The Study of Bacterial Growth

Binary Fission

In binary fission one cell becomes two, the parent cell enlarges, duplicates its chromosome, and then starts to pull its cell envelope together in the center of the cell using a band of protein. The cell wall eventually forms a complete central septum. This process divides the cell into two daughter cells.

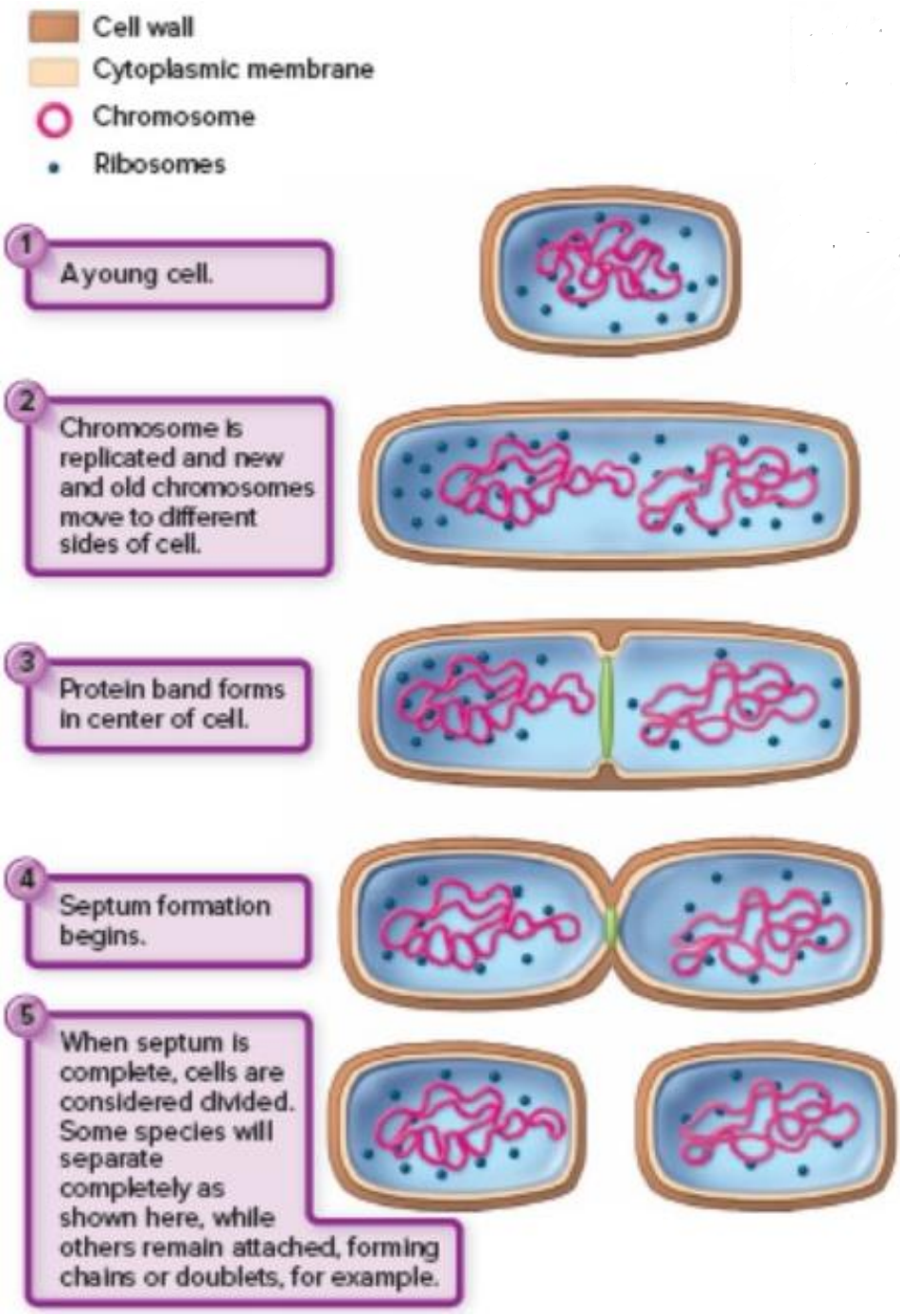

Fig. 1: Steps in binary fission of a rod-shaped bacterium

Cell Generations and Generation Time

- When one cell eventually separates to form two cells, we say that one *generation* has occurred,
- The time required for a complete fission cycle, from one parent cell to two new daughter cells, is called the **generation,** or **doubling, time.**
- In bacteria, each new fission cycle, or generation, increases the population by a factor of 2, or doubles it.
- The length of the generation time is a measure of the **growth rate** of an organism.

The generation time of a given bacterial species is variable and depends on **nutritional** and **genetic factors**, and on **temperature**.

- Under the best nutritional conditions, the generation time of a laboratory culture of *E. coli* is about 20 min. although some bacteria have generation times of days. For example, *Mycobacterium leprae,* the cause of Hansen's disease, has a generation time of 10 to 30 days, as long as that of some animals.
- Environmental bacteria commonly have generation times measured in months.
- Most pathogens have relatively short doubling times. ***Salmonella enteritidis*** and ***Staphylococcus aureus***, bacteria that cause foodborne illness, double in 20 to 30 minutes, which is why leaving food at room temperature even for a short period has caused many cases of foodborne disease.

In nature, microbial cells probably grow much slower than their maximum rates observed in the laboratory. Why?

This is because the conditions and resources necessary for optimal growth in the laboratory are often not present in a natural habitat, and unlike growth in laboratory pure cultures, microbes in nature coexist with other

microbes in microbial communities and must compete with their neighbors for resources and space.

Figure 2 shows several quantitative characteristics of growth: The cell population size can be represented by the number 2 with an exponent **(2^1, 2^2, 2^3, 2^4)**; the exponent increases by one in each generation; and the number of the exponent is also the number of the generation. This growth pattern is termed **exponential.** Because these populations often contain very large numbers of cells, it is useful to express them by means of exponents or logarithms.

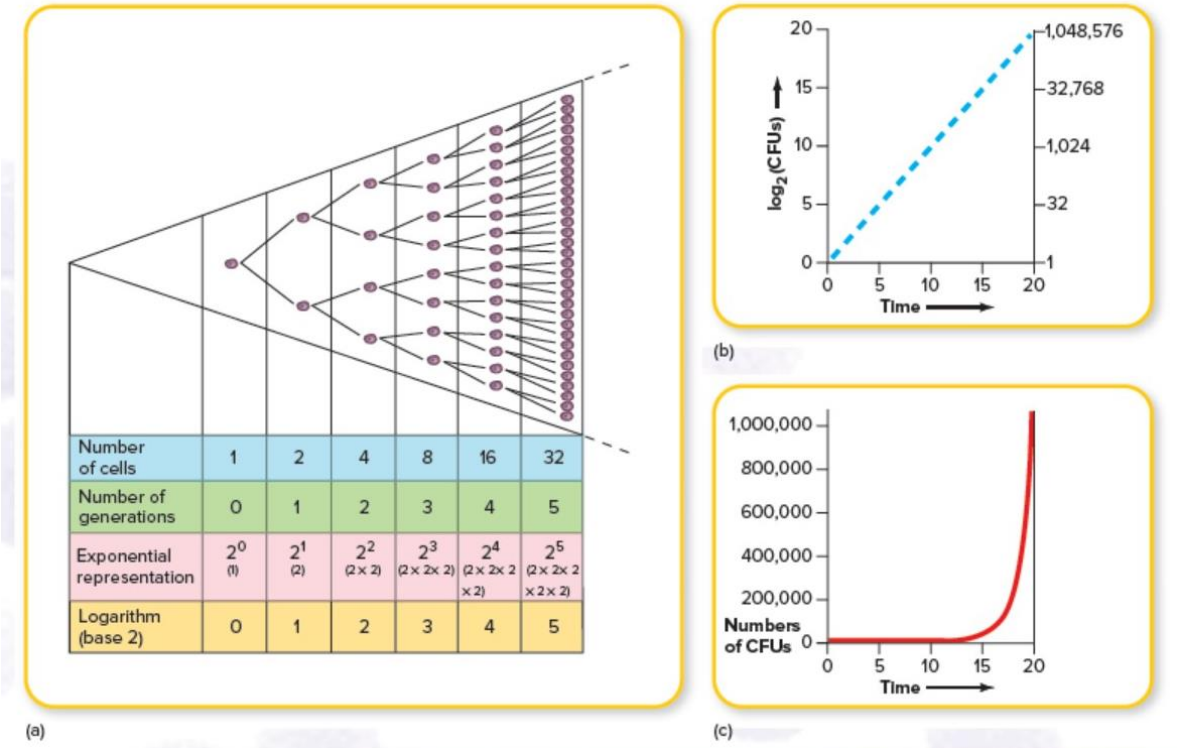

Fig.2: The mathematics of population growth.

(a) Starting with a single cell, if each product of reproduction goes on to divide by binary fission, the population doubles with each new cell division or generation. This process can be represented by logarithms using exponents or by simple numbers. (b) Plotting the logarithm of the cells produces a straight-

line indicative of exponential growth, whereas (c) plotting the cell numbers arithmetically gives a curved slope.

An easier way to calculate the size of a population over successive generations is to use this equation**:** **$Nt = (N)2^n$**

Nt is the total number of cells in the population (final number).

N is the starting number,

the exponent **n** denotes the generation number, and 2^n represents the number of cells in that generation.

Q/ Calculate the no. of cells **(Nt)** of *Staphylococcus aureus* will be present in an egg salad sandwich after it sits in a warm car for 4 hours. We will assume that N is 10 (number of cells deposited in the sandwich while it was being prepared).

Phases of Growth

When a few bacteria are inoculated into a liquid growth medium and the population is counted at intervals, it is possible to plot a **bacterial growth curve** that shows the growth of cells over time (**Figure 3**). There are four basic phases of growth: the lag, log, stationary, and death phases.

The Lag Phase

This period of little or no cell division is called the **lag phase,** and it can last for 1 hour or several days. The microbial population is undergoing a period of intense metabolic activity involving, synthesis of enzymes and various molecules.

The Log Phase (exponential growth phase)

The cells begin to divide and enter a period of growth, or logarithmic increase**.** Cells reproduce most actively, because the generation time is constant, a logarithmic plot of growth during the log phase is a straight line and the cells are most active metabolically.

The Stationary Phase

In this phase the growth rate slows, the number of microbial deaths balances the number of new cells, and the population stabilizes. The depletion of nutrients, accumulation of waste products, and harmful changes in pH may all play a role.

The Death Phase (logarithmic decline phase)

The number of deaths eventually exceeds the number of new cells formed, this phase continues until the population is diminished to a tiny fraction of the number of cells in the previous phase or until the population dies out entirely. The speed with which death occurs depends on the relative resistance of the species and how toxic the conditions are.

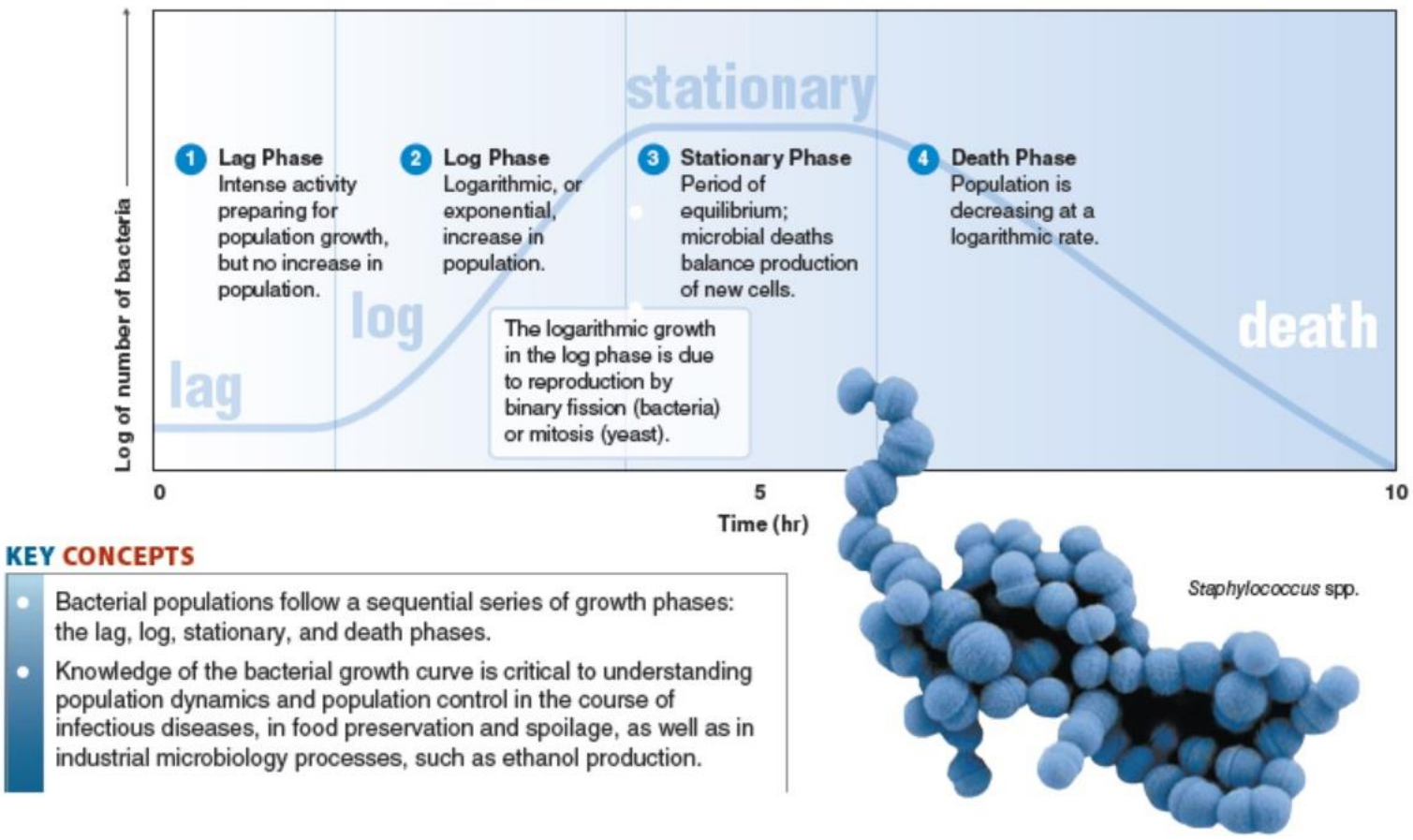

Fig 3: Bacterial growth curve

Direct and indirect Measurement of Microbial Growth

The growth of microbial populations can be measured in a number of ways. Some methods measure cell numbers; other methods measure the population's total mass, which is often directly proportional to cell numbers. Population numbers are usually recorded as the number of cells in a milliliter of liquid or in a gram of solid material.

Indirect methods for bacterial counting

Viable Plate Counts The most frequently used method of measuring bacterial populations**. Advantage of this method:** It measures the number of viable cells. **One disadvantage**: may be that it takes some time, usually 24 hours or more, for visible colonies to form. This can be a serious problem in some applications, such as quality control of milk, when it is not possible to hold a particular lot for this length of time.

Plate counts assume that each live bacterium grows and divides to produce a single colony. This is not always true, because bacteria frequently grow linked in chains or as clumps. plate counts are often reported as **colony-forming units (CFU).**

A plate count is done by either the pour plate method or the spread plate method.

The pour plate method:

This technique has some drawbacks

1- Heat-sensitive microorganisms may be damaged by the melted agar and will therefore be unable to form colonies.

2- Colonies that form beneath the surface of a pour plate are not satisfactory for such tests. To avoid these problems,

3- the **spread plate method** is frequently used instead: This method positions all the colonies on the surface and avoids contact between the cells and melted agar.

When a plate count is performed, it is important that only a limited number of colonies develop in the plate. When too many colonies are present, some cells are overcrowded and do not develop; these conditions cause inaccuracies in the count.

The U.S. Food and Drug Administration convention is to count only plates with 25 to 250 colonies, but many microbiologists prefer plates with 30 to 300 colonies. To ensure that some colony counts will be within this range, the original inoculum is diluted several times in a process called **serial dilution**

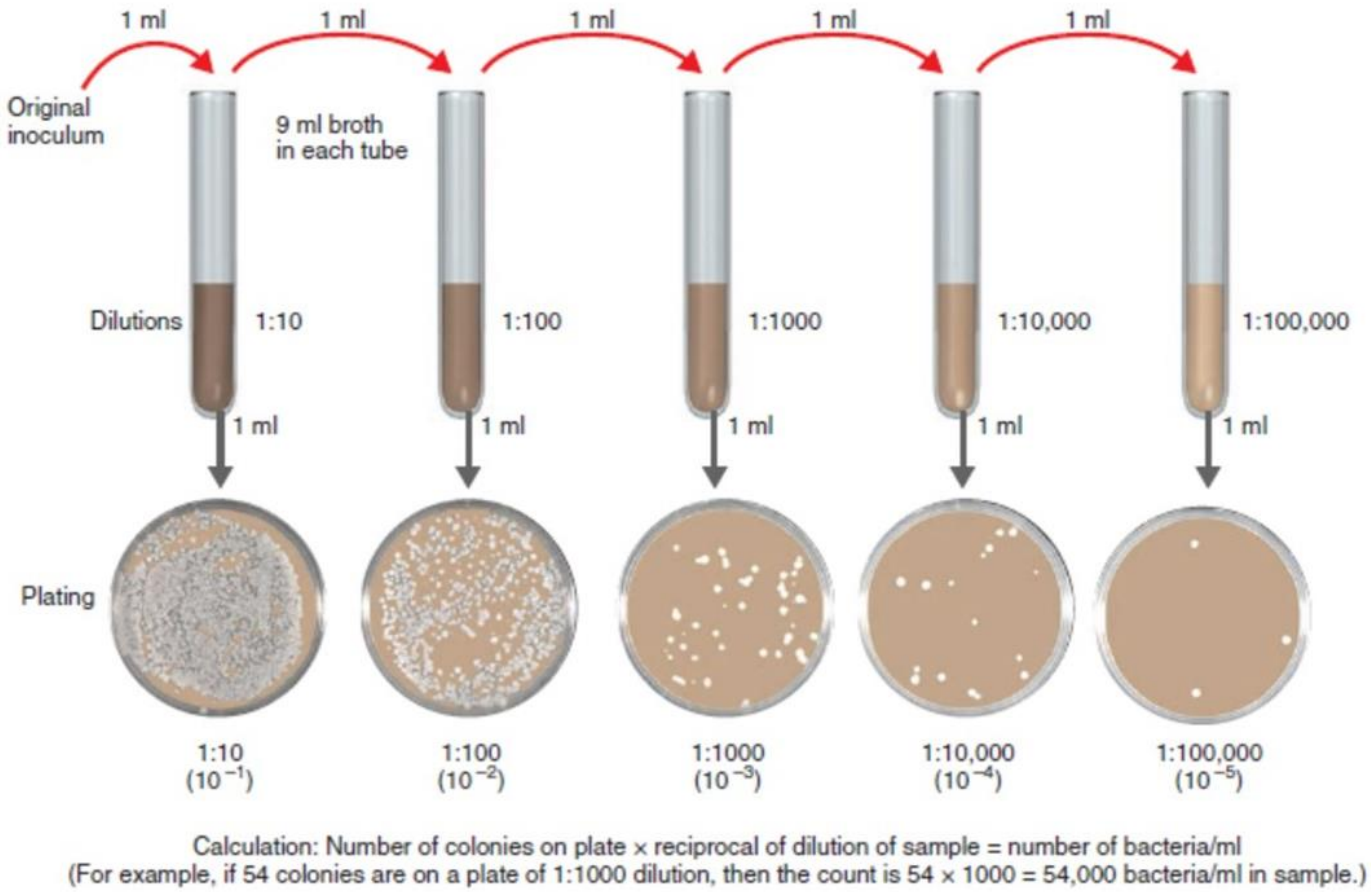

Fig 4: Serial dilution

Filtration

When the quantity of bacteria is very small, as in lakes or relatively pure streams, bacteria can be counted by **filtration** methods. This method is applied frequently to detection and enumeration of coliform bacteria, which are indicators of fecal contamination of food or water.

The Most Probable Number (MPN) Method

Another method for determining the number of bacteria in a sample is the **most probable number (MPN) method,** This statistical estimating technique is based on the fact that the greater the number of bacteria in a sample, the more dilution is needed to reduce the density to the point at which no bacteria are left to grow in the tubes in a dilution series.

The MPN method is useful when

- The microbes being counted will not grow on solid media (such as the chemoautotrophic nitrifying bacteria).
- It is also useful when the growth of bacteria in a liquid differential medium is used to identify the microbes (such as coliform bacteria, which selectively ferment lactose to acid, in water testing).

Metabolic Activity

Another indirect way to estimate bacterial numbers is to measure a population's *metabolic activity.* This method assumes that the amount of a certain metabolic product, such as acid or CO2, is in direct proportion to the number of bacteria present.

Dry Weight

For filamentous bacteria, actinomycetes and molds.

Turbidity

For some types of experimental work, estimating **turbidity** is a practical way of monitoring bacterial growth. As bacteria multiply in a liquid medium, the

medium becomes turbid, or cloudy with cells. The instrument used to measure turbidity is a *spectrophotometer* (or colorimeter).

Direct Measurement of Cell Numbers

Direct Microscopic Count

In the method known as the **direct microscopic count,** a measured volume of a bacterial suspension is placed within a defined area on a microscope slide. Because of time considerations, this method is often used to count the number of bacteria in milk.

A specially designed slide called a *Petroff-Hausser cell counter* is used in direct microscopic counts.

Advantages of this methods

It is easy, inexpensive, and relatively quick. It also gives information about the size and morphology of microorganisms.

Disadvantages

- Motile bacteria are difficult to count by this method,
- dead cells are counted as live ones.
- In addition, high concentration of cells is required to be countable,

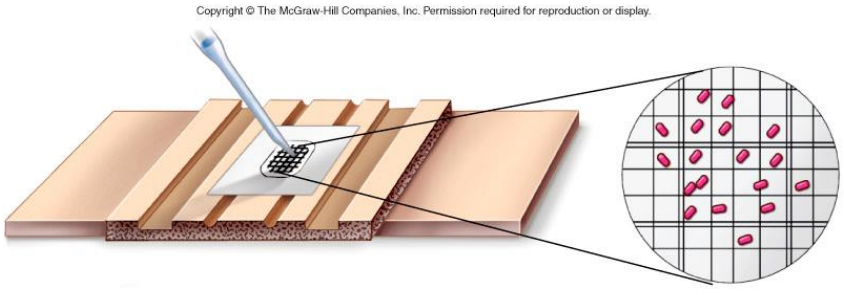

Fig 5: Direct microscopic count of bacteria.

Coulter counter- electronically scans a culture as it passes through a tiny pipette. A Coulter counter uses an electronic sensor to detect and count the

number of cells in a measured volume of liquid. These instruments are used in some research laboratories and hospitals.

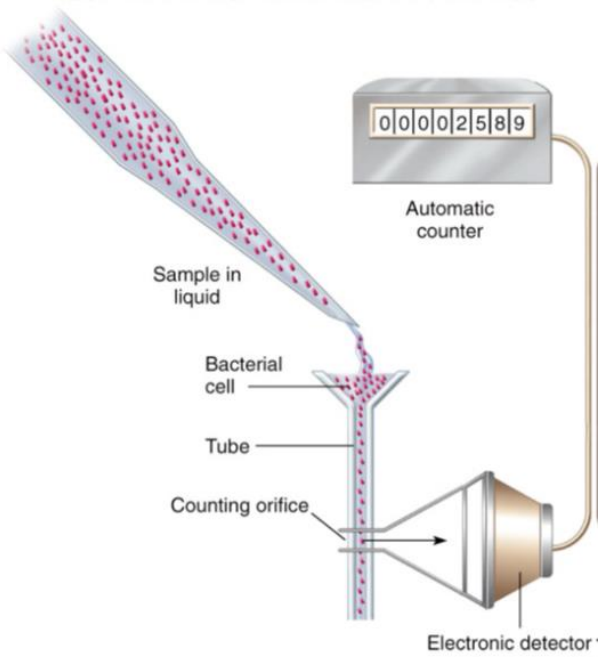

Fig 6: Coulter counter

Flow cytometer using a LASER beam measures cell size and differentiates between live and dead cells. Although flow cytometry can be used to count bacteria in natural samples without the need for culturing them. it requires fluorescent labeling.

Real-time Polymerase Chain Reaction allows to quantify bacteria and other microorganisms that are present in environmental or tissue samples without isolating or culturing them.