Measurement of Bacterial Growth

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Purposes

- The total **bacteria count is** one of the key indicators in the field of hygiene management. because the number of microorganisms shouldn't exceed certain guide values.
- Preparing a suitable number of bacteria for laboratory experiments E.g. electro/ chemical competent cells

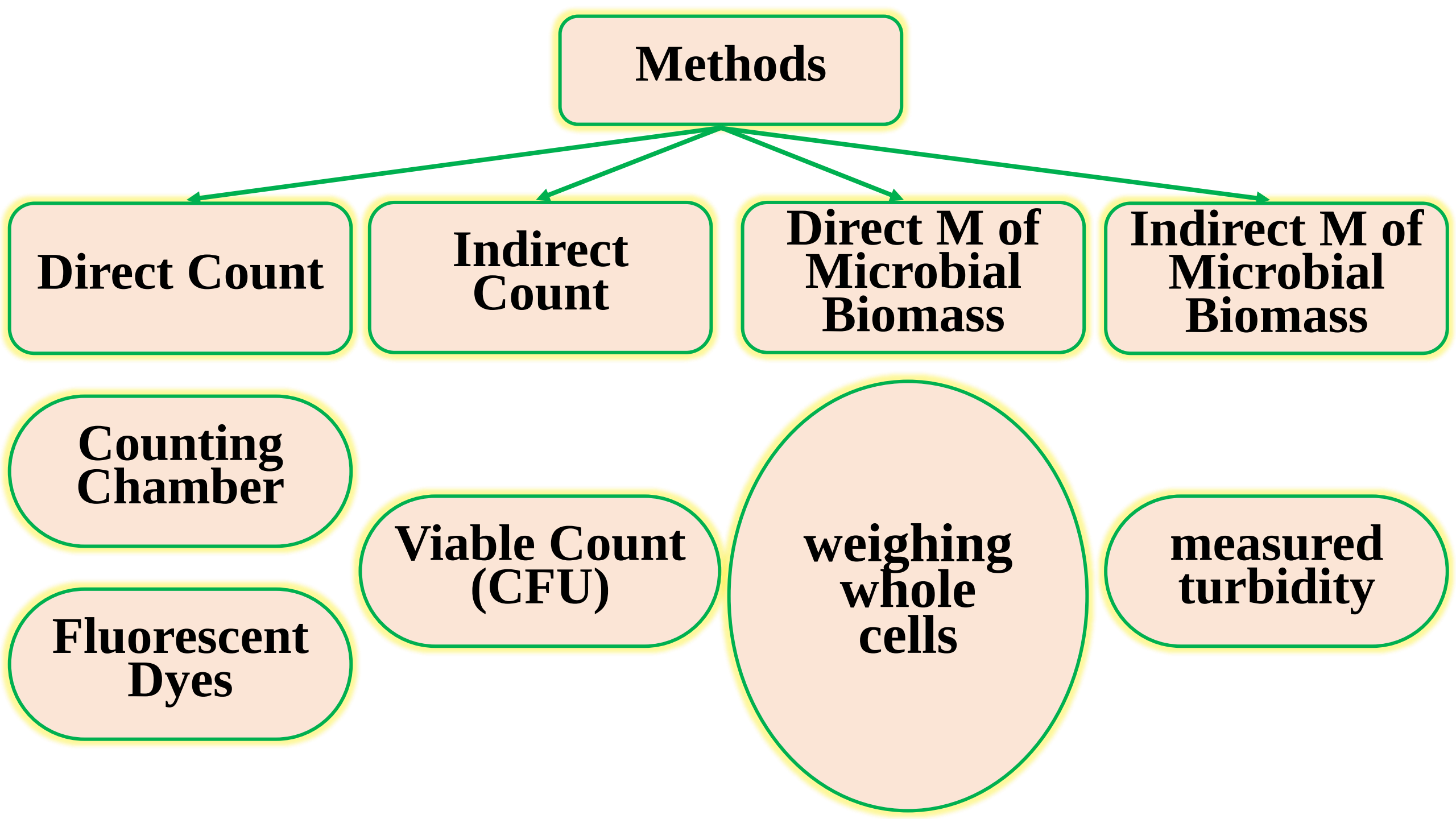
Bacterial sample for protein experiment

Starting bacterial culture from knowing point of bacterial growth curve

- Antibiotic sensitivity test
- etc

Objectives

- To learn the different techniques used to count the number of microorganisms in a sample.
- To be able to differentiate between different enumeration techniques and learn when each should be used.
- To have more practice in serial dilutions and calculations.

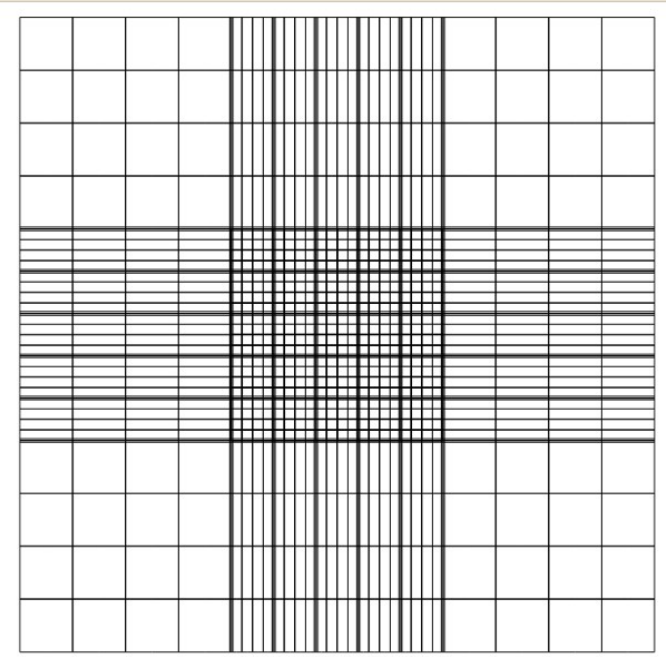

Methods
Direct Count
Indirect Count
Direct M of Microbial Biomass
Indirect M of Microbial Biomass
Counting Chamber
Fluorescent Dyes
Viable Count (CFU)
weighing whole cells
measured turbidity

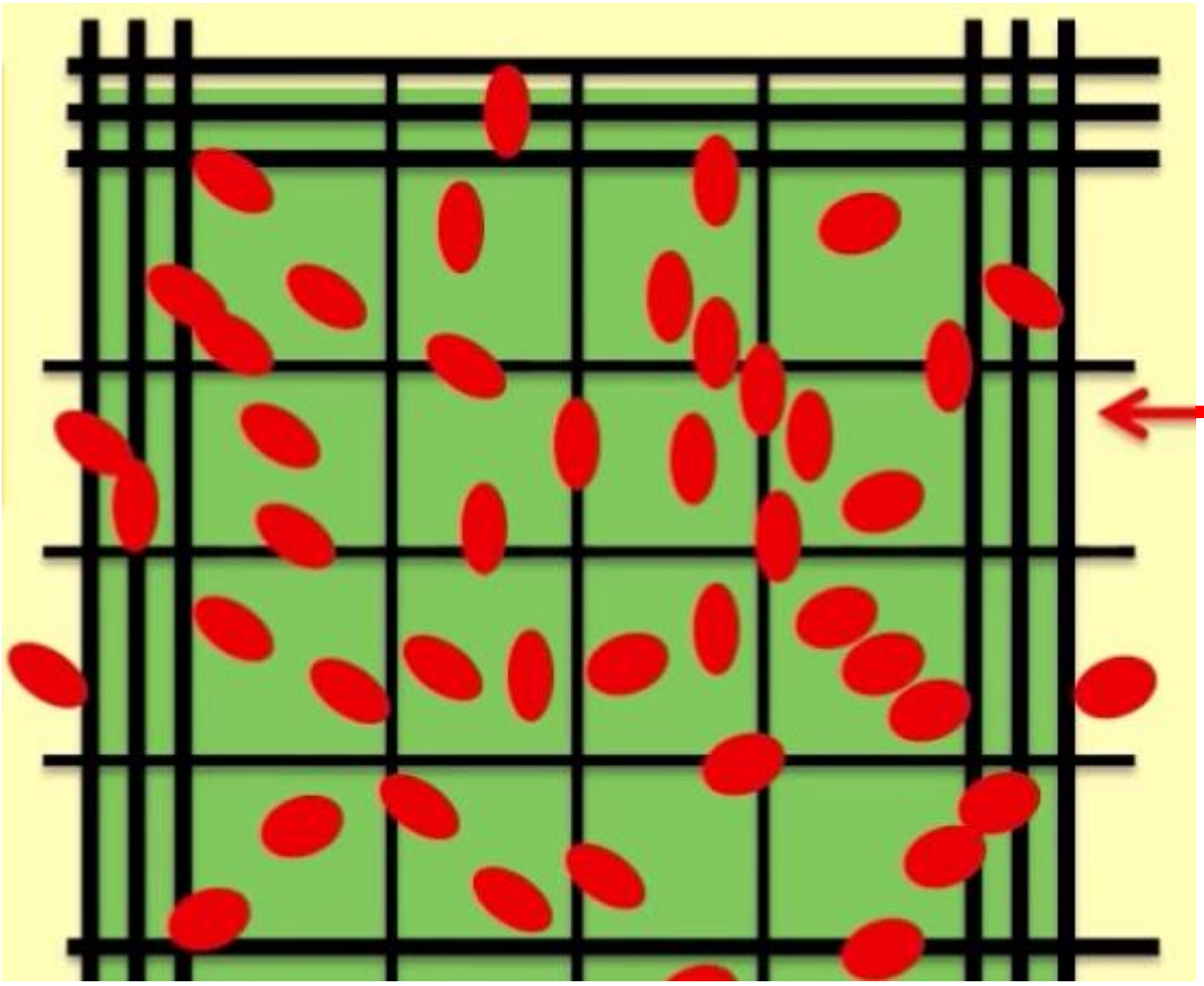
Methods

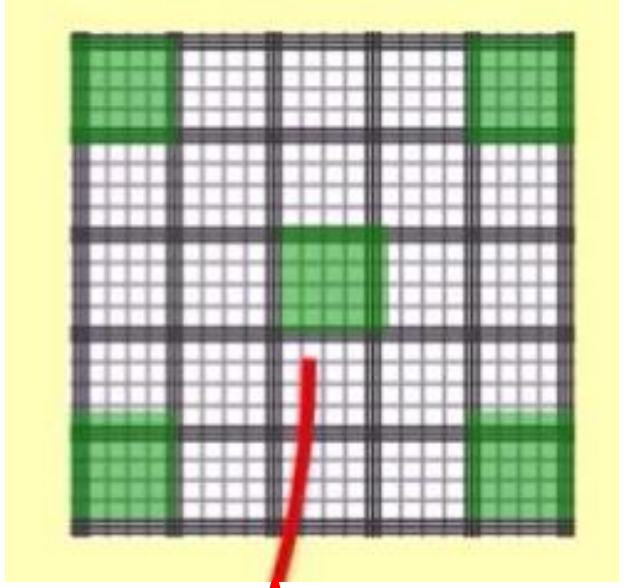
- **1. Direct Count of Cells:** Cells are counted directly under the microscope or by an electronic particle counter.

- **Direct Count Using a Counting Chamber**

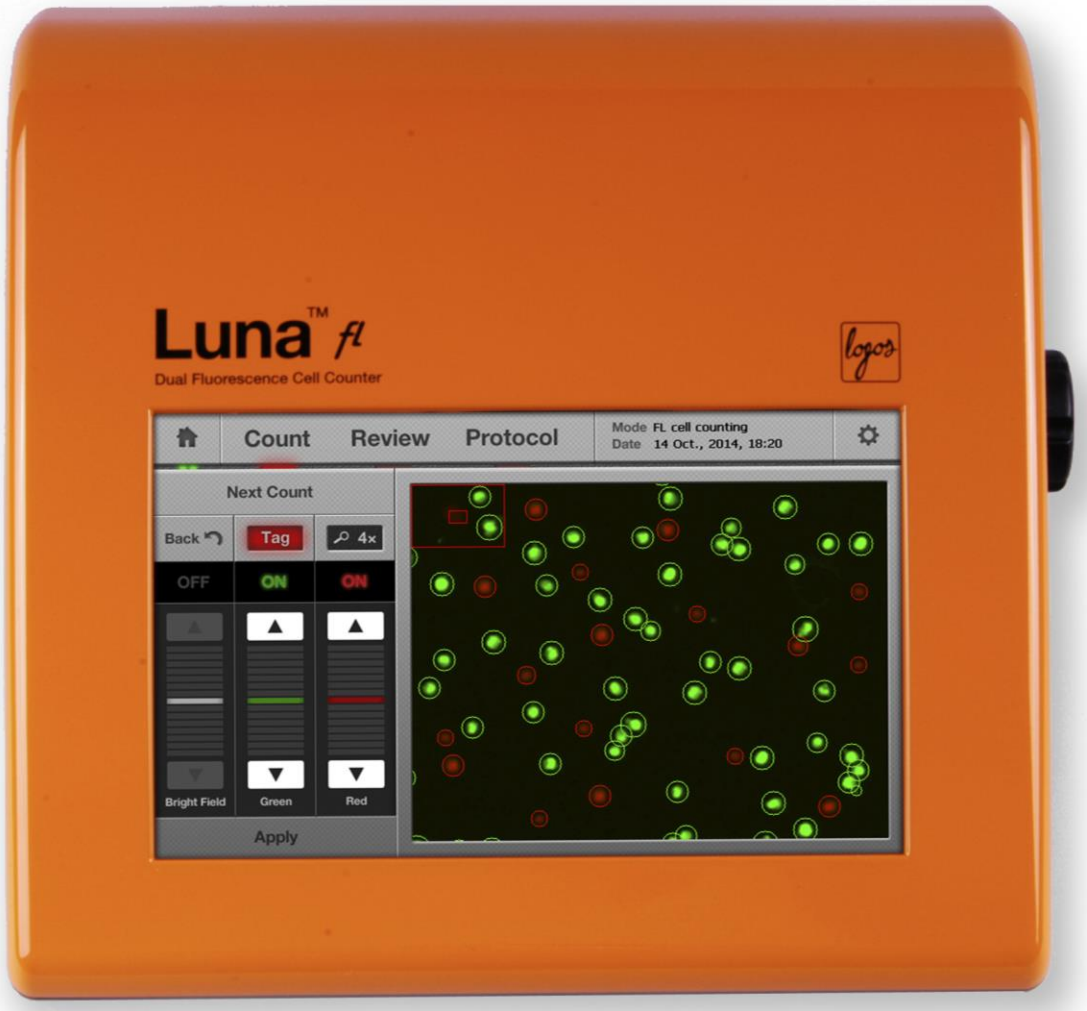
  Direct microscopic counts are performed by spreading a measured volume of sample over a known area of a slide, counting representative microscopic fields, and relating the averages back to the appropriate volume-area factors.







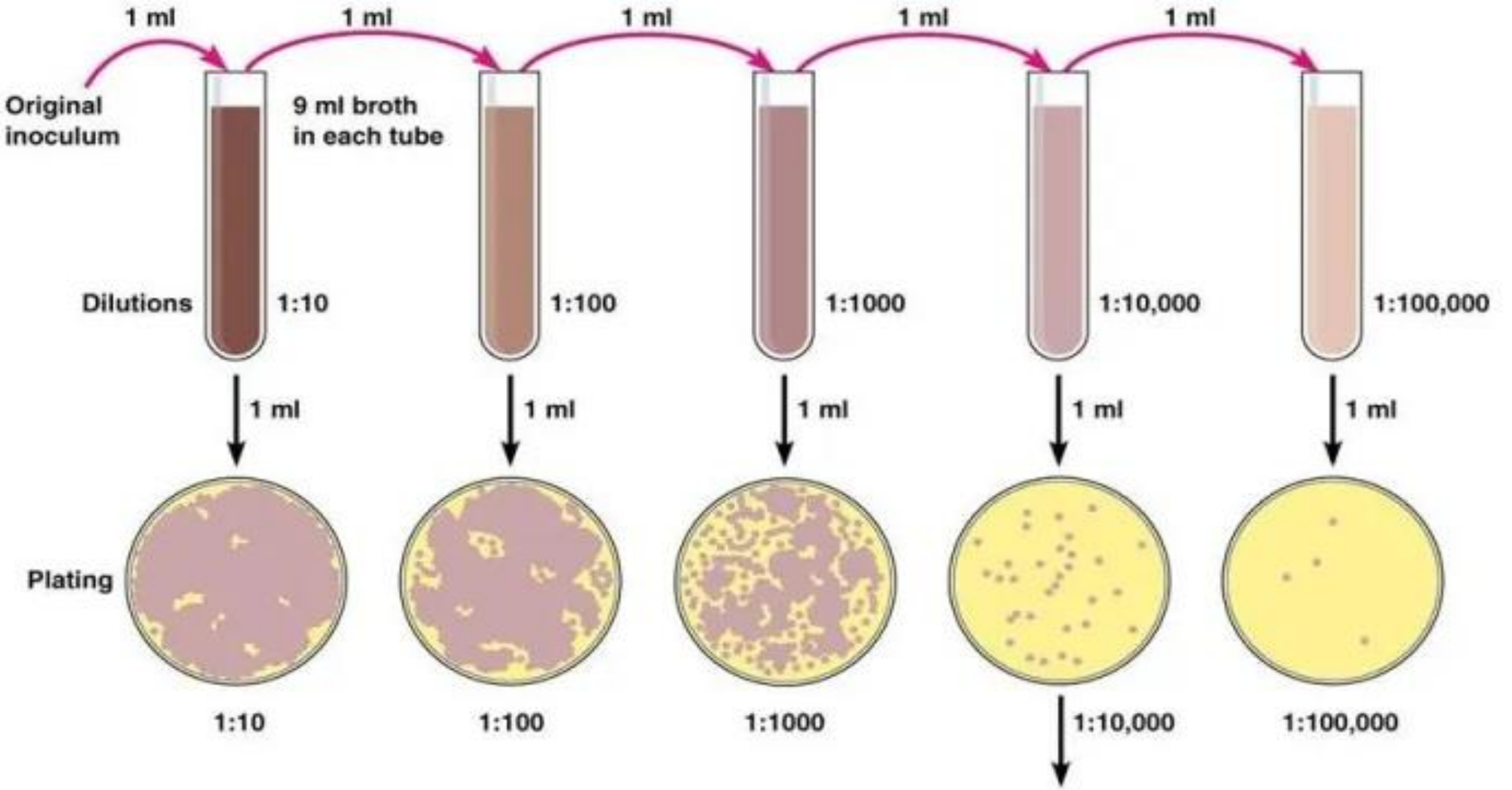
- **Direct Count Using Fluorescent Dyes**
- The most widely used fluorescent dye for counting the number of bacterial cells is **acridine orange** which stains both living and dead cells by interacting with DNA and protein components of cells. The stained cells fluoresce orange when excited near ultraviolet light.
- Trypan blue were used to counting viable cells by staining dead cells only.



- **2. Indirect Count of Cells:** Microorganisms in a sample are diluted or concentrated and grown on a suitable medium, the growth development is then used to estimate the numbers of microorganisms in the original sample.

- **Viable Count (**viable plate count) serial dilutions of a sample containing viable microorganisms are plated onto a suitable growth medium.

- Samples are serially diluted to the point of extinction, that is, to a point where there are no more viable microorganisms.



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

• 3. Direct Measurement of Microbial Biomass

- Cell mass is determined directly by weighing whole cells; biomass can be correlated with cell numbers by reference to a standard curve. Wet weight or dry weight of bacteria may be used for estimation of cell numbers.

• 4. Indirect Measurement of Microbial Biomass

- Microbial biomass is estimated by **measuring relatively constant biochemical components of microbial cells**, such as protein, ATP, lipopolysaccharides and peptidoglycan.
- biomass can also be indirectly estimated by **measured turbidity** that can then be correlated with cell numbers by reference to a standard curve.
- **Spectrophotometer**: OD e.g. OD_{600}
- **McFarland Standards**

McFarland Standard	1% BaCl2(ml)	1% H2SO4 (ml)	Approximate Cell Count Density (x10^8 cells)
0.5	0.05	9.95	1.5 x 10^8
1.0	0.1	9.9	3.0 x 10^8
2.0	0.2	9.8	6.0 x 10^8
3.0	0.3	9.7	9.0 x 10^8
4.0	0.4	9.6	12.0 x 10^8

Conclusion

- Bacteria enumeration is the process of determining the number of bacterial cells in a given sample.
- Many methods have been developed to count the numbers of bacteria in labs, but the most frequently used ones are standard plate count, turbidimetric method, and direct microscopic count.
- The techniques are essential in food and beverage industries, where counting the numbers of bacteria in the given food or beverage sample is essential to learn if they are safe to consume and are not contaminated.
- Despite making the enumeration process easier and smoother, the available techniques have several limitations.