisable to count around 40 to 70 cells to obtain an accurate cell count.
7. Calculate the cell concentration (bacterial cells per ml of our sample):

Square #	Cell count
1	
2	
3	
4	

COUNTING CHAMBER

Each of these small squares is 1/25 mm square (50 μ)

This space is one square mm. On the hemacytometer its depth is 1/10 of a mm. For content of this space, multiply by 10 to get content of one cubic mm.

IMPROVED NEUBAUER RULING

A - B - C - D ARE FIELDS USED IN DOING THE **T**otal **B**acterial **C**ount