MICROBIAL GROWTH

Introduction

- **Living organisms grow and reproduce.**
- When microbes are provided with nutrients & required environmental factors, they become metabolically active and grow.
- **Growth common refers to increase in a microbial size, population number, or both**
- **Growth also results when cells simply become longer and larger**
- involves an increase **in the number of cells** rather than in the size of individual cells.
- **The change in population in bacteria chiefly involves Binary fission.**
- **Note- it is not convenient to study growth and reproduction of individual mo because of their small size, therefore population no. is important**

2 levels of growth:

i. A cell synthesizes new components, increase its size

ii. Increase number of cells in the population Increase in cellular constituents that may result in:

- **Increase in cell number**
  - e.g., when microorganisms reproduce by budding or binary fission
- **increase in cell size**
  - e.g., some microorganisms have nuclear divisions that are not accompanied by cell divisions

❖ **Microbiologists usually study population growth rather than growth of individual cells.**

IMPORTANT POINTS

1- Bacterial Division/reproduction 2- Stages of Bacterial growth curve

3 Measurement of Microbial growth

4 Environmental factors effect microbial growth

1- Bacterial Division/reproduction

- Bacteria normally reproduce by a method called **binary fission.**
- What is Binary Fission.?

- **Binary Fission is the of process bacteria reproduction where one cell become two**
- **Ex: Bacteria, paramecium, amoeba.**

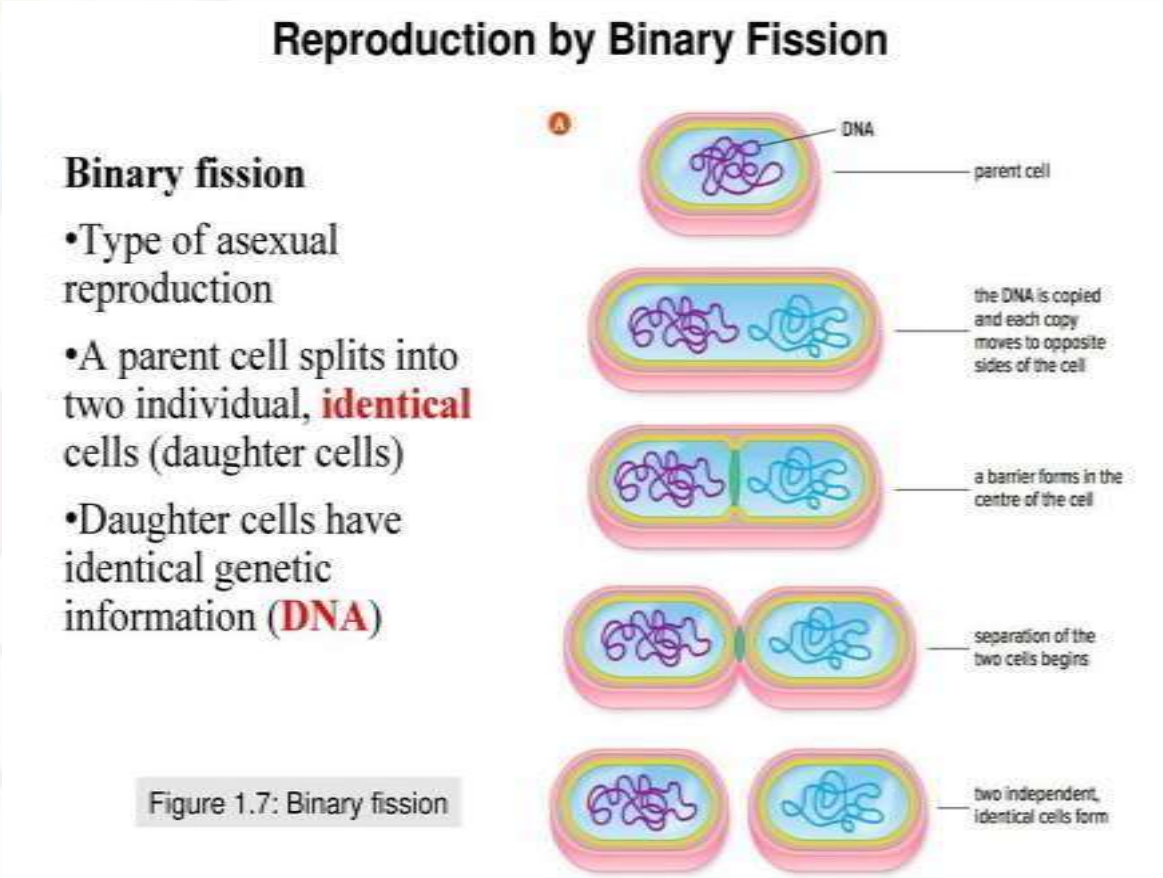


Figure 1.7: Binary fission

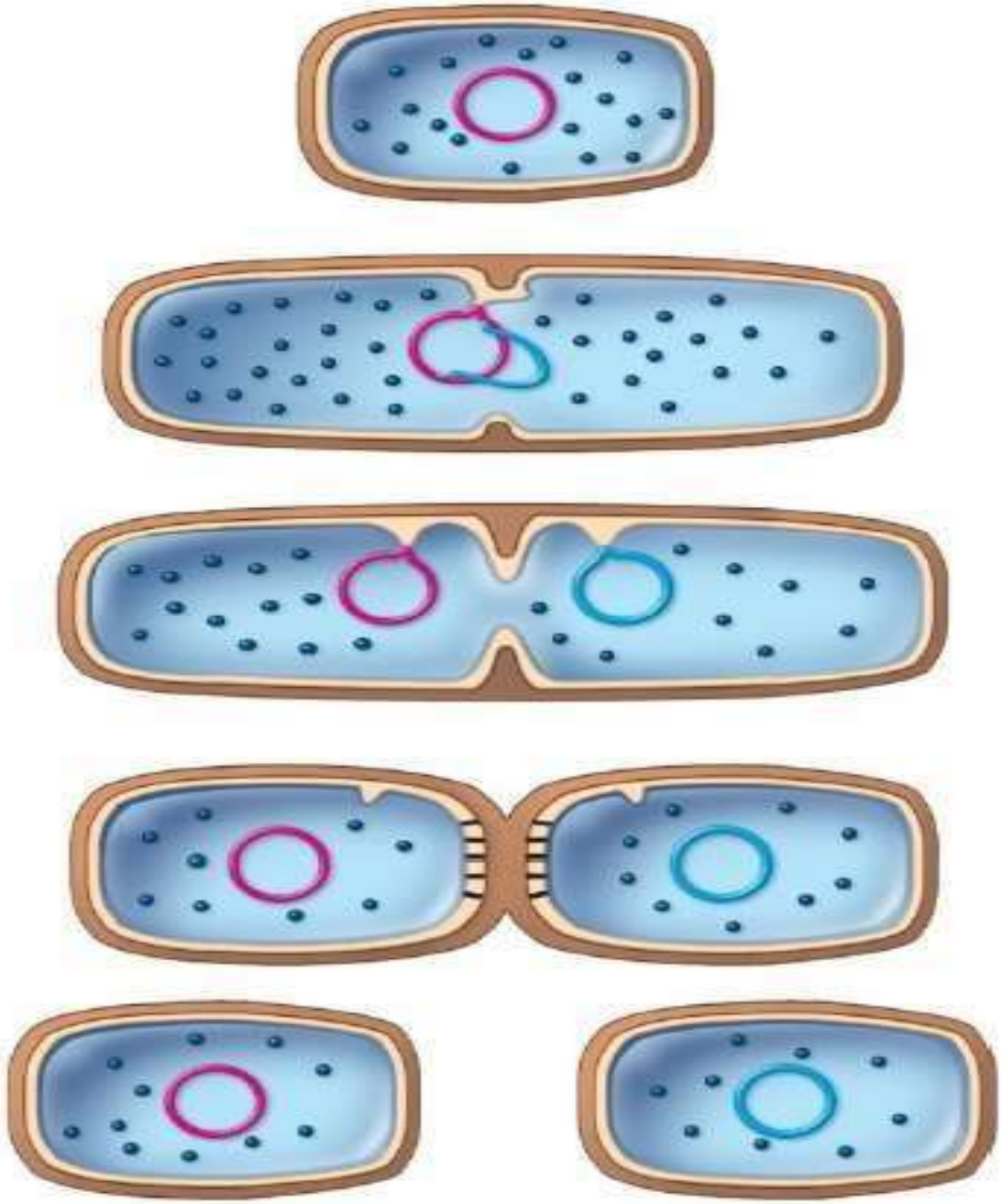
1. A young cell at early phase of cycle

2. A parent cell prepares for division by enlarging its cell wall, cell membrane, and overall volume. Midway in the cell, the wall develops notches that will eventually form the transverse septum, and the duplicated chromosome becomes affixed to a special membrane site.

3. The septum wall grows inward, and the chromosomes are pulled toward opposite cell ends as the membrane enlarges. Other cytoplasmic components are distributed (randomly) to the two developing cells.

4. The septum is synthesized completely through the cell center, and the cell membrane patches itself so that there are two separate cell chambers.

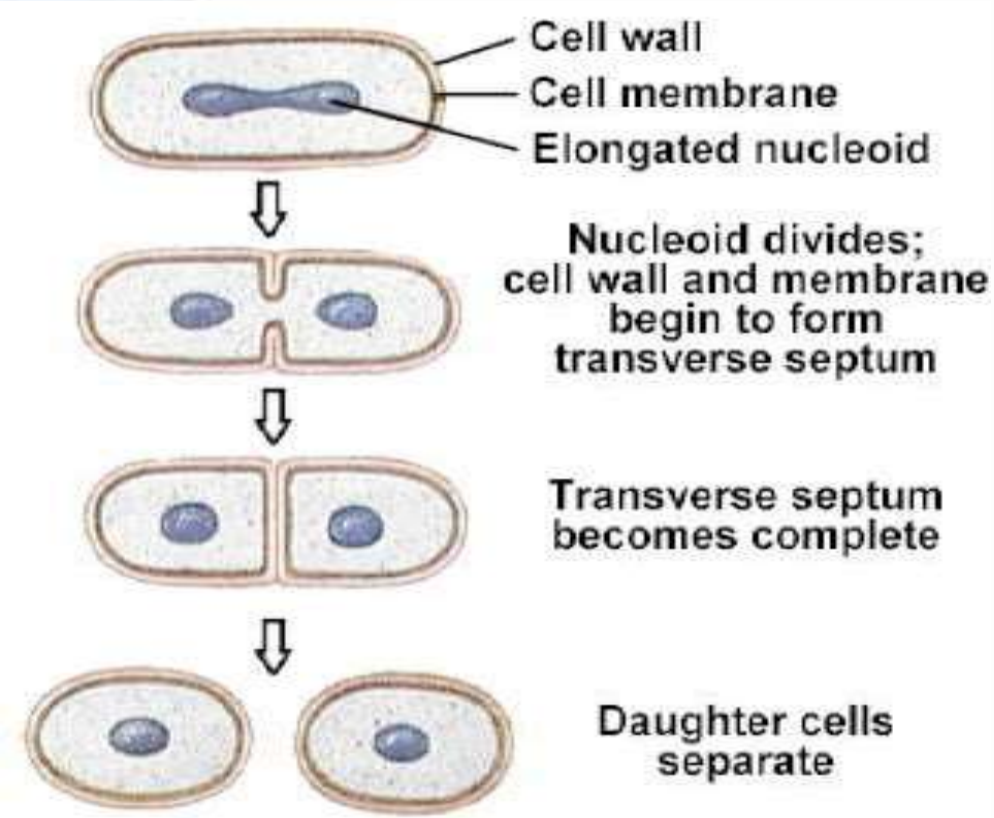
5. At this point, the daughter cells are divided. Some species will separate completely as shown here, while others will remain attached, forming chains or doublets, for example.



Stages of Binary Fission

Generation Time (Doubling time)

- **What Time is Generation**
  - The time required for a cell to divide (and its population to double) is called the **generation time**

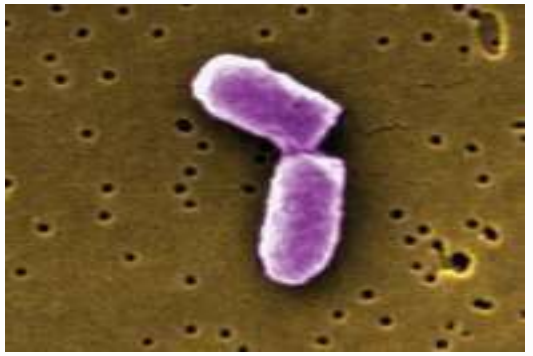


- As you seen in the picture, cell's division produces two cells, two cells' divisions produce four cells, and so on.

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*
- *Treponema pallidum---1980 mins*



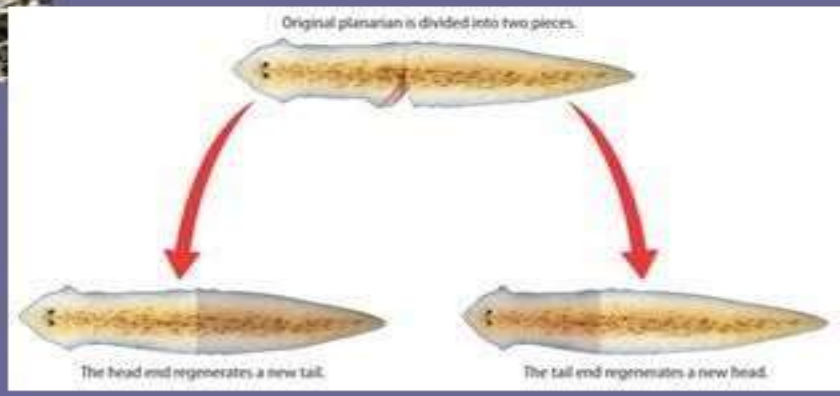
Ex) *E. coli* GT is 20 mins in optimal conditions

- After 20 generations (7 hrs) → 1 million cells
- After 30 generations (10 hrs) → 1 billion cells

Examples of asexual reproduction(animals)

- Hydra budding (this is an animal)

- Some sponges



- Planaria

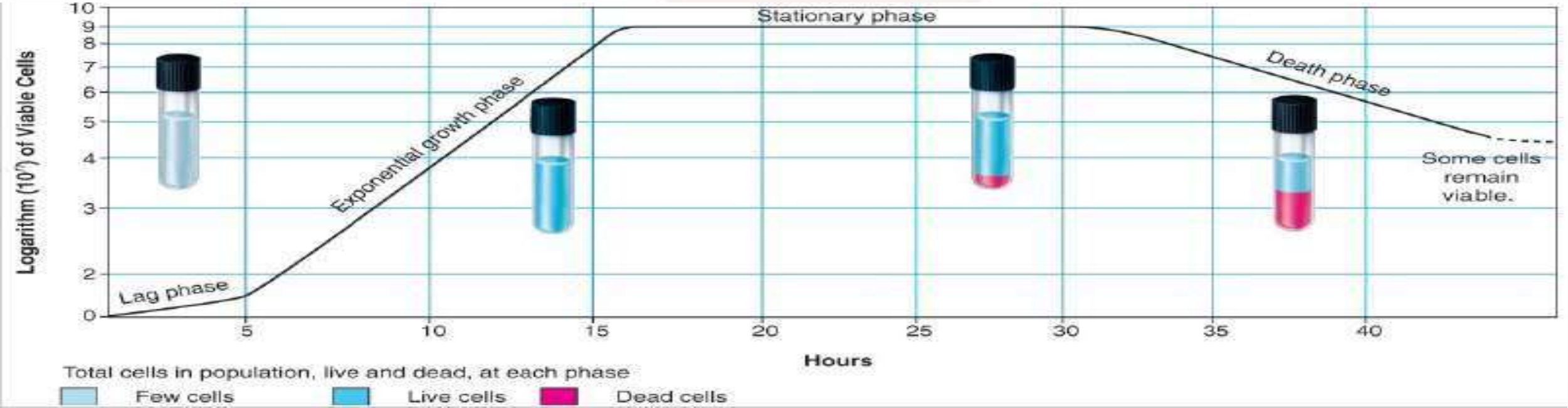
- Starfish

2-Stages in Bacterial Growth curve

- When a few bacteria are inoculated into a liquid growth medium, it is possible to plot a **bacterial growth curve** that shows the growth of cells over time.
- There are **four basic phases of growth:**
- **1- The Lag**
- **2- Log (Exponential)**
- **3- Stationary and**
- **4-Death phases.**

Bacterial growth curve

- **Batch culture (closed system)**
- **Bacteria grown in a liquid medium**
- **Single batch of medium**
- **Nutrient get exhausted and there is decline in growth**
- **When a graph is plotted as the logarithm of the no. of viable cells Vs the incubation time**



A) The Lag Phase

- **Introduction of microorganism into fresh medium**
- **Bacteria are trying to adapt to nutrients**
- **No immediate increase in cell number, no increase in net mass**
- **Lag phase-preparing to grow in size and synthesize enzymes etc.**
- **Cell is synthesizing their own components**
- **Can be very short or even absent**
- **Importance of lag phase-**

1. **Cells may be old and depleted of ATP, essential cofactors, ribosomes and these must be synthesized before growth begins.**
2. **The medium may be the different than the previous one so enzymes are required**
3. **Possibly the mo have been injured and require some time to recover.**

B) Log Phase

- Also called **Exponential phase**
- **Growth and division at maximal rate**
- **Also called balanced growth since all cell constituents are synthesized at constant rate.**
- Population number of cells undergoing **binary fission doubles at a constant** interval called **generation time**
- **Continue as long as cells have adequate nutrients & good environment**
- **All studies related to their various properties are done in this phase.**

Example of Exponential growth

Time[a]	Division Number	2^n	Population ($N_0 \times 2^n$)
0	0	$2^0 = 1$	1
20	1	$2^1 = 2$	2
40	2	$2^2 = 4$	4
60	3	$2^3 = 8$	8
80	4	$2^4 = 16$	16
100	5	$2^5 = 32$	32
120	6	$2^6 = 64$	64

Demonstration of balanced and unbalanced growth

- **Shift-up experiment-** bacteria when transferred from nutritionally poor medium to richer medium the lag phase is longer
- **Shift-down experiment-** bacteria when transferred from nutritionally rich medium to poorer medium the lag phase is shorter

C) Stationary Phase

- **Reproduction rate is balanced by the death rate and population remains constant**
- **There is exhaustion of some nutrients and accumulation of some toxic materials**
- In this stage microbial death is equal to microbial growth e-g Death=growth.
- Period of equilibrium
- Metabolic activity of surviving cells slows down which stabilizes the population
- Cause of discontinuity of exponential growth is not always clear
- May play a role: exhaustion of nutrients, accumulation of waste products and
- harmful changes in pH

Chemostat – continuous culture used in industrial fermentation

Possible reasons for entry into stationary phase

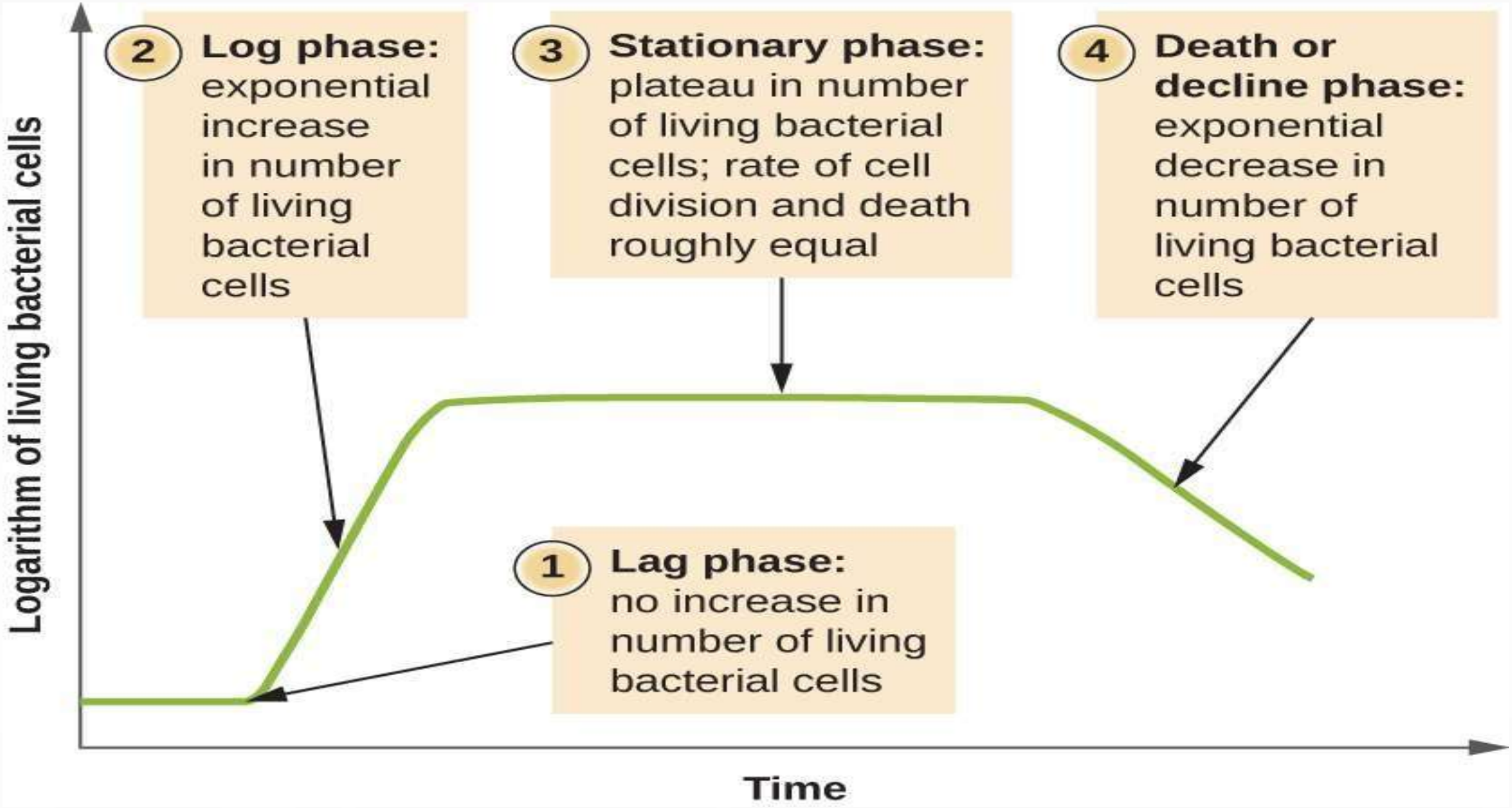
- Nutrient limitation
- Limited oxygen availability
- Toxic waste accumulation
- Critical population density reached

D) Death Phase

- Decline in the number of viable cells
- cells dying, usually at exponential rate
- Death; loss of ability to reproduce
- in some cases, death rate slows due to accumulation of resistant cells
- Slower than log phase
- Also known as **Logarithmic Decline Phase**
- Continues until a small fraction of the population is diminished
- Some population dies out completely
- Others retain surviving cells indefinitely while others only retain for a few days

Characteristics of death phase-

- Nutrient deprivation
- Waste material accumulation
- Irreversible loss of the ability to reproduce
- The death can be logarithmic and can vary with environmental conditions such as the growth medium employed and the mo involved
- Resistant bacteria show extended survival
- Generally bacteria die in log phase


2 Log phase: exponential increase in number of living bacterial cells
3 Stationary phase: plateau 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
Logarithm of living bacterial cells
Time

3- Measurement of Microbial Growth

- Can measure changes in number of cells in a population
- Can measure changes in mass of population
- Microbial growth to determine **growth rates and generation times** can be measured by different methods.

Measurement of Cell Numbers

- **What are the lab equipment's used to measure bacteria cell numbers?**
- There are two types:-

1 Total count/Direct cell counts:-

a)Counting chambers method/ Haemocytometer method

b) Breed method/direct microscopic method

c) Electronic counters methods

d) Proportional counter method

2 Viable cell counts/Indirect method

- **Plating methods (spread, pour plate)**
- **Membrane filtration methods**

A) Breed method/direct microscopic method

Protocol:

- A known volume of microbial cell suspension **(0.01 ml)** is spread uniformly over a glass slide covering a **specific area (1 sq. cm).**
- The smear is then fixed by heating, stained, examined under oil immersion lens, and the cells are counted.
- Customarily, cells in a few microscopic fields are counted because it is not possible to scan the entire area of smear.
- The counting of total number of cells is determined by calculating the total number of microscopic fields per one square cm. area of the smear.

- **The total number of cells can be counted with the help of following calculations:**

(a)Area of microscopic field = πr^2 Where,

r=(oil immersion lens) = 0.08 mm.

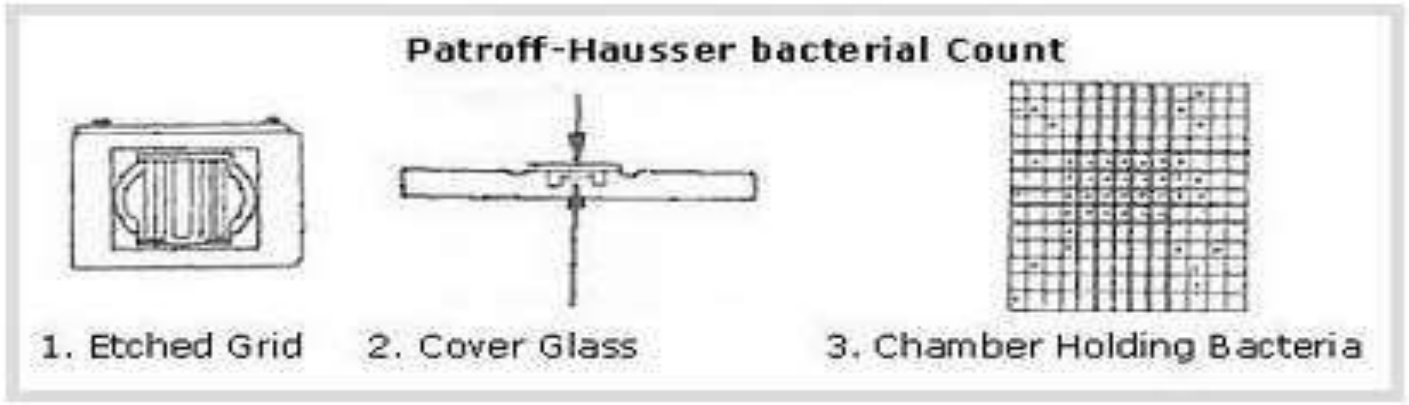
Therefore, Area of the microscopic field under the oil- immersion lens= $\pi r^2 = 3.14 \times (0.08 \text{ mm})^2 = 0.02$ sq. mm.

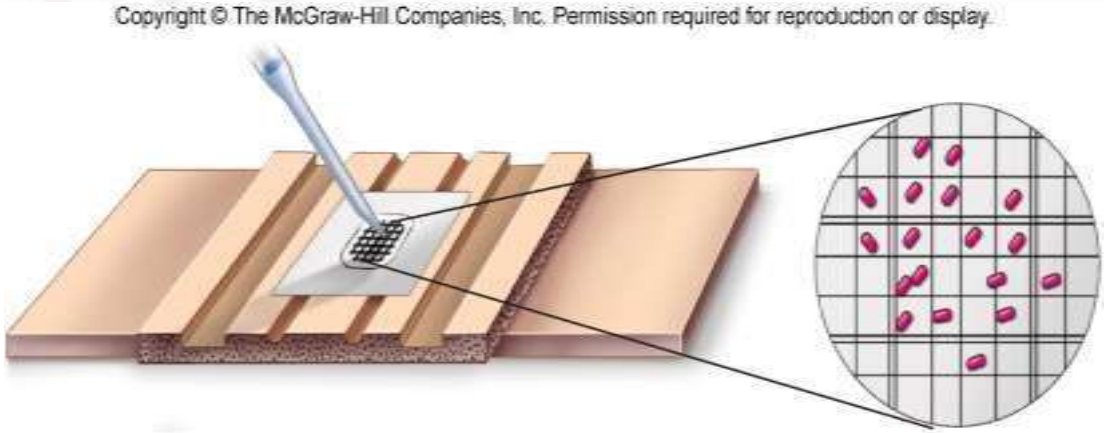
(b)Area of the smear one sq. cm. = 100 sq. mm. Then, the no. of microscopic fields = 100 / 0.02= 5000

(c)No. of **cells 1 sq. cm.** (or per 0.01 ml microbial cell suspension) = **Average no. of microbes per microscopic field x 5000**

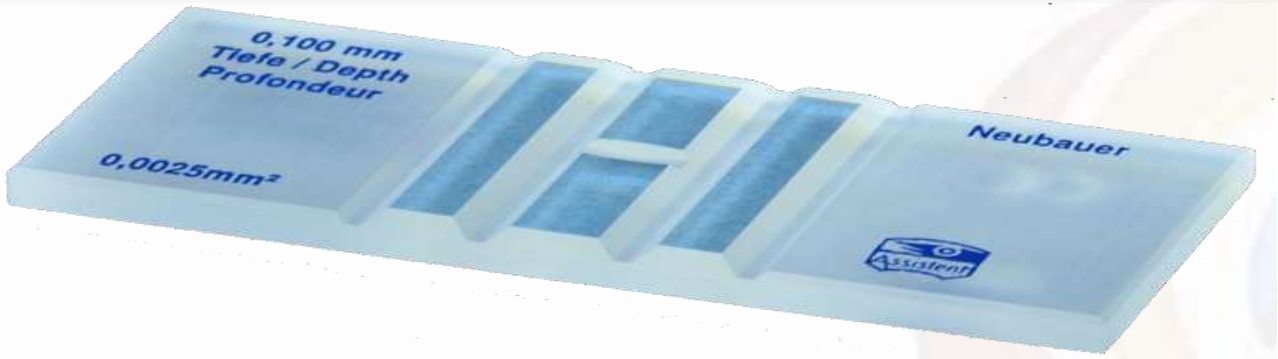
B) Counting chambers/ Petroff-Hausser counting chamber

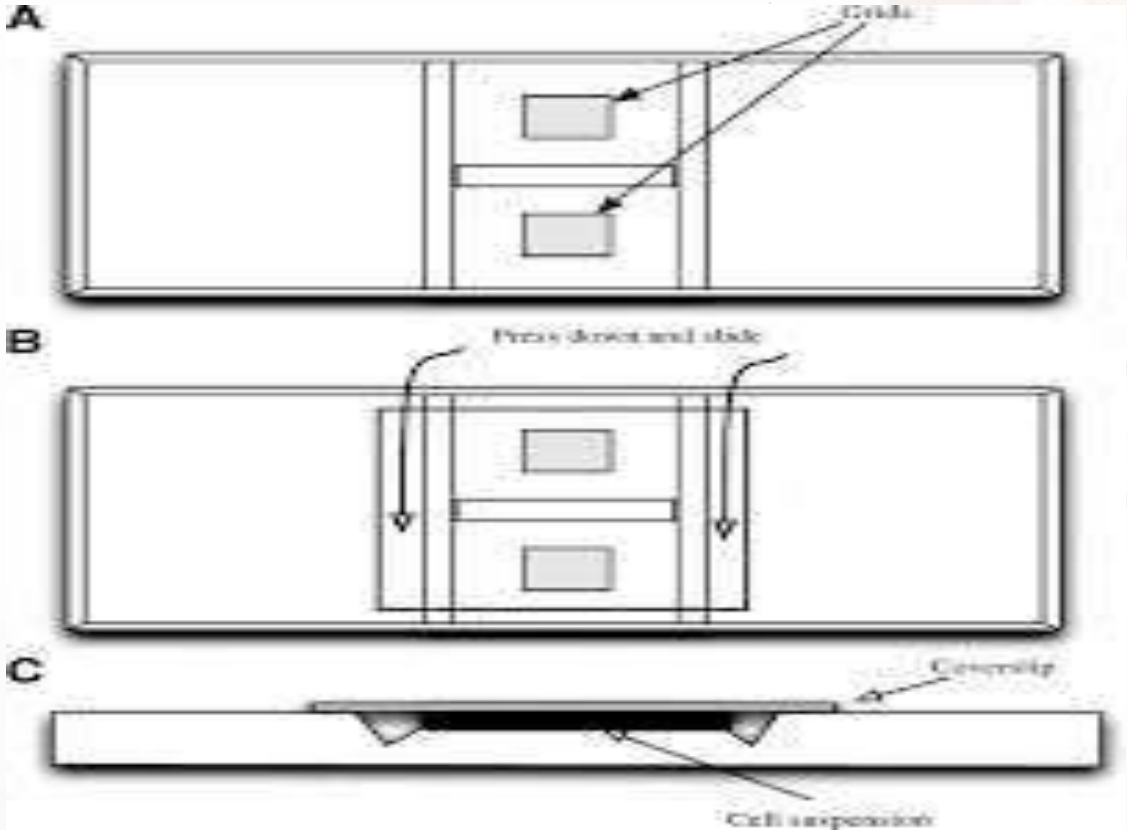
- **Easy, inexpensive, and quick**
- Useful for counting both **Eucaryotes(Haemocytometer) and Procaryotes (Petroff- Hausser counter)**
- More easily counted if they are stained or if phase I contrast of florescence microscope is employed.
- Cannot distinguish living from dead cells
- Very small cells are usually missed.
- Calculated taking the count of number of bacteria per unit area of grid and multiplying it by a conversion factor (depending on chamber volume and sample dilution used).

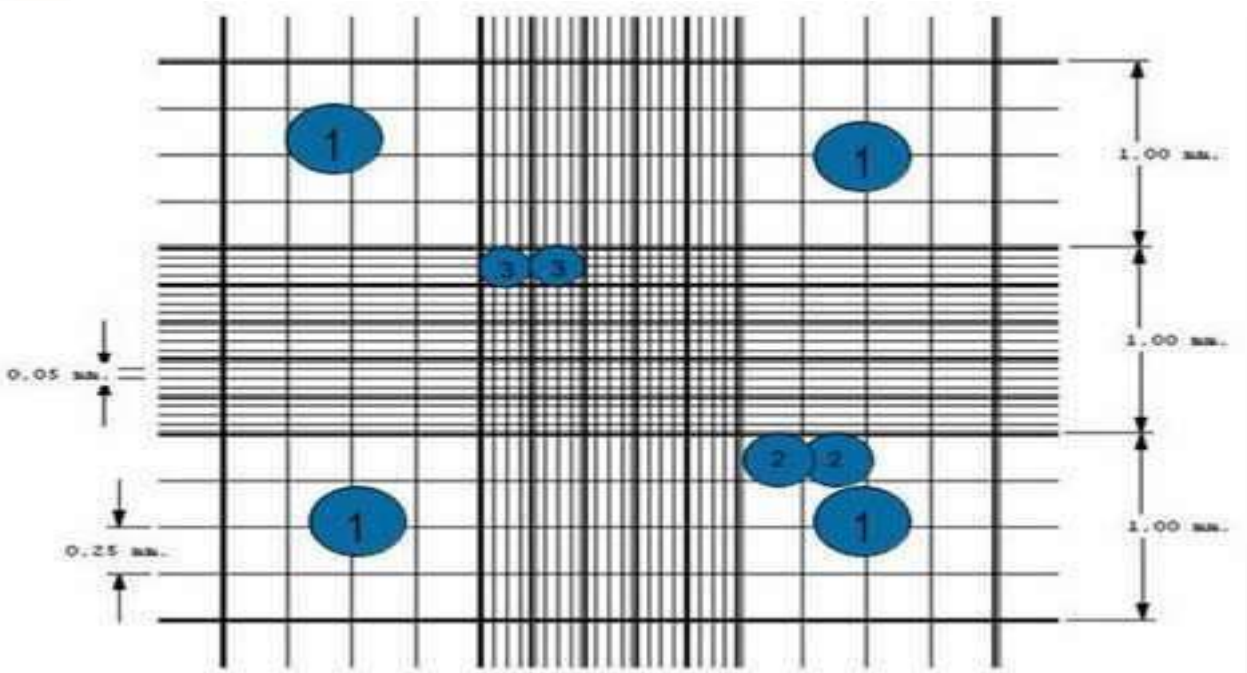




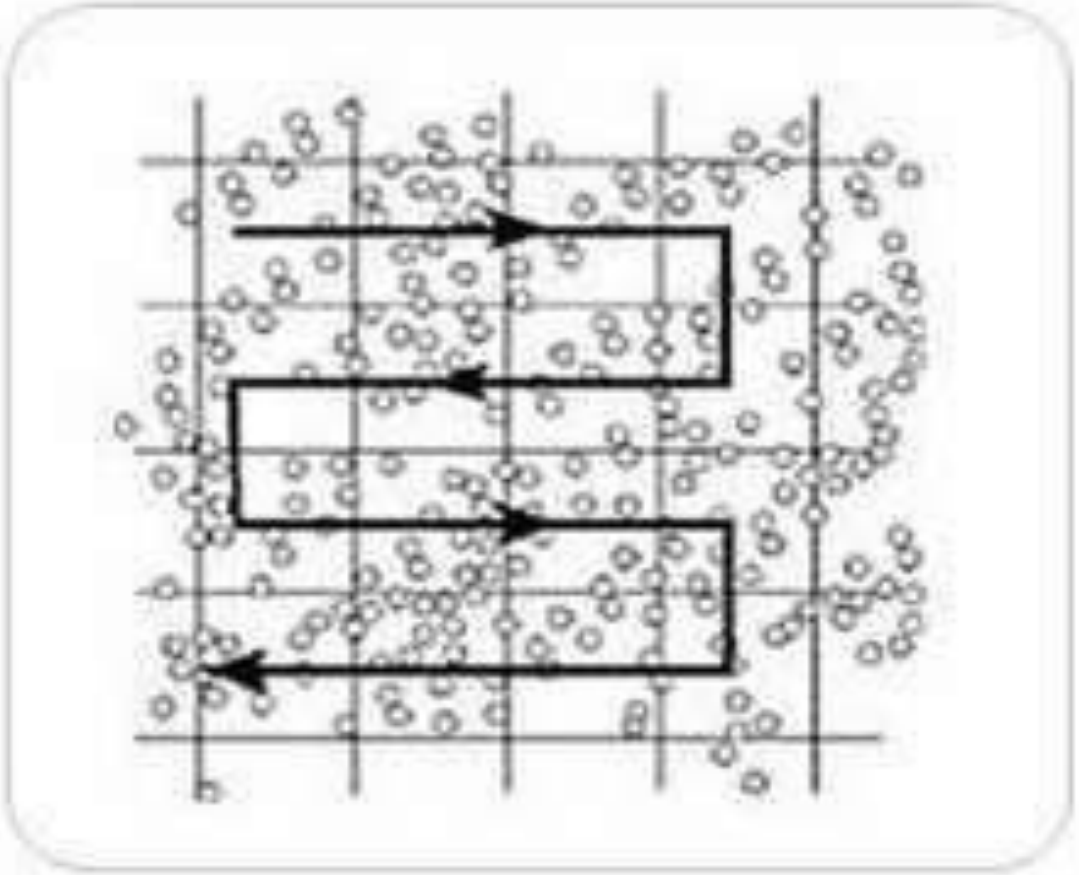
Neubauer’s slide/Haemocytometer

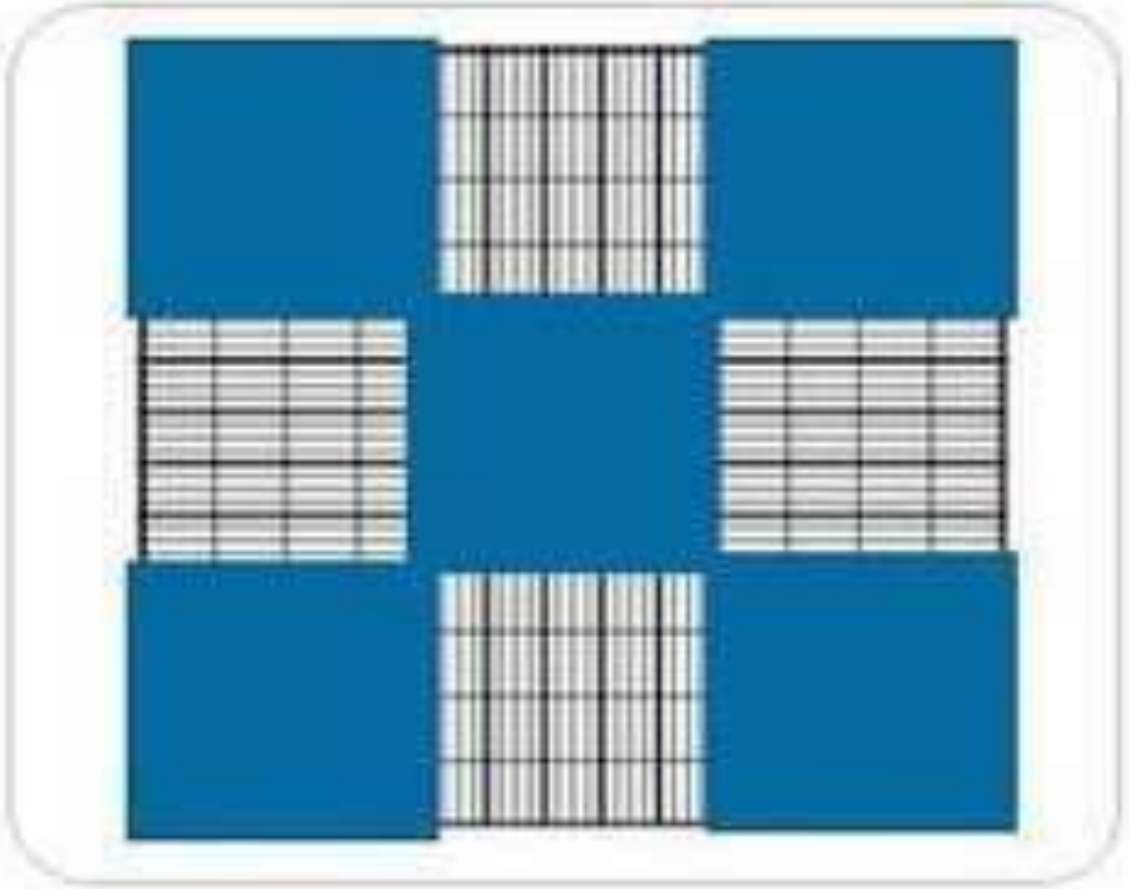






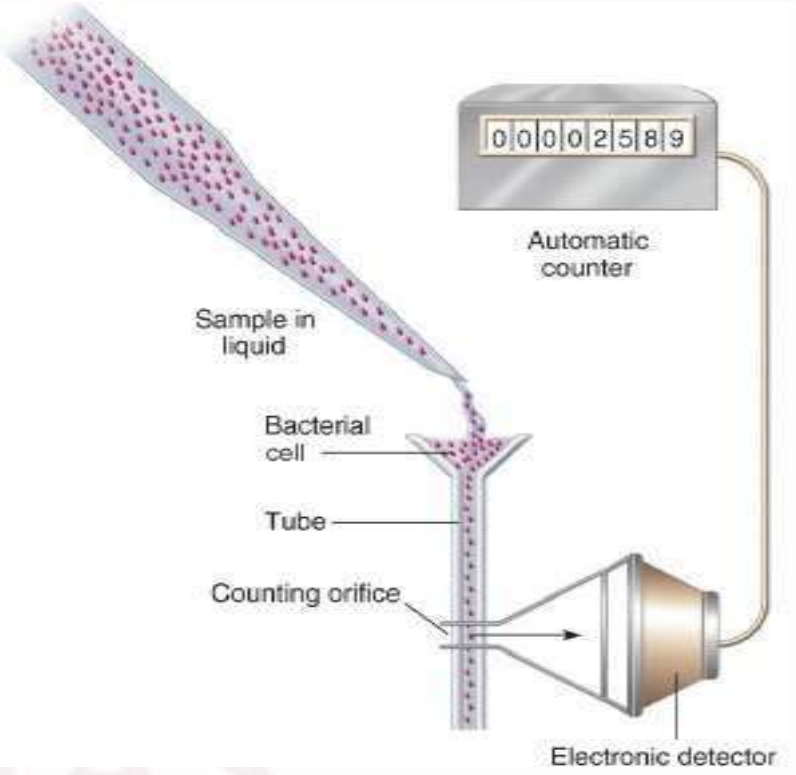
Reading style





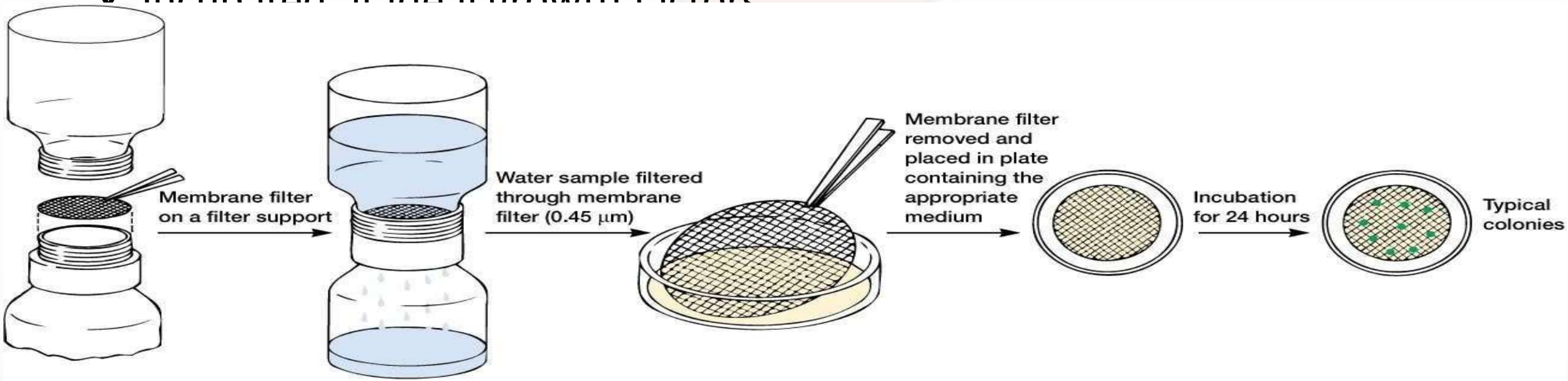
C) Electronic counters

- Electronic instrument
- As Coulter counter
- Microbial suspension forced through small hole or orifice or capillary tube
- The diameter of this tube is so microscopic that allows only one cell to pass at a time.
- Can count thousands of cells in a few seconds.
- Movement of microbe through orifice/capillary tube impacts electric current that flows through orifice
- **Cannot distinguish living from dead cells**
- **Counts even dust particles.**
- **Suspension must be free of any foreign particles.**
- **Not for prokaryotic cells**
- Quick and easy to use
- Useful for large quantity
- Microorganisms and blood cells.



D) Membrane filters

- ✓ A diluted suspension of microorganism/Cells is filtered through special membrane that provides dark background for observing cells
- ✓ Cells are retained on filter
- ✓ The disc is placed in a culture media in a petri plate
- ✓ Incubated at ideal growth factors



Advantages

- A large volume of the sample can be analysed
- Various types of microorganism can be detected by using selective media in the plate

2. Viable cell count

Standard Plate Count/plate count technique

- Assignment

Important links

- http://www.biologydiscussion.com/microorganisms/measurement-of-cell-numbers-and-cell-mass-_of-microorganisms/55160
- https://www.slideshare.net/AbdulRehman944/m_ethods-of-measuring-microbial-growth
- http://upendrats.blogspot.com/2010/02/measur_ement-of-microbial-growth.html
- http://library.open.oregonstate.edu/microbiology/chapter/environmental-factors/
- https://www.onlinebiologynotes.com/factor-_affecting-bacterial-growth/