Growth of Bacteria & Cultivation

Prof. Dr. Lamyaa Kadhim

Medical Bacteriology

LEARNING OBJECTIVES

1- Bacterial Division/reproduction

2- Stages of Bacterial growth curve

3- Measurement of Microbial growth

4-Environmental microbial growth factors

5-Bacterial culture methods

What do we mean by Bacterial Growth?

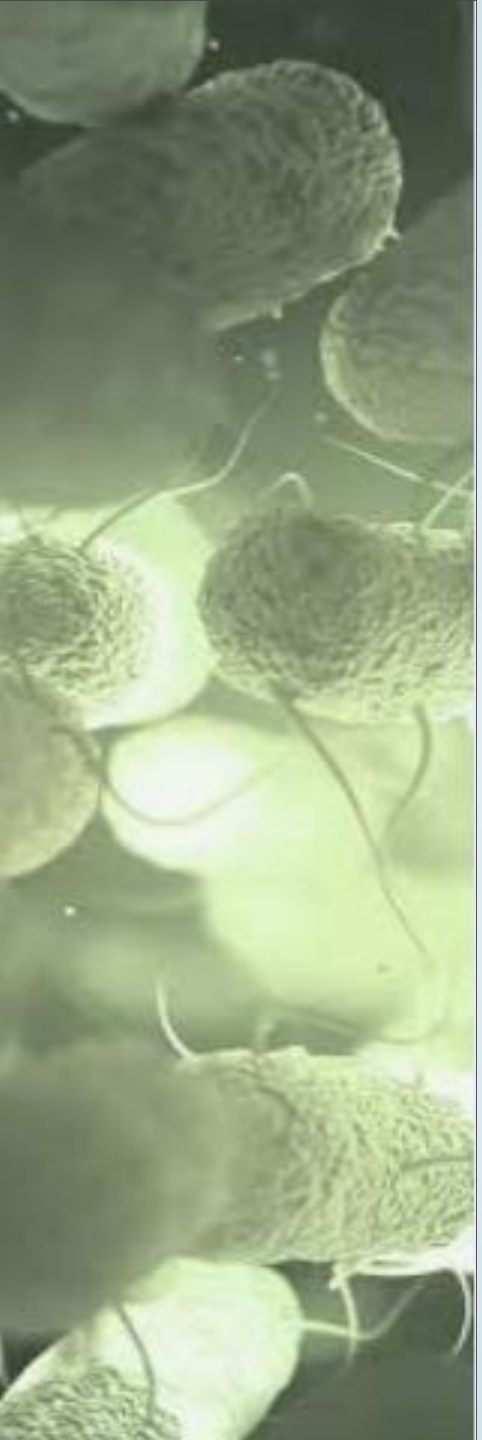
Bacterial growth is an increase in all cell components, which ends in multiplication of the cell leading to an increase in population.

Bacterial Growth and Survival

The survival of any microbial group within an environment depends on 2 factors:

1- The availability of Nutrients in the media.
2- The ability of the microbe to consume these nutrients.

The survival and growth of bacteria is evaluated by studying isolated bacteria under optimal condition in laboratories.



The requirement for bacterial growth

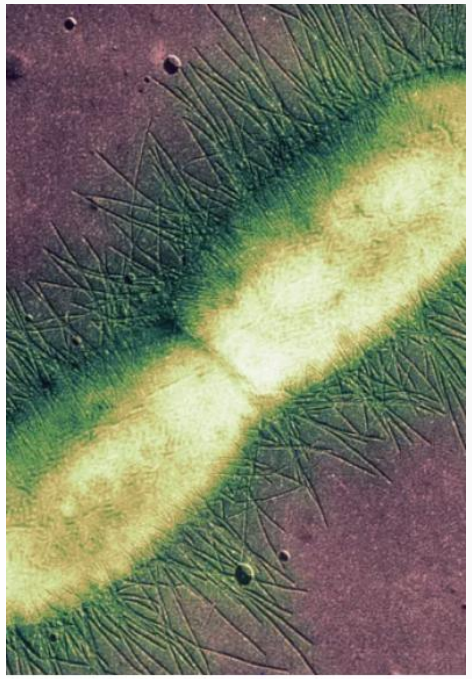
Physical aspects

-Tempersture
-PH
-Osmotic pressure
-Oxgen requirement

Chemical requirements

Carbon, Nitrogen, Sulfur, Phosphorus, Trace elements, and organic growth factors

Bacterial Division



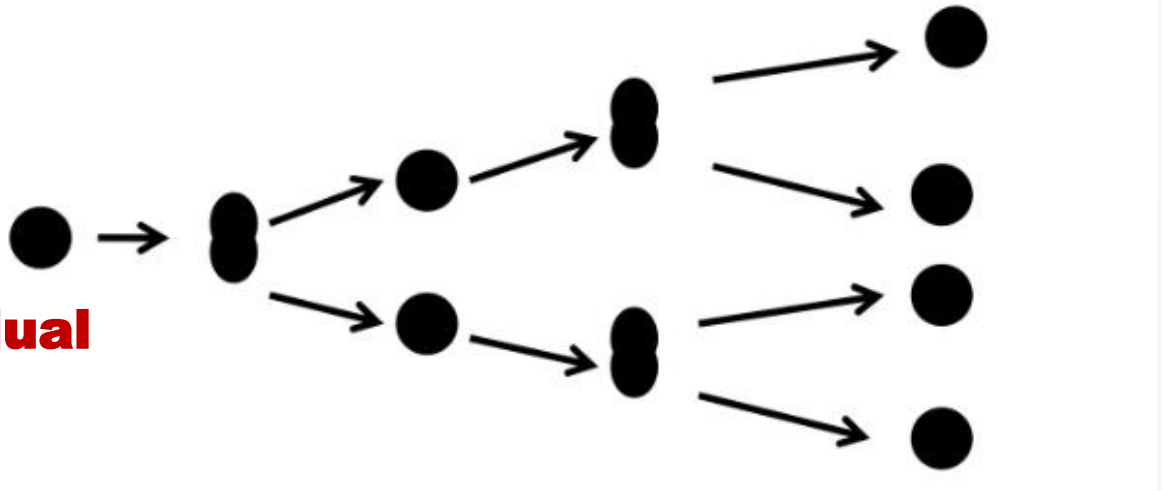
- Micro-organisms multiply by asexual process of cell fission.

- Bacteria divide by binary fission where individual cells enlarge and divide to yield two progeny of approximately equal size.

- **binary fission** involves:

1-an increase in the size of the cell

2-an increase in the number of individual cells



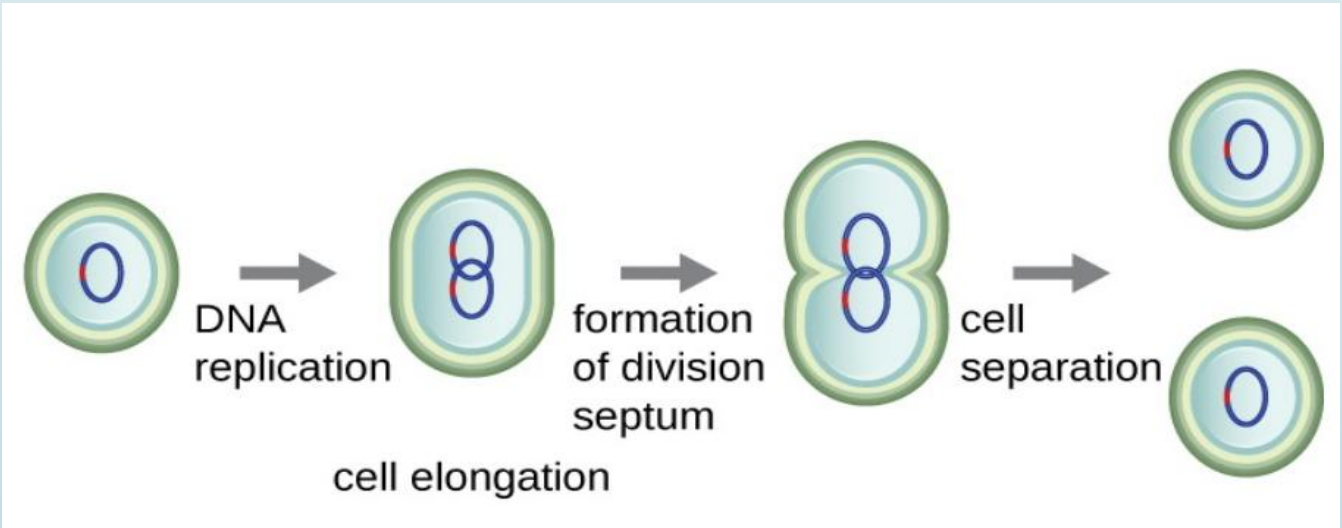
How binary fission occurs?

1 – DNA replication

2 – Elongation of the bacterial cell

3 – Inward growth of the Plasma membrane and cell wall forming a septum between the two copies of DNA.

4 – Complete splitting will result in two bacterial cells



Important Terms

- **Generation Time or Doubling Time:**

The time required for a bacterium to give rise to two daughter cells under optimum conditions, is known as the generation time or doubling time.

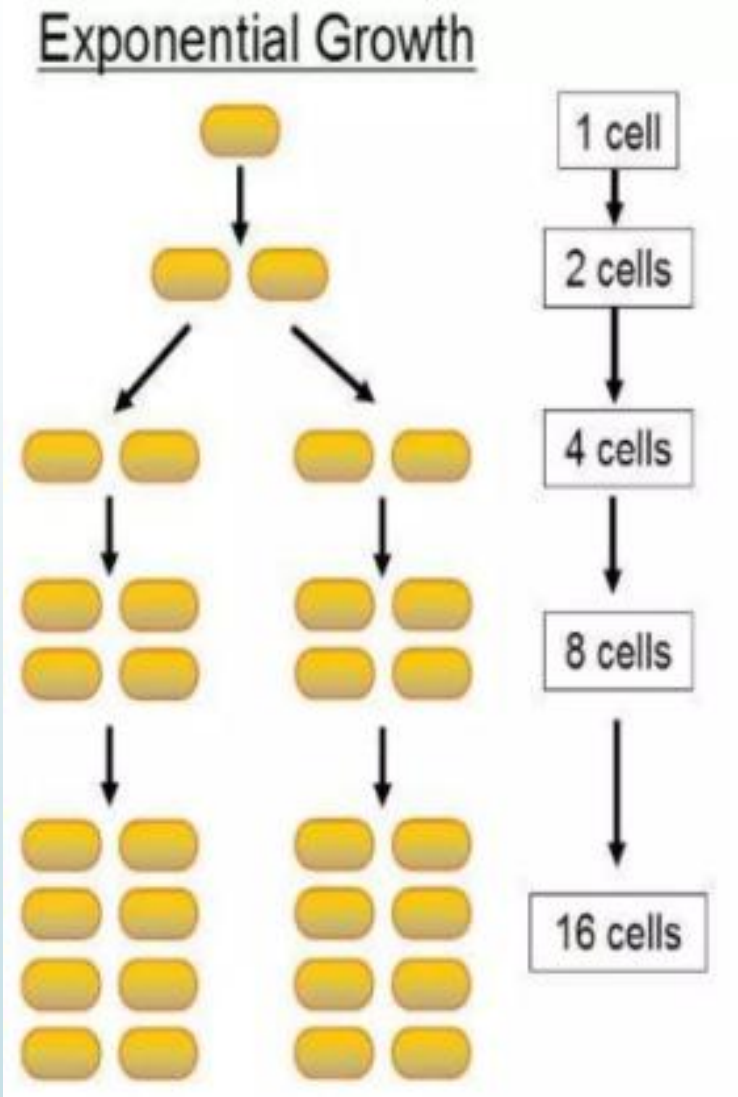
-Doubling time varies from one bacterial species to another and depends on the growth conditions

Bacterial Generation (Doubling) Time

Examples';

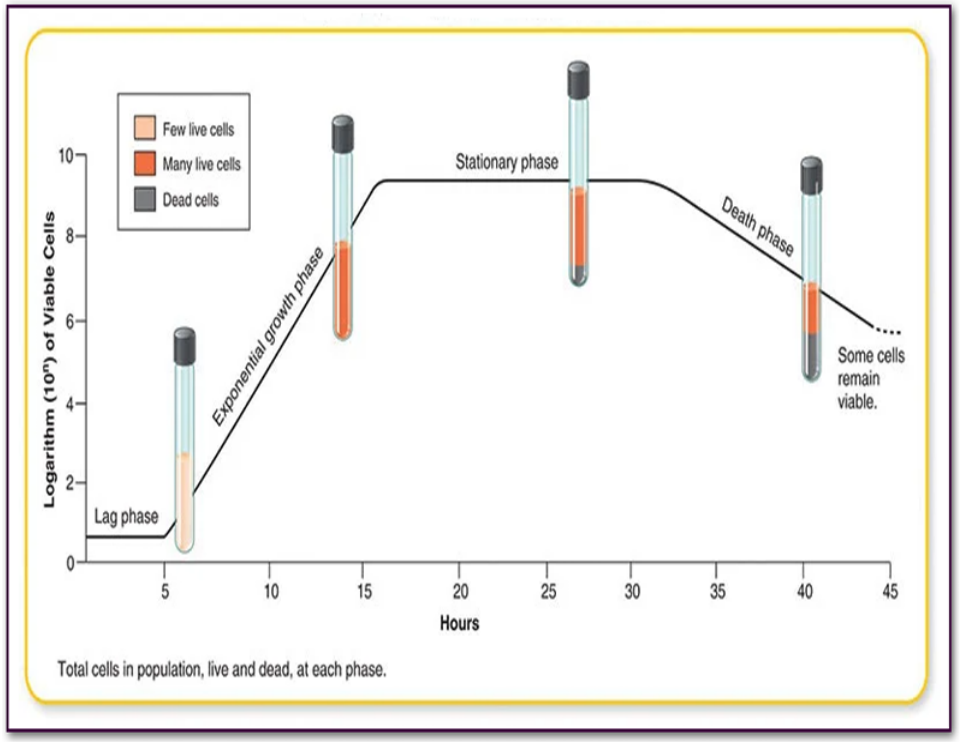
- *Escherichia coli and other medically important bacteria -------20 mins*
- *Staphylococcus aureus----27-30mins*
- *Bacillus subtilis------25-27mins*
- *Mycobacterium tuberculosis-----18 hours*
- *Mycobacterium leprae------14 days*

E. coli (*Escherichia coli*) is a type of bacteria that lives in the digestive tracts of humans and animals. It grows by splitting in half, through a process called binary fission.



Bacterial Growth Curve

When a few bacteria are inoculated into a liquid growth medium, it is possible to plot a bacterial growth curve that describes the density of cell populations in liquid culture over time.



Phases of Bacterial Growth Curve

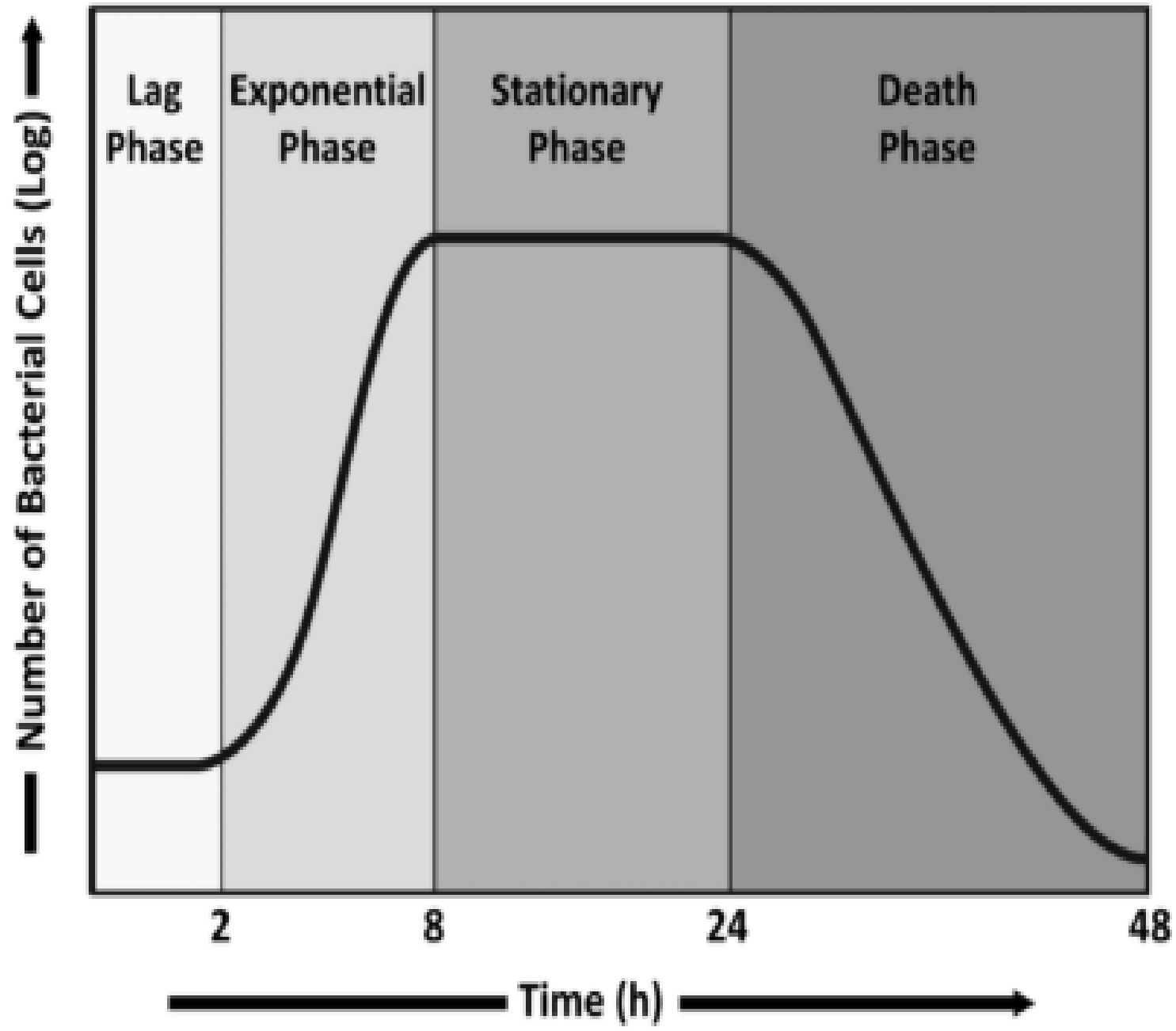
- The bacterial growth curve shows 4 distinct phases:

- **1-Lag phase**

- **2-Log (exponential) phase**

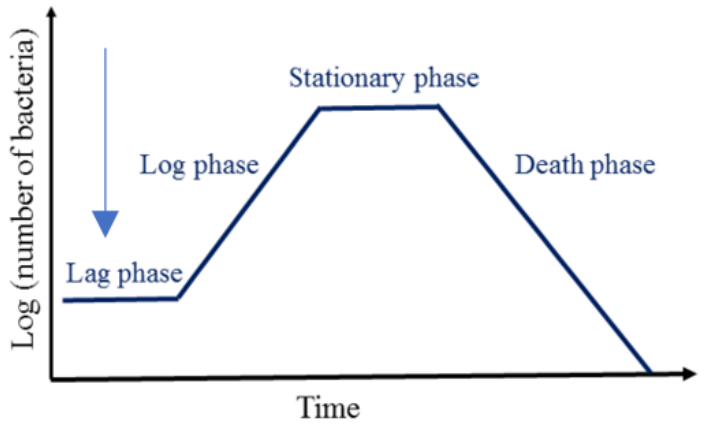
- **3-Stationary phase**

- **4-Decline (Death) phase**



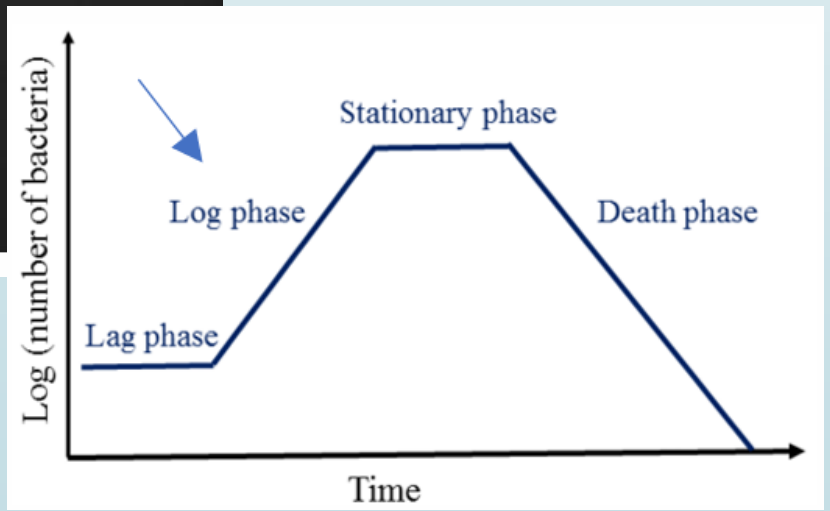
Lag Phase

- Bacteria are first introduced into an environment or media.
- Bacteria are checking out their surroundings.
- Cells are very active metabolically.
- Shape of cells change very little.
- This phase last for 1 Hour to several days.



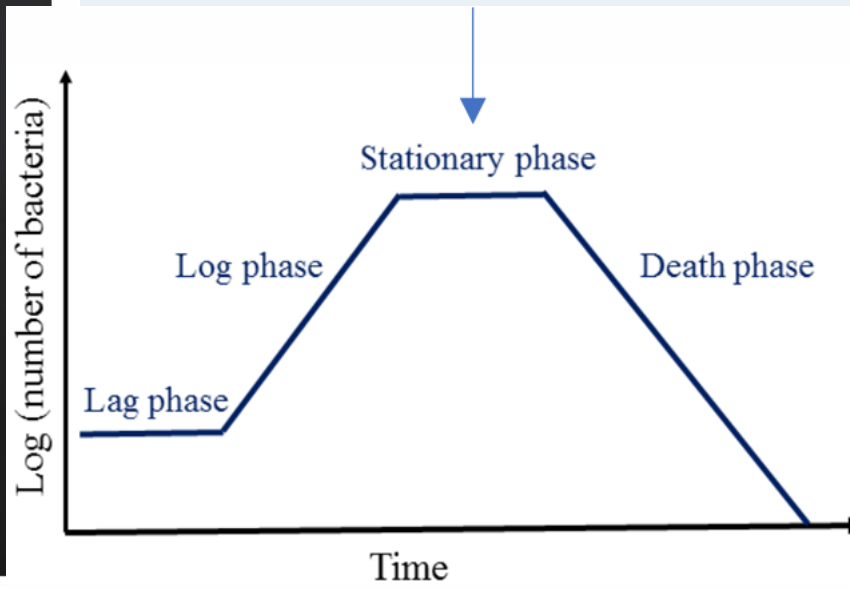
Log Phase :

- Rapid cell growth
- Exponential growth
- Population double every generation
- Microbes are sensitive to adverse conditions
  - antibiotics
  - antimicrobial agents



Stationary Phase :

- In this phase
- Death rate = rate of reproduction
- Cells begin to encounter environmental stress
- lack of nutrients
- Lack of water
- not enough space
- metabolic wastes
- Oxygen
- PH



*Endospores would be formed now

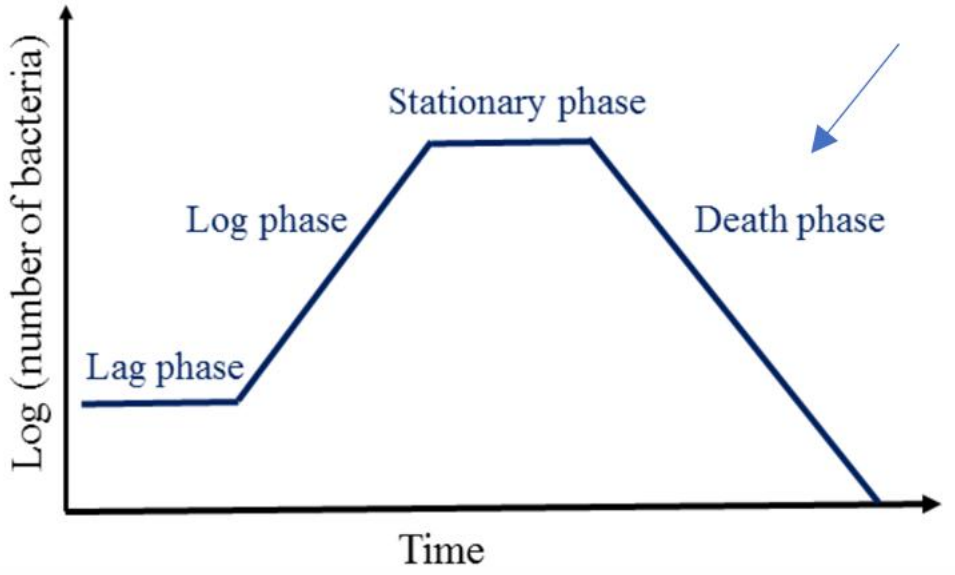
Death Phase :

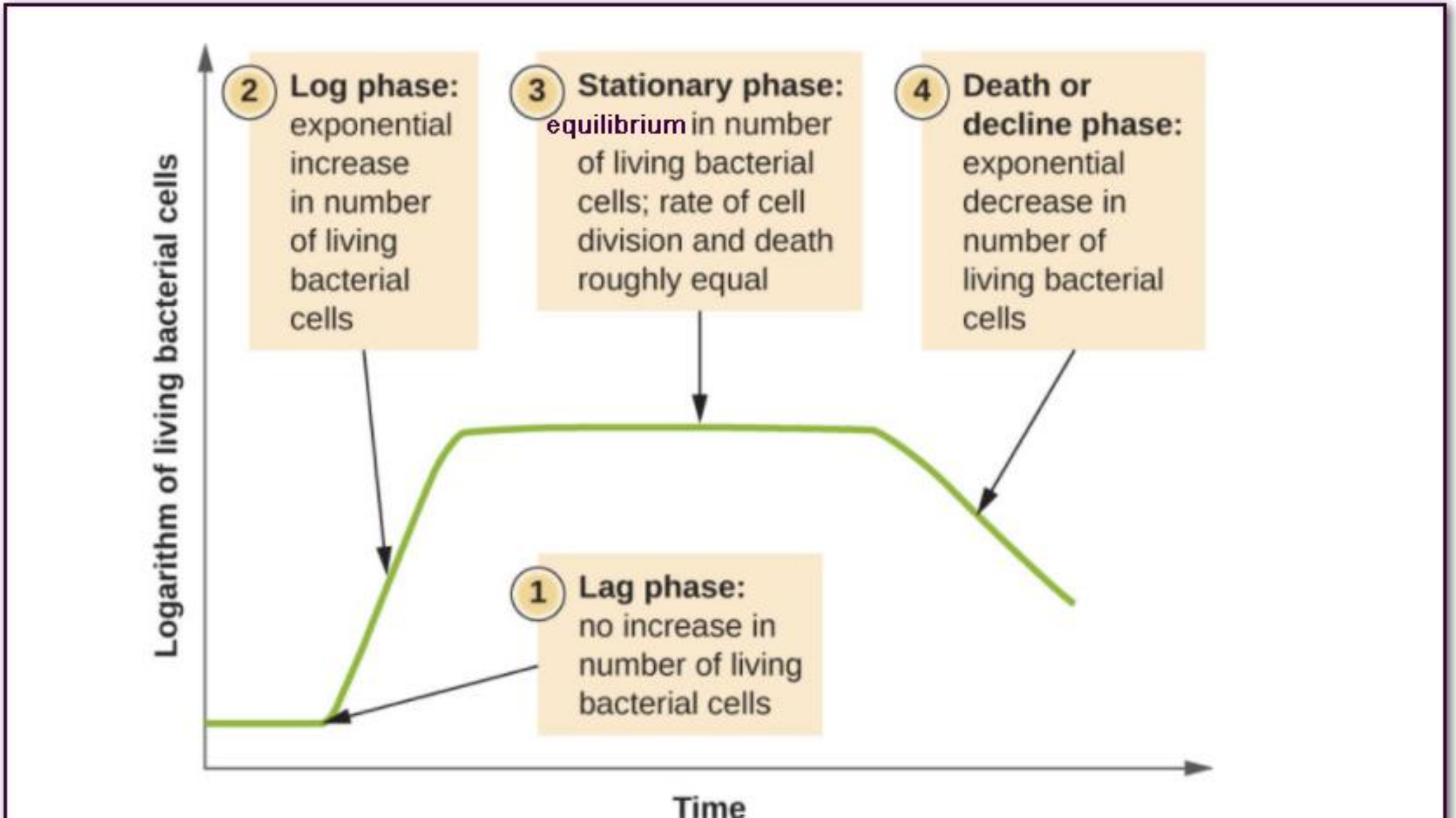
- In this phase
- Death rate > rate of reproduction
- Due to the limiting factors in the environment.

Death occurs because of:

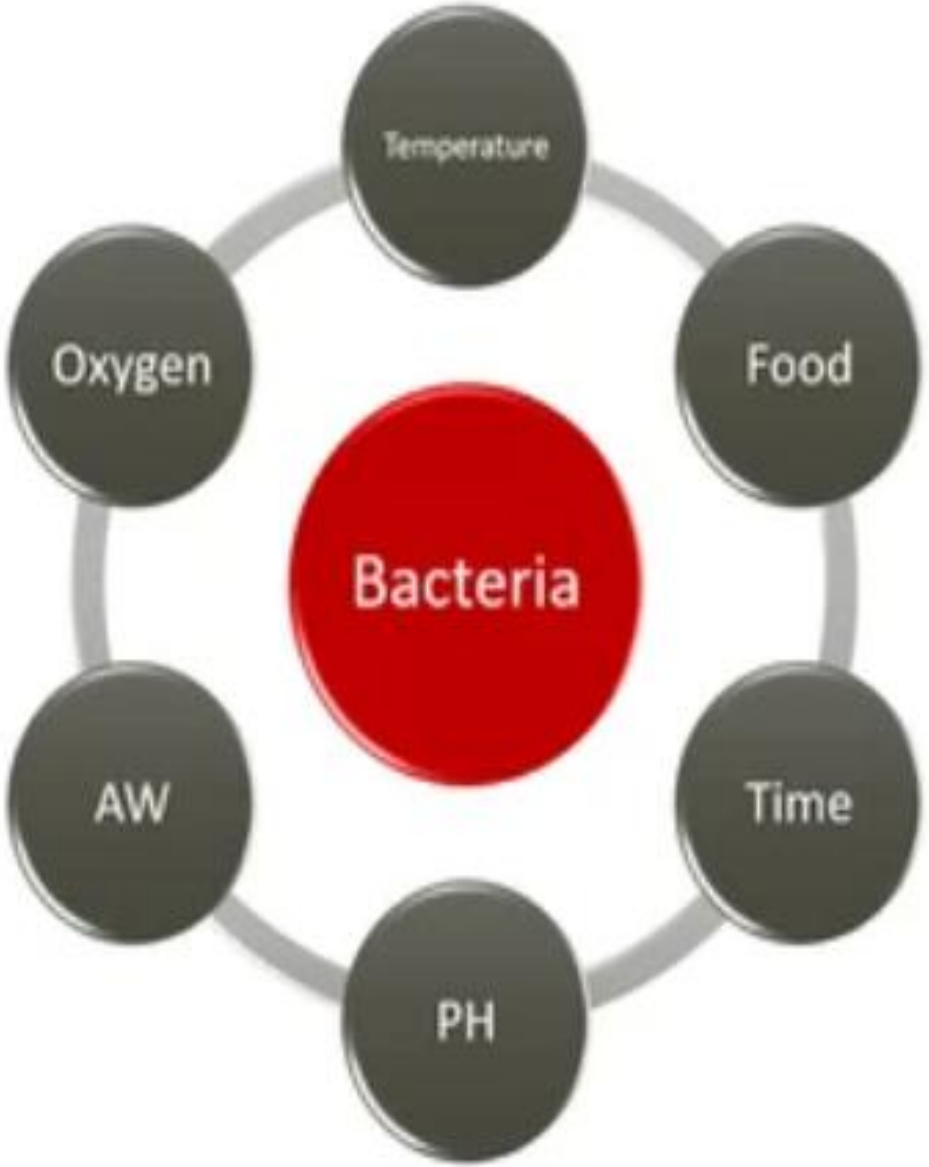
.Accumulation of toxic products and autolytic enzymes

- Nutrient deprivation
- Waste material accumulation




Logarithm of living bacterial cells
Time
2 Log phase: exponential increase in number of living bacterial cells
3 Stationary phase: equilibrium in number of living bacterial cells; rate of cell division and death roughly equal
4 Death or decline phase: exponential decrease in number of living bacterial cells
1 Lag phase: no increase in number of living bacterial cells

Factors affecting bacterial growth



1-Lack of food,water or nutrients space.

2-Accumulation of metabolic wastes.

3-Lack of oxygen.

4-Changes in pH.

5-Temperature.

Measurement of Bacterial Growth

Estimating the number of bacterial cells in a sample, known as a bacterial count.

The number of bacteria in a clinical sample serves as an indication of the extent of an infection.

Quality control of drinking water, food, medication, and even cosmetics depend on estimating of bacterial counts to detect contamination and prevent the spread of disease.

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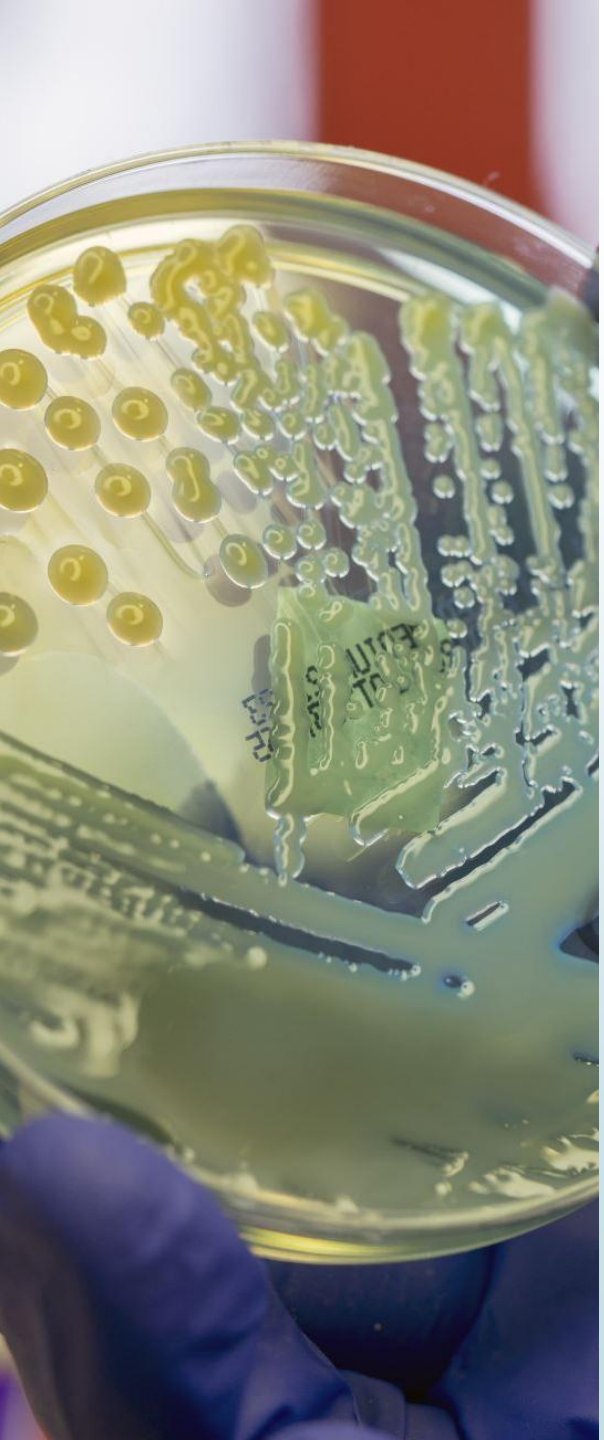


Bacterial Count

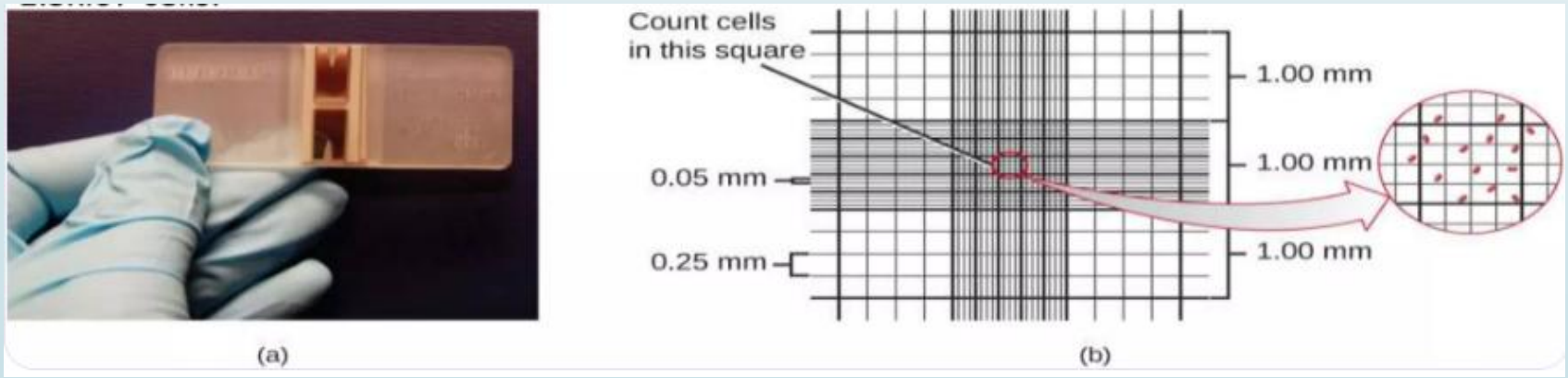
- Bacteria in a culture medium or clinical specimen can be counted by several methods:

- 1.**Total count**: This is **total number** of bacteria present in a specimen irrespective of whether they are living or dead.

- 2. **Viable count**: This measures **only viable (living) cells** which can grow and produce a colony on a suitable medium.

(Total Cell Count)
Direct Microscopic Method

Total cells (both live and dead) of liquid sample are counted by using a special microscope glass slide called Haemocytometer.

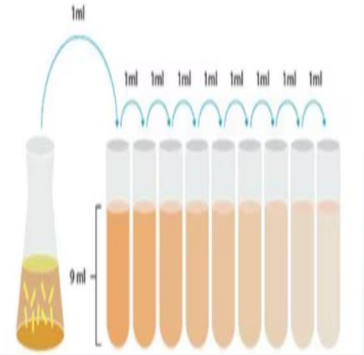


Determination of Viable Count

Serial Dilution

In dilution method, the suspension is diluted to a point beyond which unit quantities do not yield growth when inoculated into suitable liquid media.

Determination of Viable Count

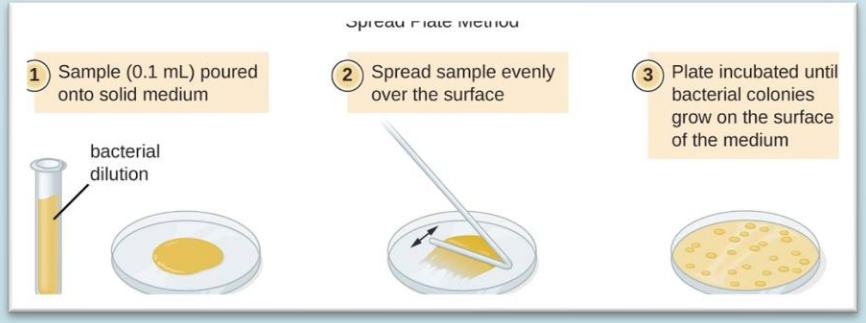


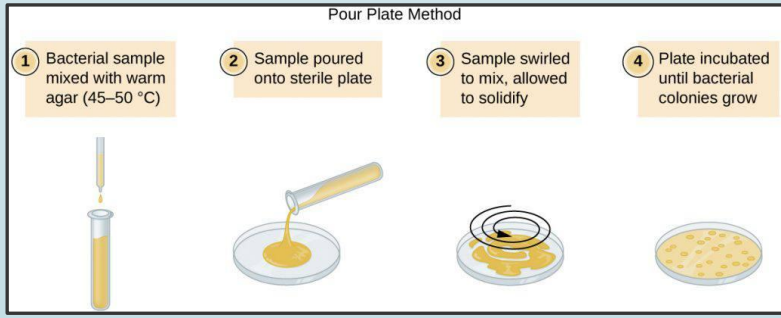
Direct Methods of Measurement

- **Plating method**: In the plating method, appropriate dilutions are inoculated on solid media by two ways of forming plate count :

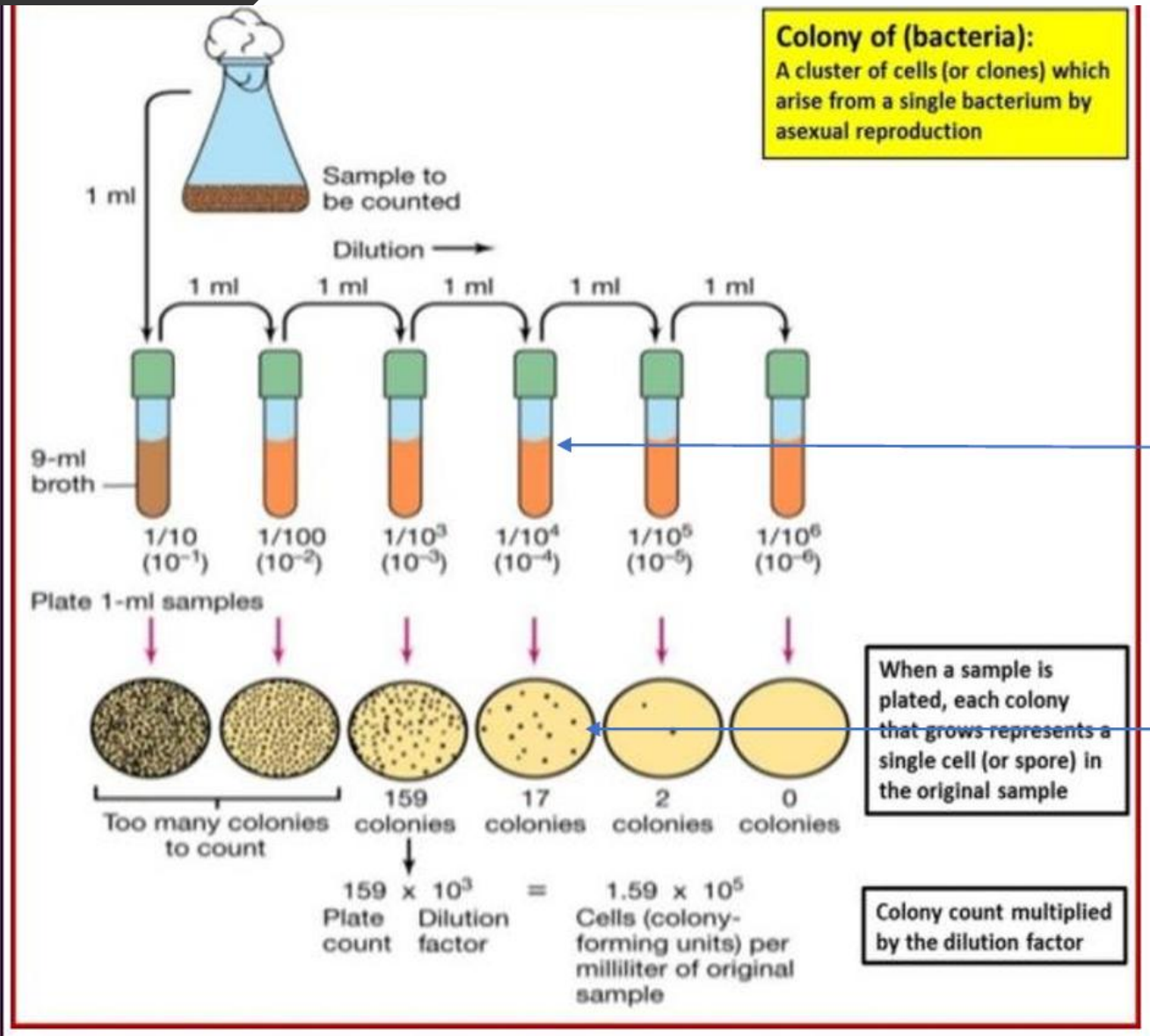
i. **Pour-plate method**

ii. **Spread plate method**-in which serial dilutions are dropped on the surface of dried plates and colony counts obtained





Viable cell count is measured by the following equation:
Number of bacteria / mL = Number of colonies x Dilution of sample



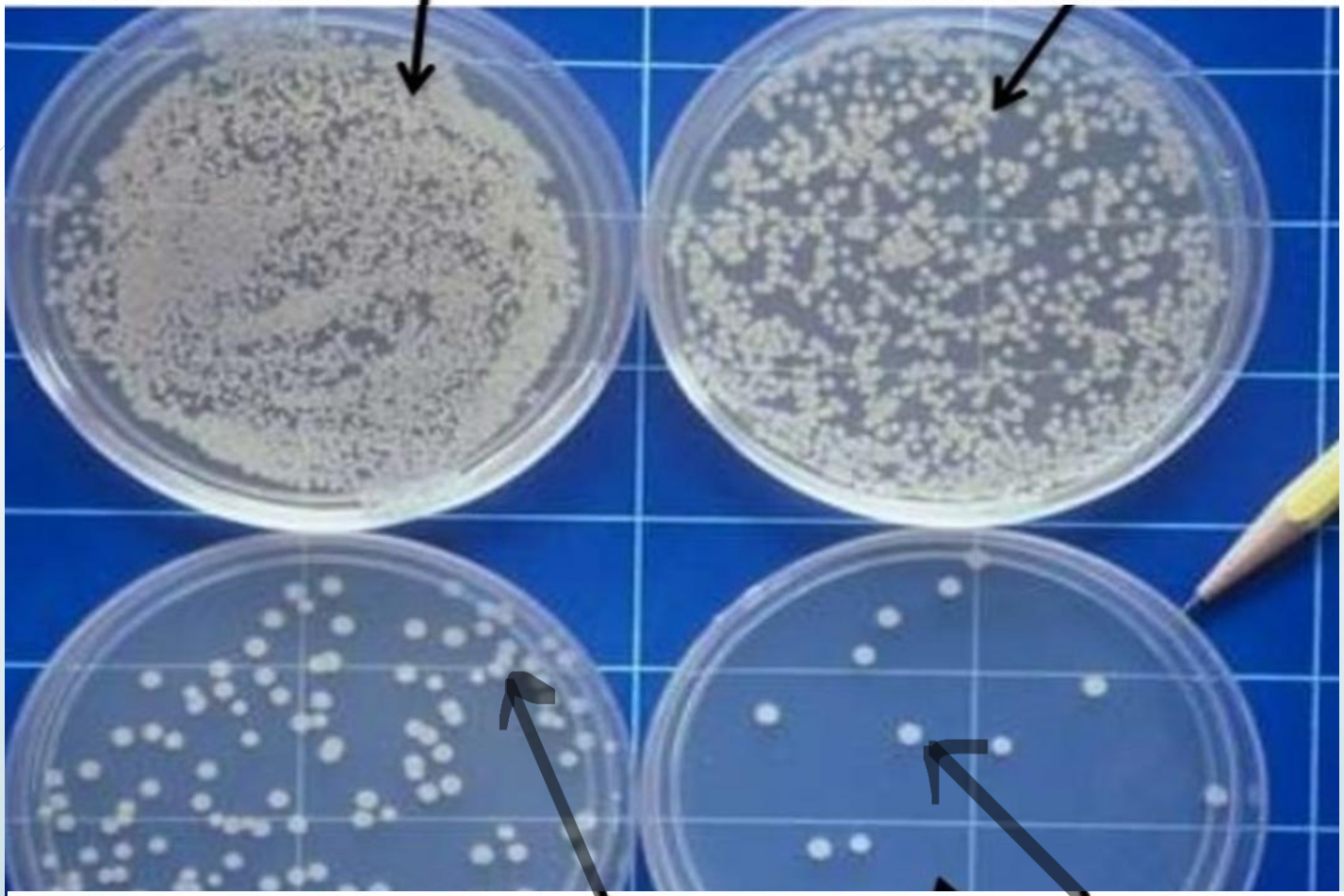
Dilution Factor = 10^4 = 10,000

Colonies = 17

Bacterial count in original solution =
17 x 10^4 = 1.7 x 10^5 CFU/mL

Too much growth, hard to count colonies

Growth not as much, but still hard to count colonies



OK to count but there are some colonies that are not single colonies, they are so close together

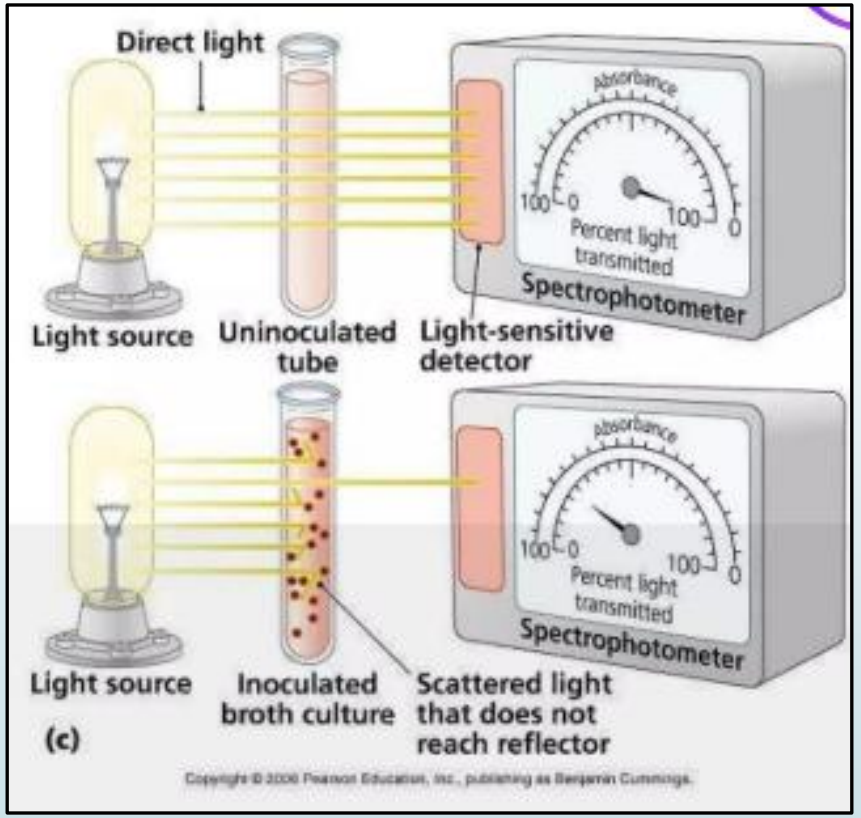
Definitely the best plate to estimate the CFU

Indirect Methods of Measurement

Turbidimetric method

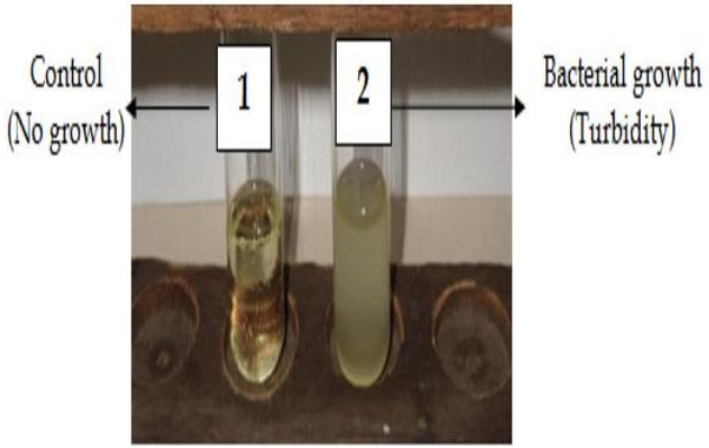
- In this method the growth of bacteria is measured by volume of turbidity formed.
- This technique is based on the amount of light absorbed through a suspension of bacteria.

-The laboratory instrument used to measure turbidity is called a spectrophotometer.



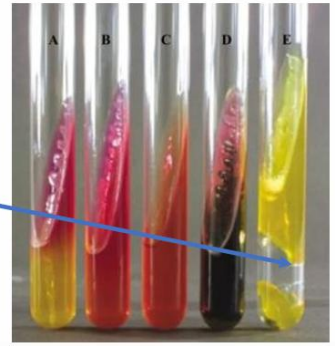
-What are the signs of bacterial growth in culture media?

1. **Turbidity** :which can be detected by naked eyes or spectrophotometry.



2. Color changes :some microorganisms are capable of producing pigments .

3. Gas production :some bacteria produce gasses .



4. Change in pH :due to acid production by bacteria .

Bacterial cultivation purposes

Culture methods employed depend on the purpose for which they are intended.

- Isolation of a pure strain
- To study morphology of bacteria
- Testing the antibiotic sensitivity
- Genetic and molecular study (DNA extraction)
- Maintenance and storage of stock culture

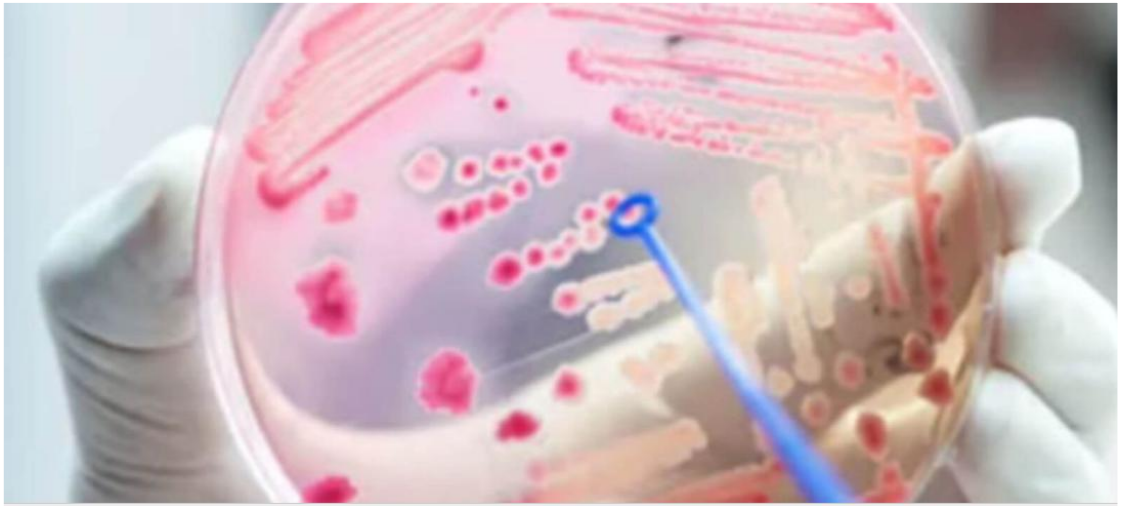
CULTURE MEDIA

The main types of culture media are:

1. Basic media
2. Enriched and enrichment media
3. Selective media
4. Differential media
5. Transport media

Culture Methods

- Streak culture
- Lawn culture
- Stroke culture
- Stab culture
- Pour plate method




THANK
YOU!