Practical Microbiology

Cihan University
Medical Laboratory Analysis

Lab 9: Microbial counting methods (viable, direct & spectrophotometric)

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MSc: Microbiology

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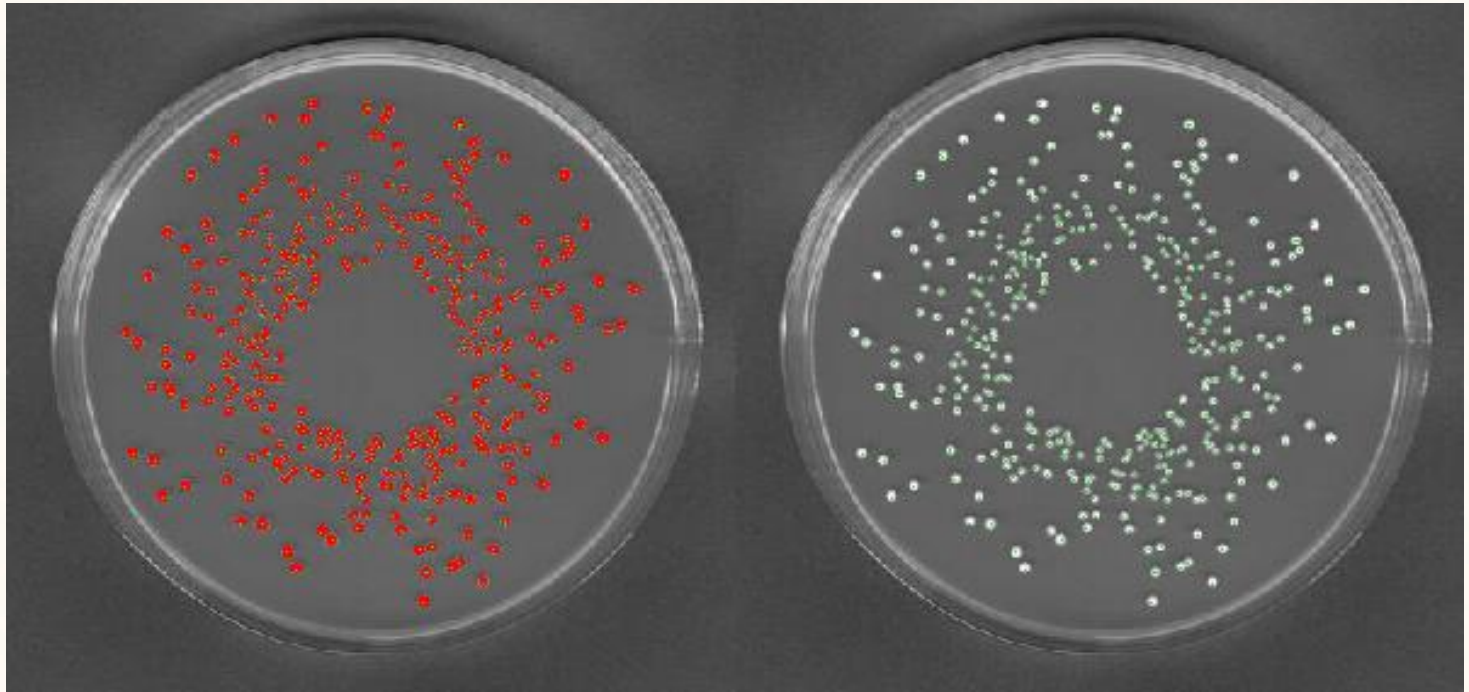
Objective

Each student should be able to:

- ✓ Count the bacterial cells in different samples.
- ✓ Know the methods in bacterial counting:
- ✓ Direct microscopic count method.
- ✓ Standard plate count method.
- ✓ Spectrophotometric method.

Bacterial counting

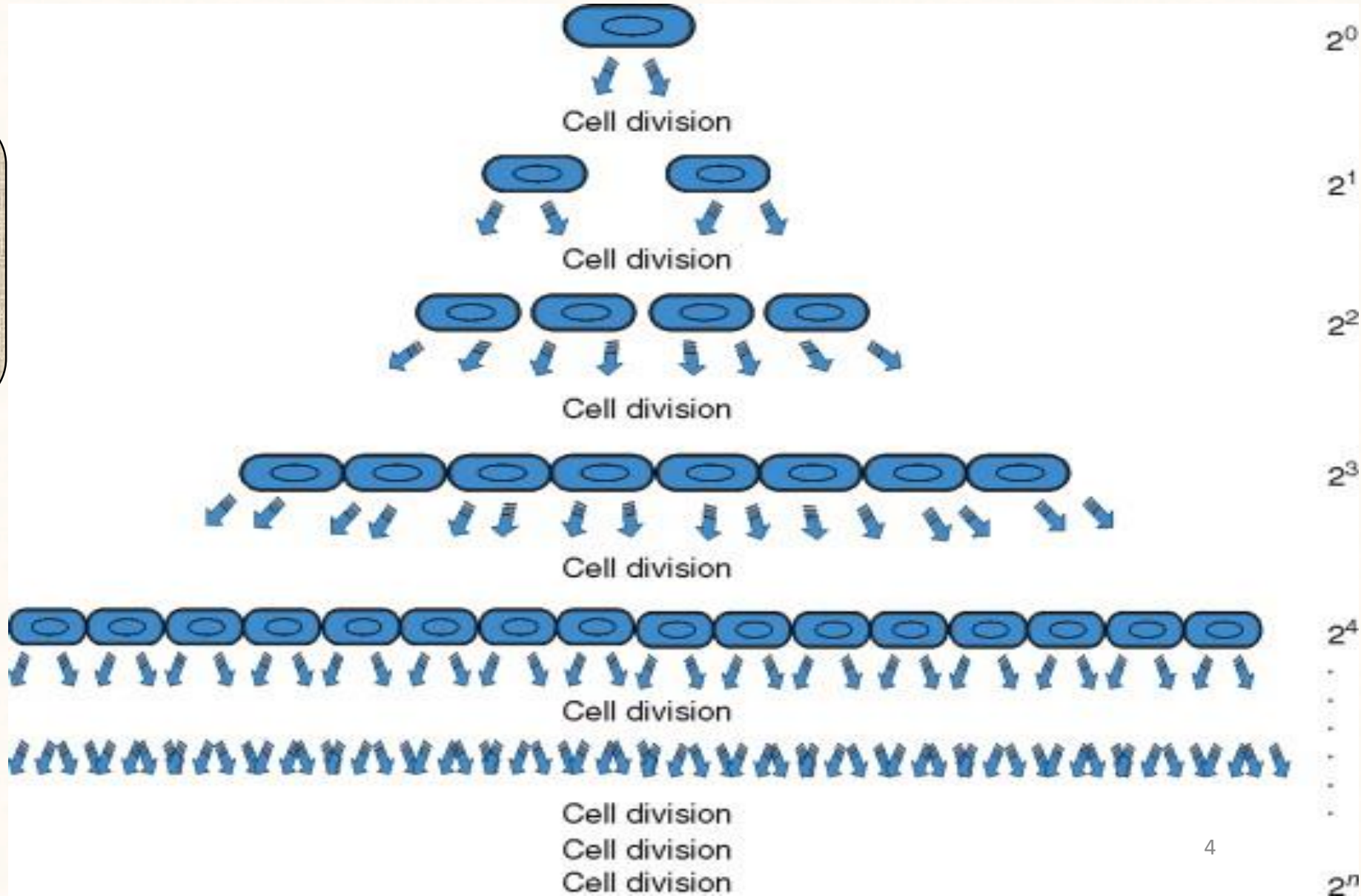
- Is the enumeration of bacteria especially in the Environmental samples, clinical samples, dairy microbiology, food microbiology, and water microbiology.
- Bacterial growth may be determined based on **increase in the number of cells**.
- Enumeration methods may yield either **total counts or viable counts.**
- **Total count** is a count of cells including **dead and live** cells.
- **Viable count** is a count of only those cells that are alive in the sample.



Bacterial counting

- The time it takes for a population of bacterial cells to double in number is called the **generation time.**

Generation time differs significantly from one bacterium to another.



Bacterial counting

➢Different samples could be used in bacterial counting:

- ✓Clinical samples or body fluids (urine, CSF).
- ✓Broth culture of bacteria.
- ✓Environmental samples (soil).
- ✓Water (sewage, tap water, drinking water).
- ✓Foods (vegetables, canned food).
- ✓Drinkable items (fruit juice, soft drink, milk, etc…).

Methods in bacterial counting

Direct methods

- ✓ Direct Microscopic Count (Petroff-Hausser chamber).
- ✓ Standard plate count (viable count).
- ✓ Most probable number (MPN).
- ✓ Breed count method.
- ✓ Direct epiflourocent filter technique.
- ✓ Membrane filter technique.
- ✓ Flow cytometry.

Indirect methods

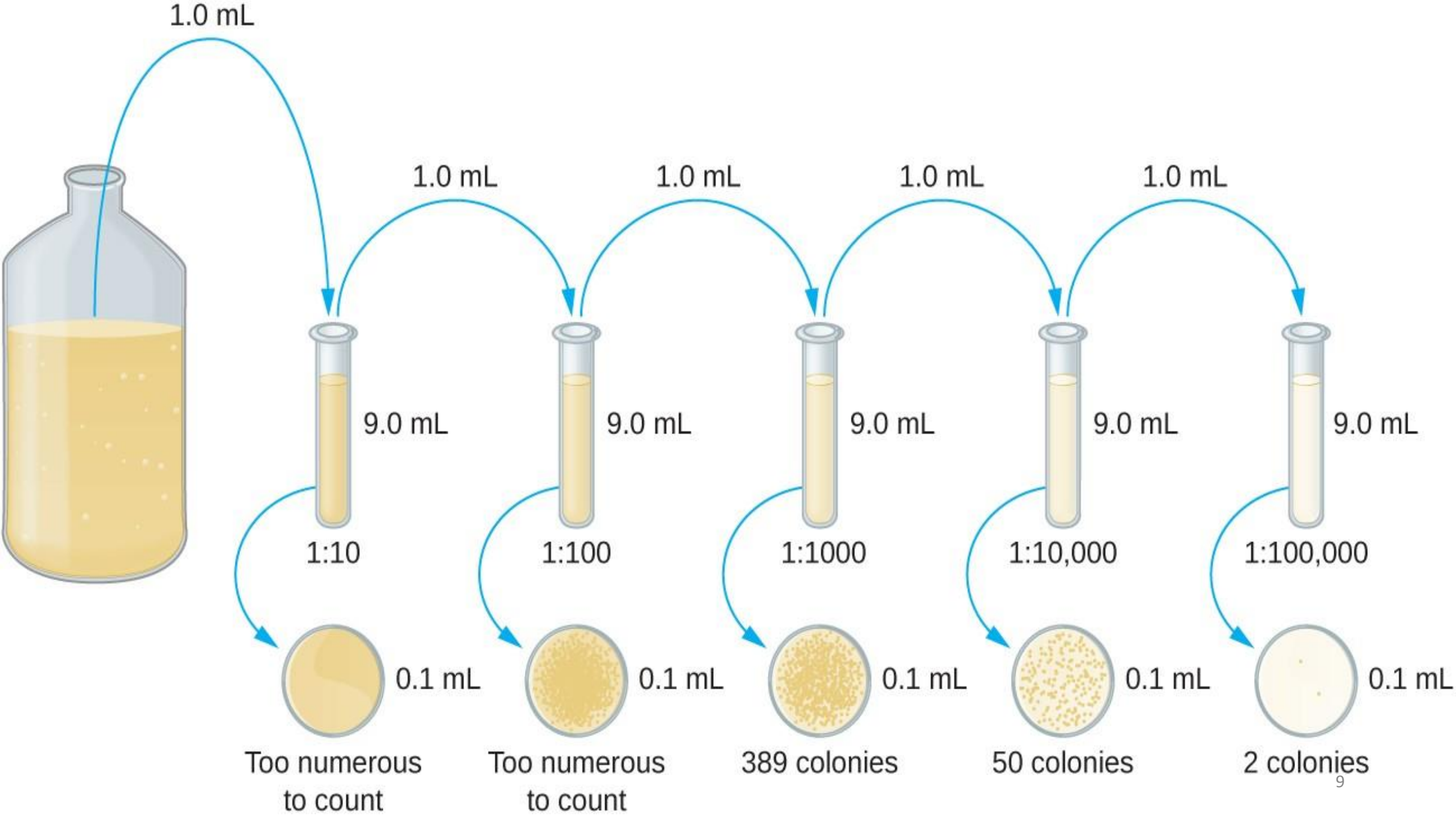
- ✓ Spectrophotometric method.
- ✓ Dry weight.
- ✓ ATP bioluminescence.
- ✓ Electrical impedance.

Standard plate count (viable count)

- A **viable** cell is defined as a cell which is able to divide and form a population (or colony).
- The standard plate count method consists of diluting a sample with sterile saline or phosphate buffer diluent until the bacteria are dilute enough to count accurately.
- **Serial dilution:** a set of dilutions in a mathematical sequence, as to obtain a culture plate with a countable number of separate colonies.
- The final plates in the series should have between 30 and 300 colonies.

Procedure

- Prepare eight test tubes each must contain 9ml of sterile normal saline.
- Transfer one ml of the original sample to the first tube by sterile pipette to form dilution number (1:10) or 10^{-1} (this is tenfold dilution).
- Transfer 1ml from 10^{-1} tube to second tube to form 10^{-2} dilution number, repeat the same step to remaining tubes to form dilutions 10^{-3}, 10^{-4},…..
- Take 0.1 ml from each dilution (shaking by vortex) and spread on the surface of several **Nutrient agar**, invert the plates & incubate at 37C^{o} for 24 or 48 hours.


1.0 mL
1.0 mL
1.0 mL
1.0 mL
1.0 mL
9.0 mL
9.0 mL
9.0 mL
9.0 mL
9.0 mL
1:10
1:100
1:1000
1:10,000
1:100,000
0.1 mL
0.1 mL
0.1 mL
0.1 mL
0.1 mL
Too numerous to count
Too numerous to count
389 colonies
50 colonies
2 colonies

- Fewer than **30** colonies are not acceptable for statistical reasons, and more than **300** colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct **c**olony-**f**orming **u**nits (CFUs).
- **Choosing plates that have colony between 25 and 250 colonies.**
- The number of bacteria in 1 ml of the original sample can be calculated as follows:
  - Total No. of Bacteria (CFU)/ml= Average No. of colonies x1/dilution or (Reciprocal of Dilution).
  - **Suppose that you count 150 colonies in 10^{-5} dilution.**

$$\textbf{CFU/ml} = 150 \times \frac{1}{\text{dilution } (10^{-5})}$$

$$= 150 \times 10^{5}.$$

So CFU/1ml= 150 x 10 x 10^{5}.

Colony counter

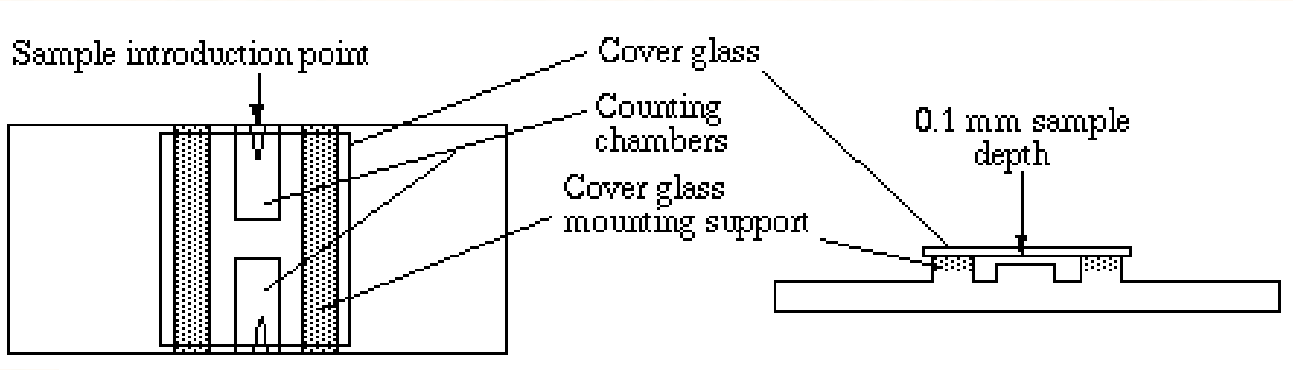
Some tips

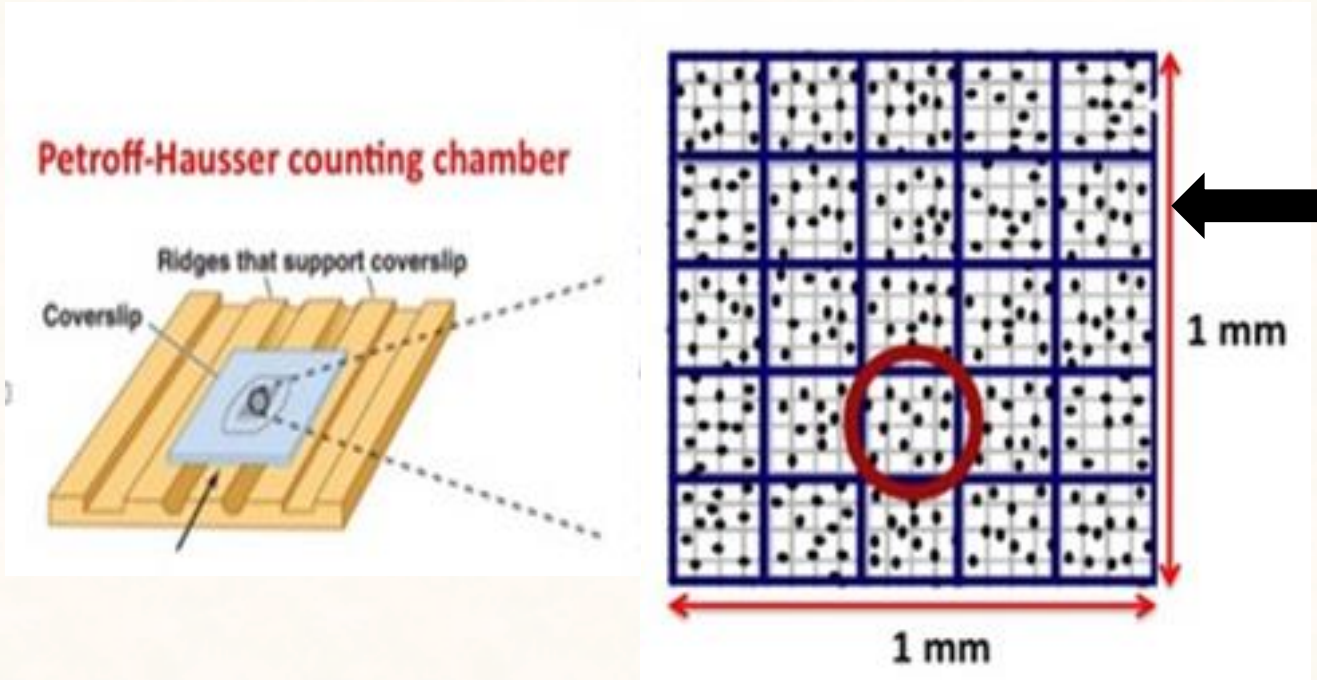
- ✓Some bacteria tend to stick together (two or more bacteria will give rise to one colony).
- ✓If a sample has many different kinds of bacteria, it is not possible to have a medium or conditions that support various necessary growth conditions (blood agar & chocolate agar).
- ✓Only viable organisms are counted.
- ✓Only plates with colony numbers between 30-300 are useable.
- ✓Use aseptic technique.
- ✓Dilutions to be done accurately.

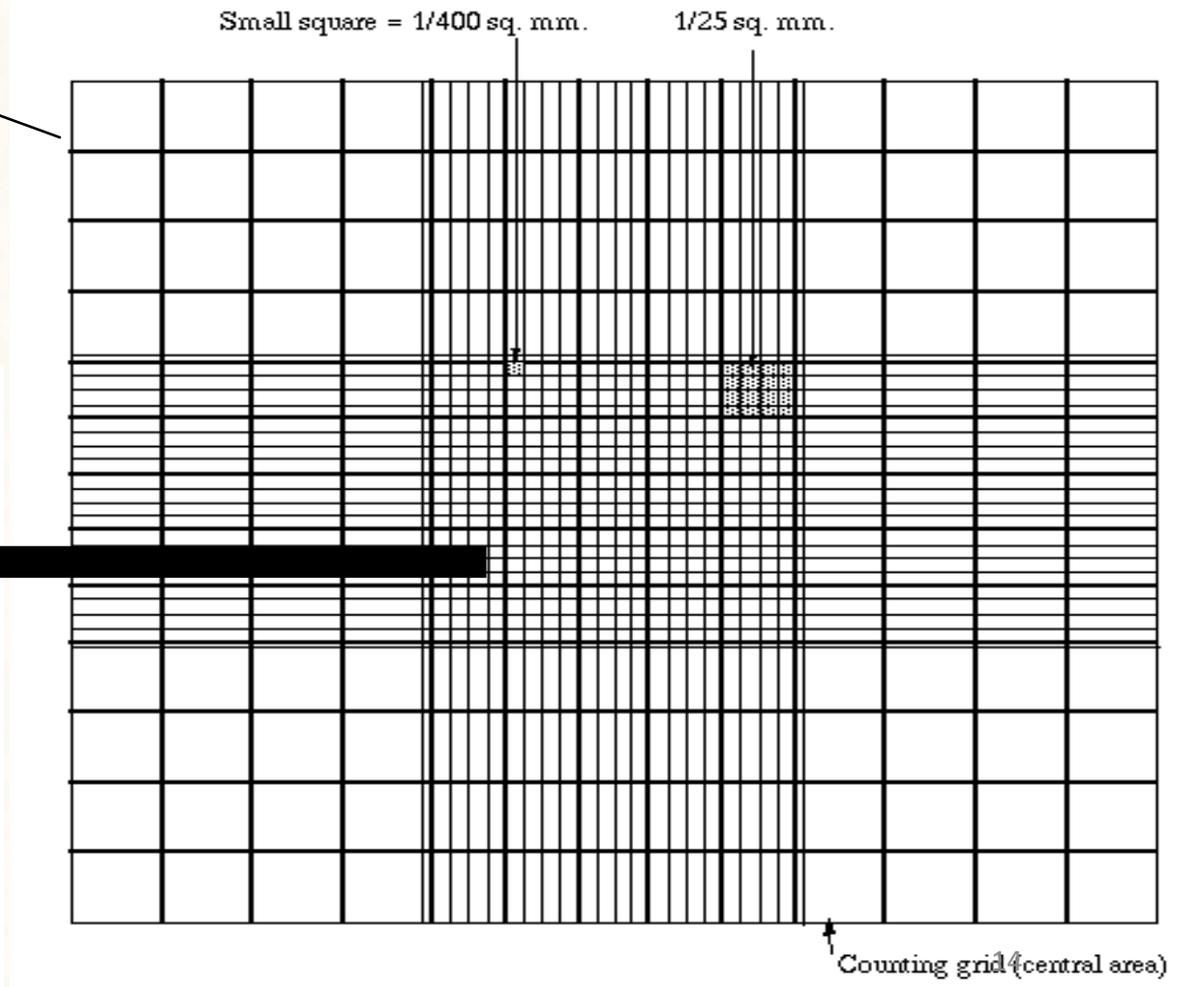
Direct Microscopic Count (Petroff-Hausser Chamber)

- In this method, a suspension of bacteria about 10 ul placed on a slide that has been ruled into squares and is designed to hold a specific volume of liquid.
- Sample could be diluted 1/10.
- By counting the bacteria that appear on the grid areas, the number of organisms in the sample can be calculated. It is a much faster test than the plate count.
- The drawback of this method is viable and nonviable organisms appear the same under a microscope.

- Chamber composed 9 big squares.
- Central big square used for bacterial counting which composed of 25 small squares each with 16 smaller ones.
- One big square is 1mm length, 1mm width.
- Space between the chamber to cover slide is 0.1mm.







Cells calculation per 1 ml

- Suppose we count **150** cells in **5** small squares.
- Area of 1 large square= length × width= 1mm × 1mm= $1mm^2$
- 1 big square composed of 25 small squares, so area of 1 small= 1/25= $0.04mm^2$.
- Volume of one small square= $0.04mm^2 \times 0.1mm$= **0.004**mm^3.
- No of cells/ $1mm^3$= total counted cells/ no. of squares× volume of 1 small square.
- No. of cells/$1mm^3$= **150**/**5**× **0.004**= 150/0.02= 7500 cells.
- To find the cells in $1cm^3$ ⟹ $1cm^3$= 1000 mm^3 so $7500 \times 1000 = 75 \times 10^5$ in $1cm^3$
- So $1cm^3$=1ml
- No. of cells per 1ml= 75×10^5/ml.

Spectrophotometric (turbidity) method

- Is an instrument which measures an amount of light (photon) that a sample absorbs when it passes through the sample. To know the concentration of any substance.

✓**Visible spectrophotometer: (400-700 nm).**

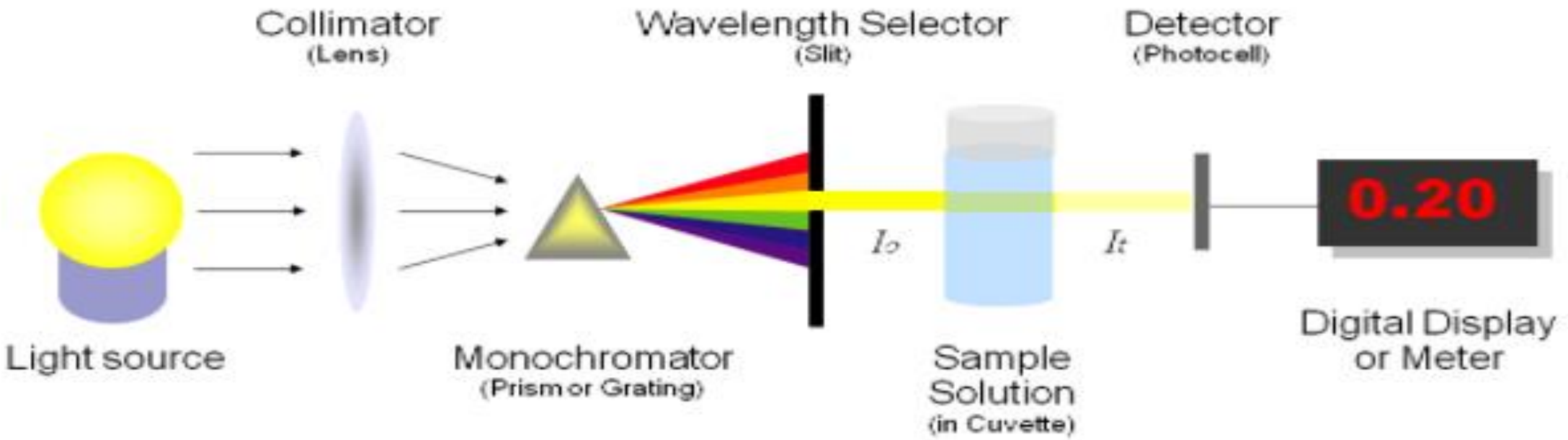
✓**UV-visible spectrophotometer: (185-700 nm).**

✓**IR spectrophotometer: (700 - 15000 nm).**

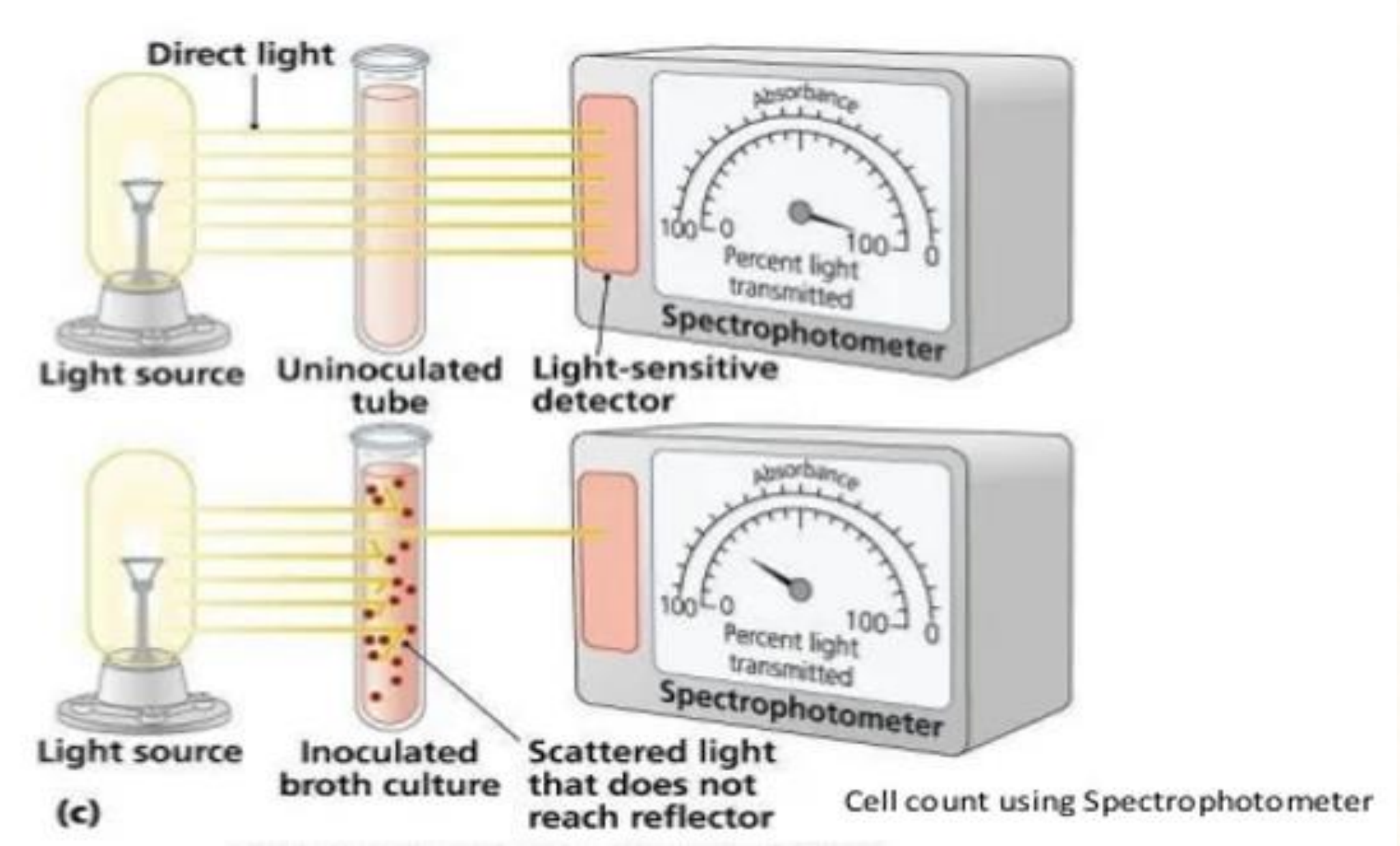
- Used to measure the **turbidity** or **optical density (O.D.)** of bacteria in a broth.
- This must be done with each bacteria because they are **different sizes**.
- sensitivity is limited to about 10^7 cells per ml for most bacteria.
- For instance, an optical density reading of **0.2 OD** for a broth culture of ***E. coli*** is nearly equal to **1 X 10^8 cells/ml.**
- For ***S. aureus*** **0.09 OD** is nearly equal to the same number.

Spectrometer

Photometer



Spectrophotometric (turbidity) method

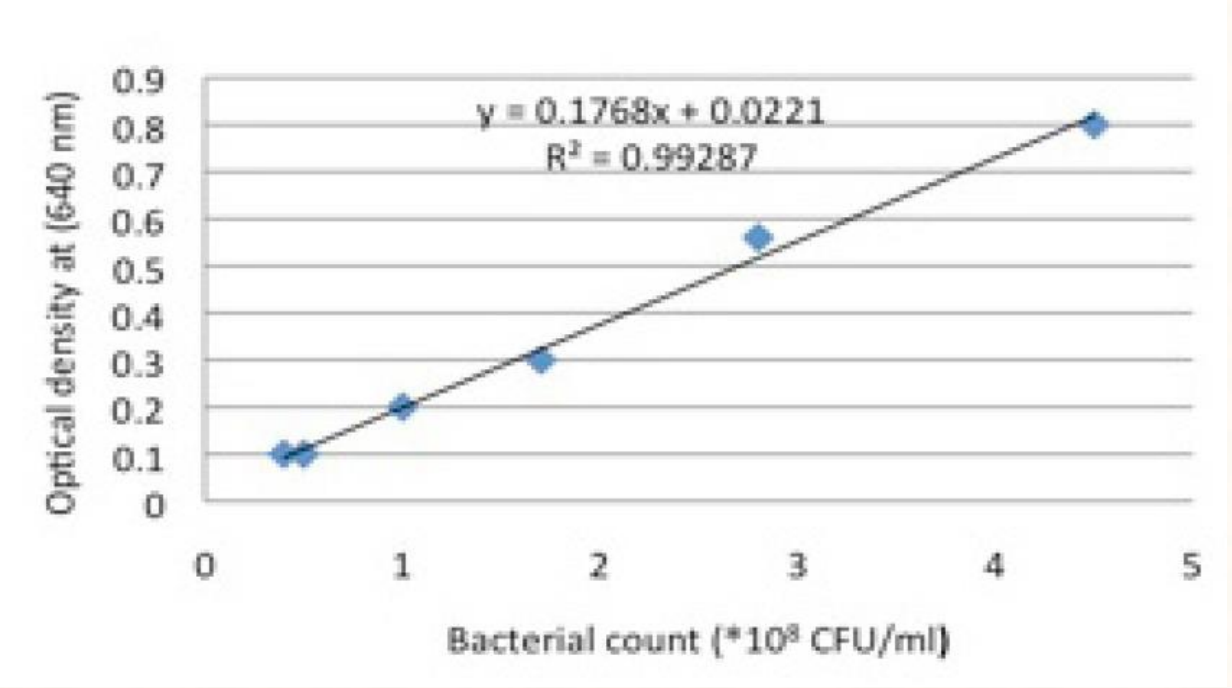


Cell count using Spectrophotometer

Procedure

- ✓Obtain an overnight fresh culture.
- ✓Add 3 ml of an overnight culture to the first polystyrene cuvette.
- ✓Add 3 ml of fresh media (without bacteria) to a second cuvette (used to zero the spectrophotometer).
- ✓Turn it on, choose the wavelength of 600 nm button and enter.
- ✓Place the cuvette with the fresh media into the sample chamber, read and zero it.
- ✓Place your first cuvette with bacterial culture into the sampling chamber and read.
- ✓Record the reading and use standard curve to know bacterial number .

Example, if you prepared a sample and got a spectrophotometer reading of 0.2 OD, you would find 0.2 on the Y-axis and read the bacterial count on the X-axis, in this case is equal to 1 x10^8 cells/ml.



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