# Microbial growth and metabolism

Assist.Prof.Dr.Sewgil S.Anwer

lec 6

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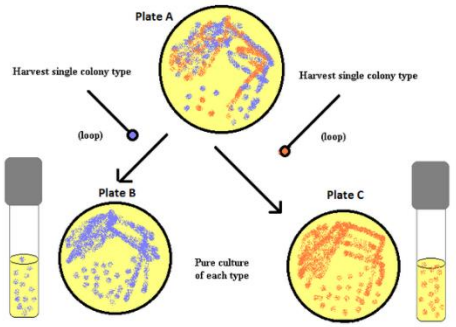
# Microbial growth and microbial growth requirement

## Microbial growth

Microbial growth is the increase in number of cells, not cell size.

## Growth in colonies

- A pure culture contains only one species or strain.
- A colony is a population of cells arising from a single cell or spore or from a group of attached cells.
- a unit used to estimate the number of viable microbial cells is colony forming unit (CFU)



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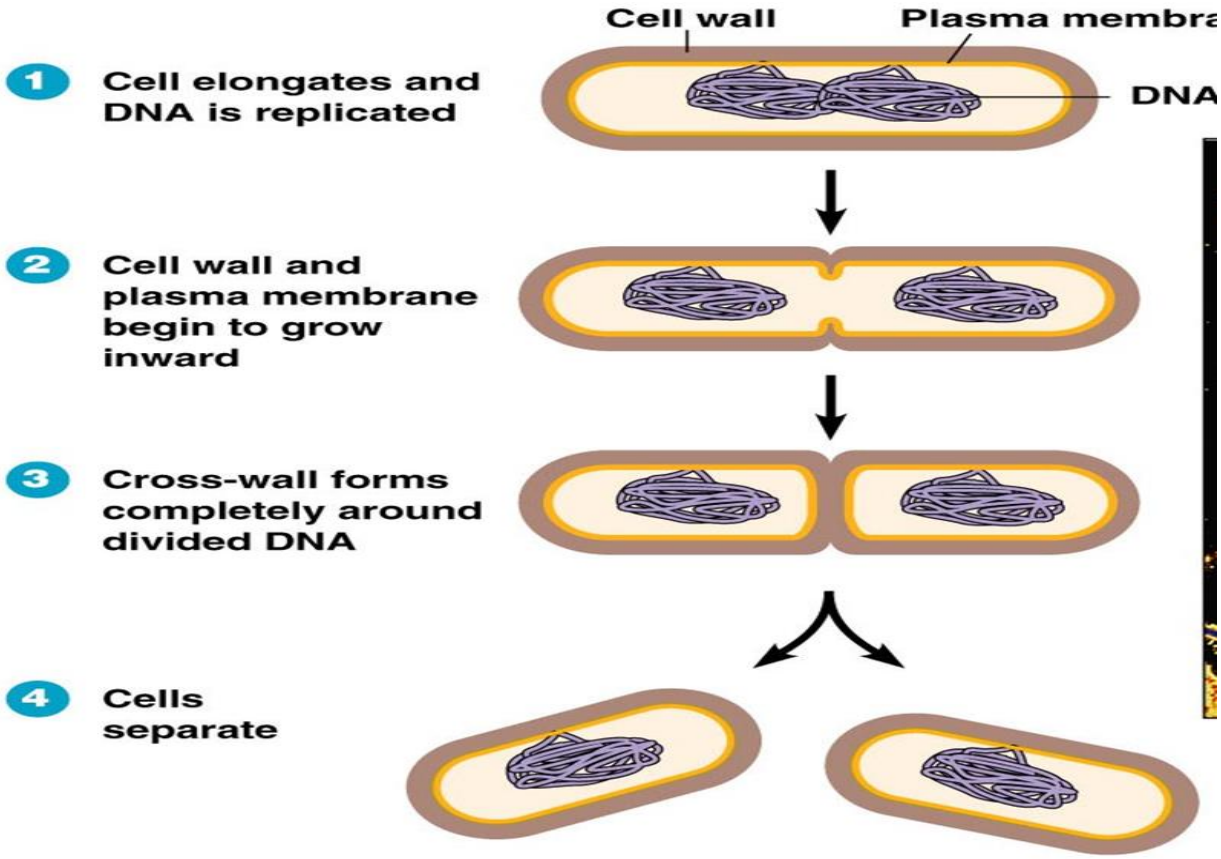



**(a)** A diagram of the sequence of cell division.

## Growth rate

The number of generation per hour.

## Generation Time

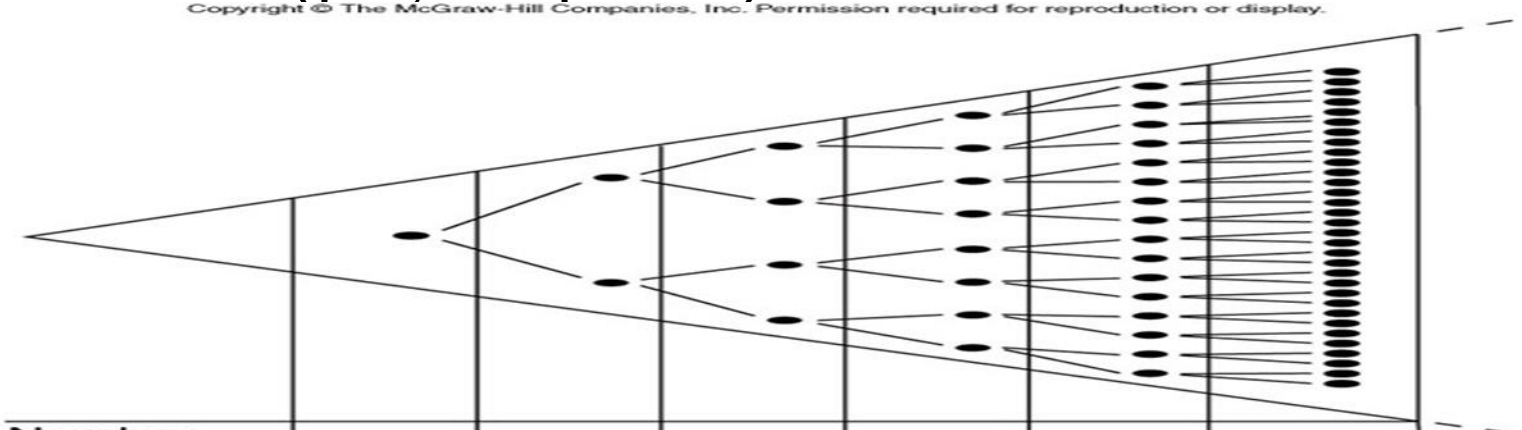
The **time required for** a cell to divide or a **population to double** is known as the generation time.

The generation time depend upon:

1- The nutrient in the medium.

2- Physical condition (pH, temp. etc.)

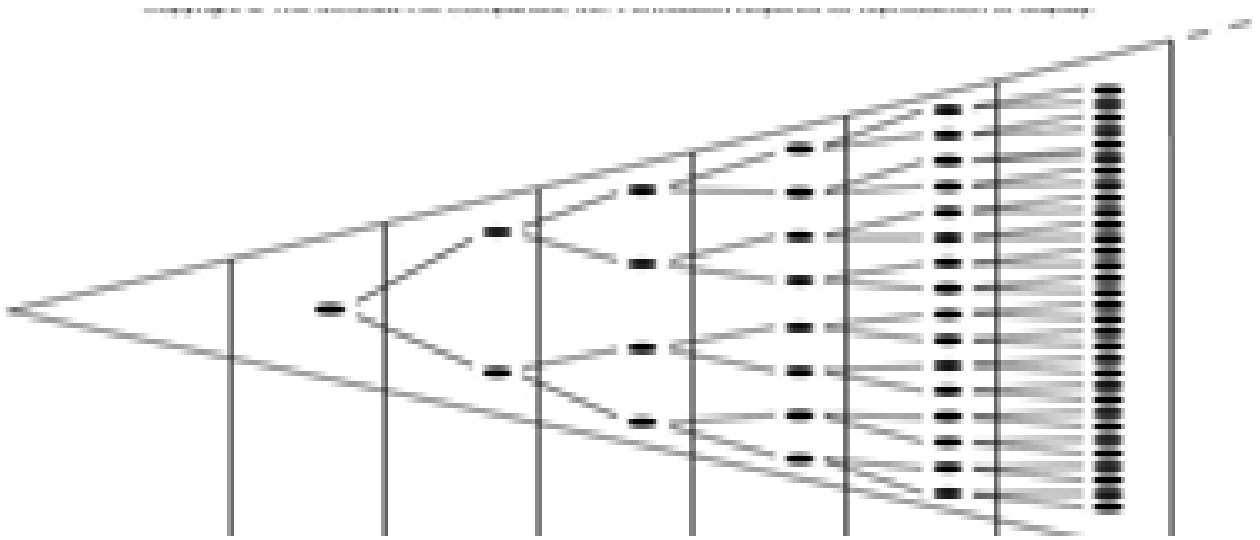




| Number of cells | 1 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|---|---|---|
| Number of generations | | 1 | 2 | 3 | 4 | 5 |
| Exponential value | | $2^1$ (2×1) | $2^2$ (2×2) | $2^3$ (2×2×2) | $2^4$ (2×2×2×2) | $2^5$ (2×2×2×2×2) |

Q/- what is the generation number of bacteria when the number of cell is 8

- If the generation number of bacteria was 4 what is the Exponential value?



## Bacterial growth curve

- All microorganisms undergo similar growth patterns

Each growth curve has 4 phases:

1- Lag phase

2- Log phase

3- Stationary phase

4- Death or decline phase

Between each phases there is a transitional phase is represent the time require by all the cell before get to inter the new phase.

## 1- Lag phase:

- The number of the population remains constant.
- The bacterial cell increase in their size beyond their normal dimensions.
- In this phase the bacterial cell is metabolizing but there is a lag in the cell division.
- Microorganism start to adapted itself to the environment.

## 2- Log phase (logarithmic phase or exponential phase):

- The bacteria multiply at the fastest rate possible under the conditions provided.
- Most research is performed on cells during log phase

- the number of cells increases as an exponential function of 2n (i.e. $2^1$ , $2^2$ , $2^3$, $2^4$ ,$2^5$ and so on).

## 3- Stationary phase:

- Growth levels off.
- Cells per volume does not increase or decrease.
- Growth rate = Death rate.

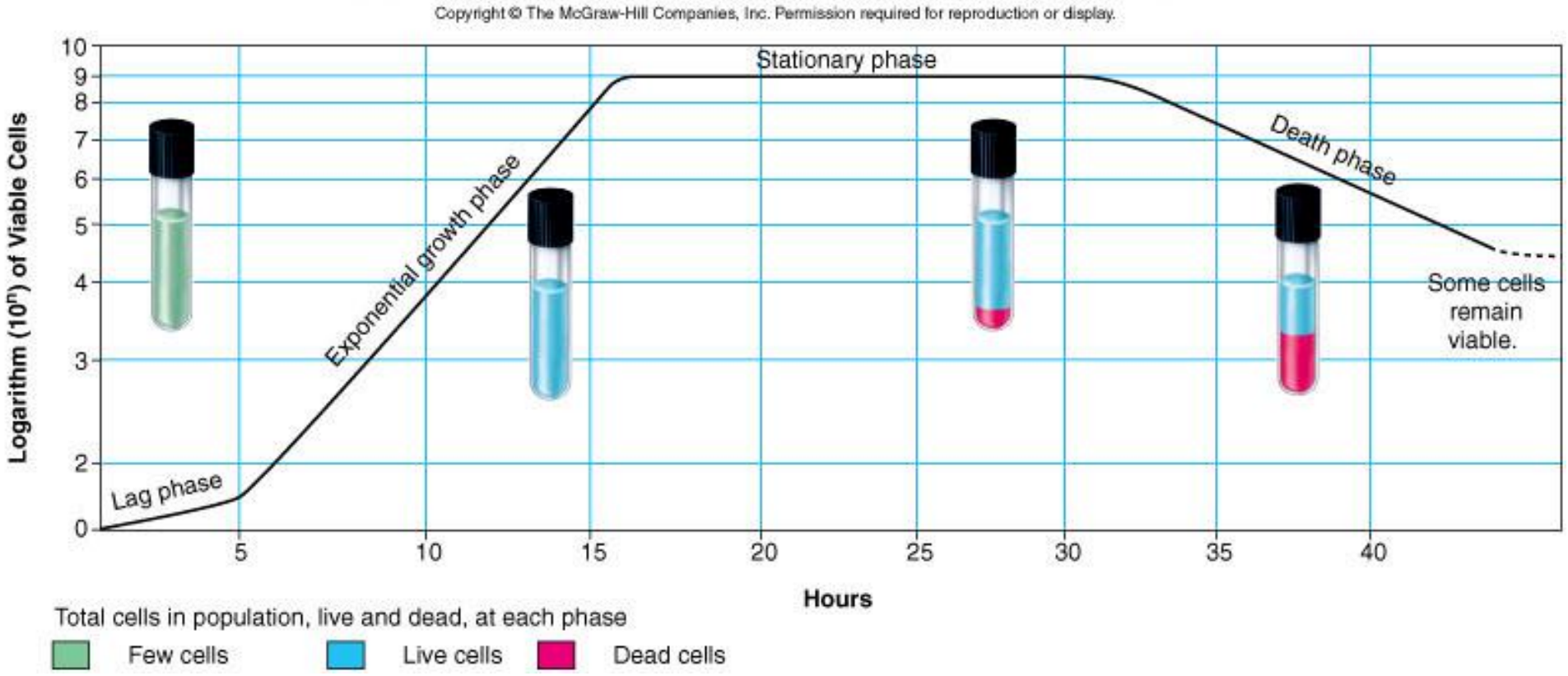
**Due to**

- Depletion of nutrients
- Increase in waste products

## 4- Death phase:

- The number of deaths exceeds the number of new cells formed
- Cells per volume decreases
- Due to
- Very low concentrations of nutrients
- Very high concentrations of waste products

# Standard Growth Curve



## Nutritional Requirements for Microorganisms:

**-Water** (preservation of a microbial culture from drying)

**-Energy:** Phototroph- Energy from sun light

Chemotroph- Energy from chemicals

Heterotroph- from carbohydrate and other organic material

**-Carbon:**

- Autotrophs- carbon from carbon dioxide- inorganic carbon
- Heterotroph- carbon from organic carbon

- e.g. Carbohydrates, lipids, protein.

- **Essential elements**

- Hydrogen (H), Sulfur (S), Oxygen (O), Phosphorous (P)
- Nitrogen (N)

- commonly supplied as ammonia (NH4)
- some microbes fix atmospheric nitrogen (N2)

**- Trace elements**

- Required in small amounts
- Copper (Cu), Zinc (Zn), Selenium (Se)

# Environmental requirements for growth

- Temperature, pH, Oxygen, Carbon dioxide, Osmotic pressure, Hydrostatic pressure

- **Temperature**

- Psychrophiles – less than 20 ºC
- Psychrotroph- 0-40 (20) ºC
- Mesophiles – 20 - 45 ºC
- Thermophiles – 45 – 80 ºC
- Extreme thermophiles – more than 85 ºC

- **pH (**- log [ H+ ])

  Low pH = acid, High pH = basic or alkaline
- Acidophiles - below pH 5.5
- Neutrophiles – at pH 6 – 8
- Alkalophiles – above pH 8

- **Molecular oxygen**

Microbe vary greatly in sensitivity to oxygen.

- Aerobes – microbes which require oxygen.
- Facultative anaerobes – microbes which can grow in presence or absence of oxygen.
- Obligate Anaerobes – which do not utilize oxygen and are killed by oxygen.
- An aerotolerant anaerobe is an organism that tolerates the presence of oxygen but does not require it for growth.
- Microaerophiles – required 3 – 15 % oxygen.

# Chemical Requirements for Growth: **Oxygen**

## $O_2$ requirements vary greatly

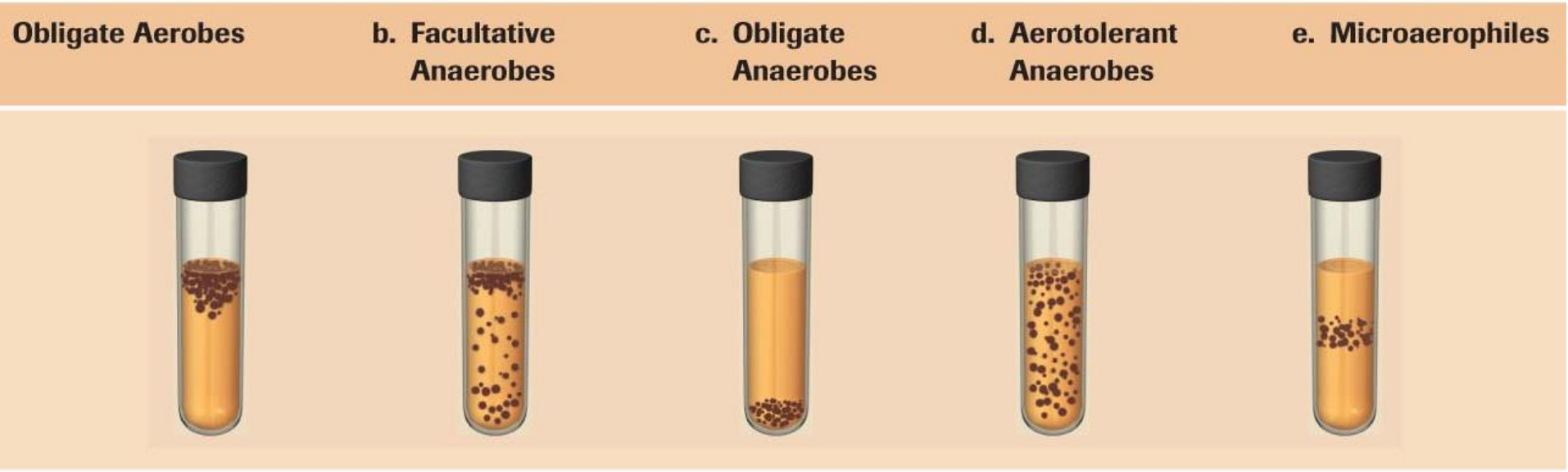



*Table 6.1:* The Effects of Oxygen on the Growth of Various Types of Bacteria

- **Carbon dioxide**

- Capnophiles : 3 – 10 % carbon dioxide
- Many microaerophiles are also capnophiles

**Capnophiles** are microorganisms that grow in the presence of high concentrations of carbon dioxide (CO2). Cultured in a candle jar

- *E.g: Campylobacter jejuni*
- Use candle jar, $CO_2$-generator packets,
- or $CO_2$ incubators

## Quantitative methods for measuring growth of bacteria:

The growth of bacteria can be determined by numerous techniques based on one or more of the following types of measurement:

1- **Cell count**

a- microscopy or by using electronic particle counter.

b- colony count or number (plate count method, MPN)

**2- Cell mass**

a- weighting (dry weight)

b- Measurement of cell nitrogen

c- Indirectly by turbidity with culture , directly without culture

3- **Cell activity-** indirectly by relating the degree of biochemical activity to the size of population Such as measurement of utilizing O2

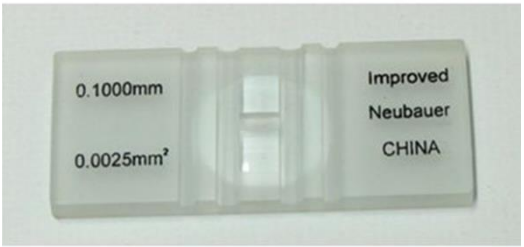
## Direct methods :

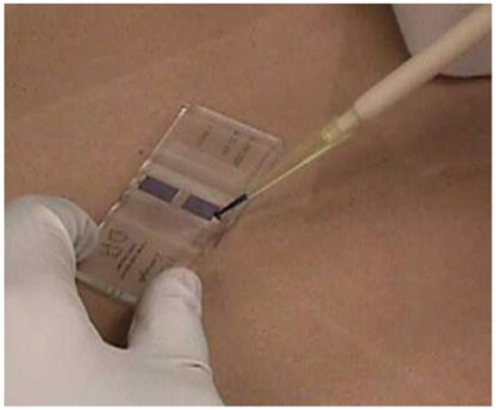
With direct methods we count individual cells or colonies that are assumed to have apart or arise in through the division of a single cell.

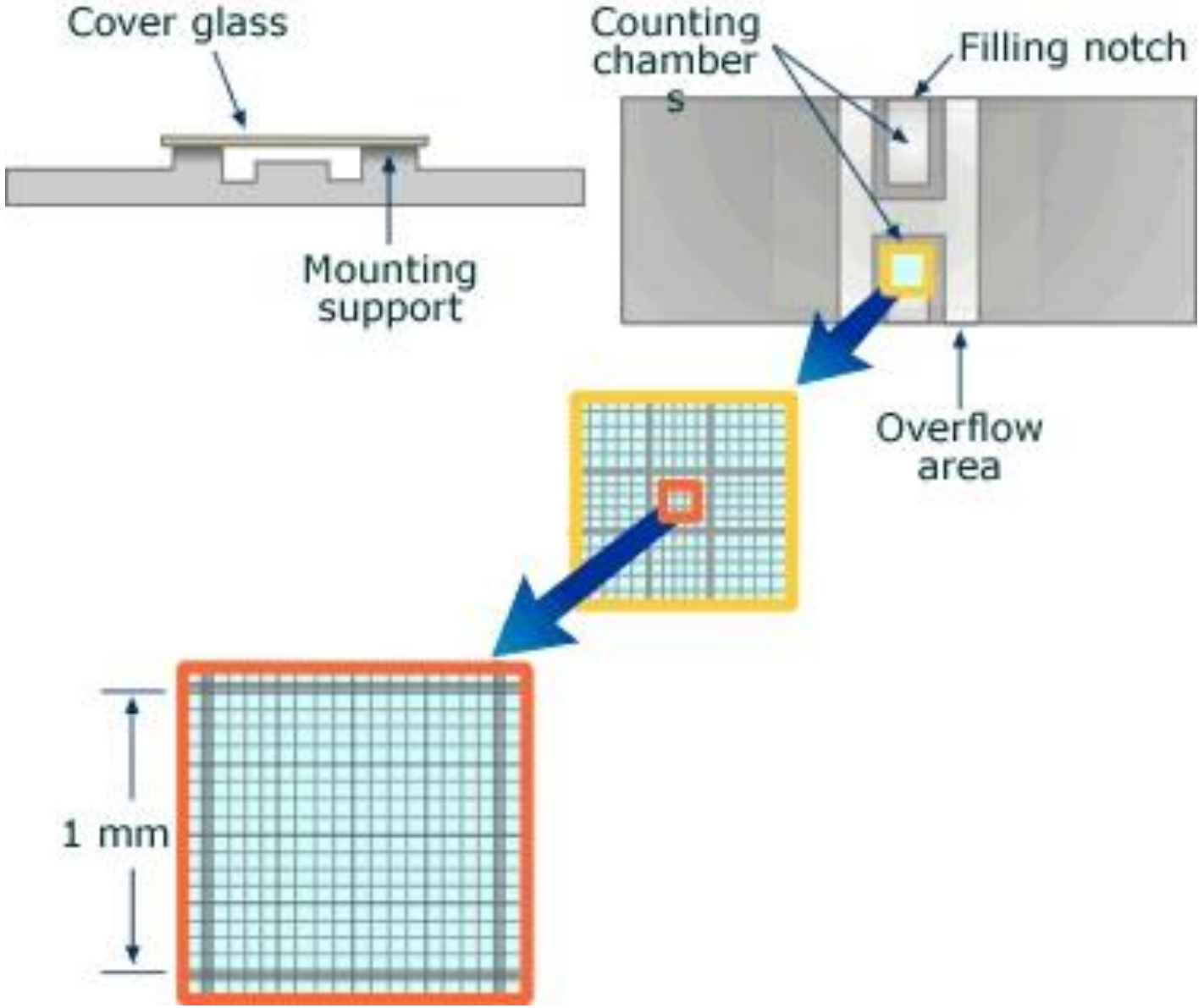
## 1- Counting Chamber (Hemocytometer) :

The hemocytometer is a specialized microscope slide used to count cells.

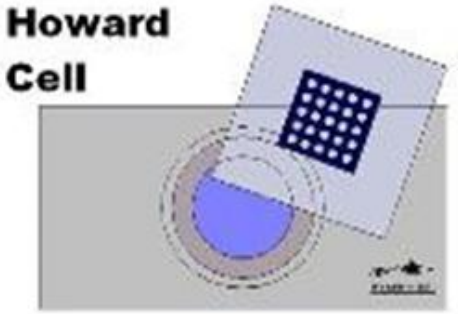
The center portion of the slide has etched grids (H) with precisely spaced lines.

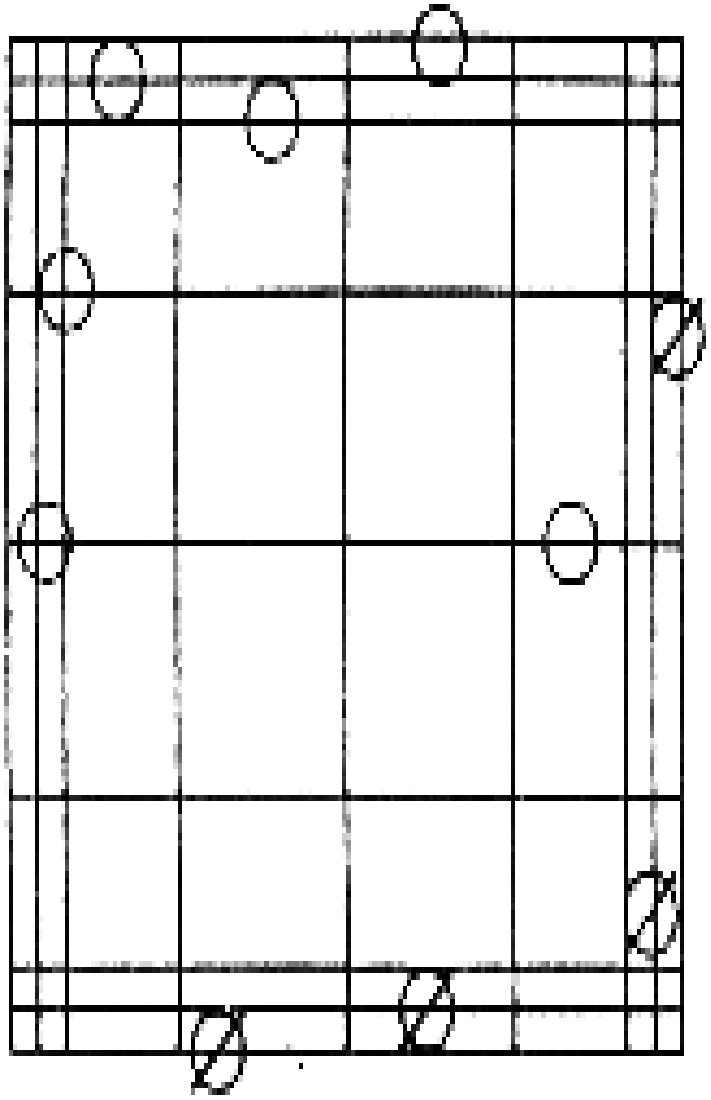



Cover glass
Mounting support
Counting chambers
Filling notch
Overflow area
1 mm

# CORNER SQUARE (ENLARGEMENT)



Count cells on top and left touching middle line (○).
Do not count cells touching middle line at bottom and right (Ø).

# Steps of Counting of cell using hemocytometer

1- percentage of viable cell= Total viable cell/ total cell *100

2-Average of cell per square = total viable/ average

3- Dilution factor =Final volume / volume of cell

4- Concentration ( viable cells/ ml)=Average of cell* dilution factor*

10000 ($10^4$)

## 2- Coulter Counter :

electronic counting (this machine detects the difference in current as individual microorganisms pass through a small orifice).

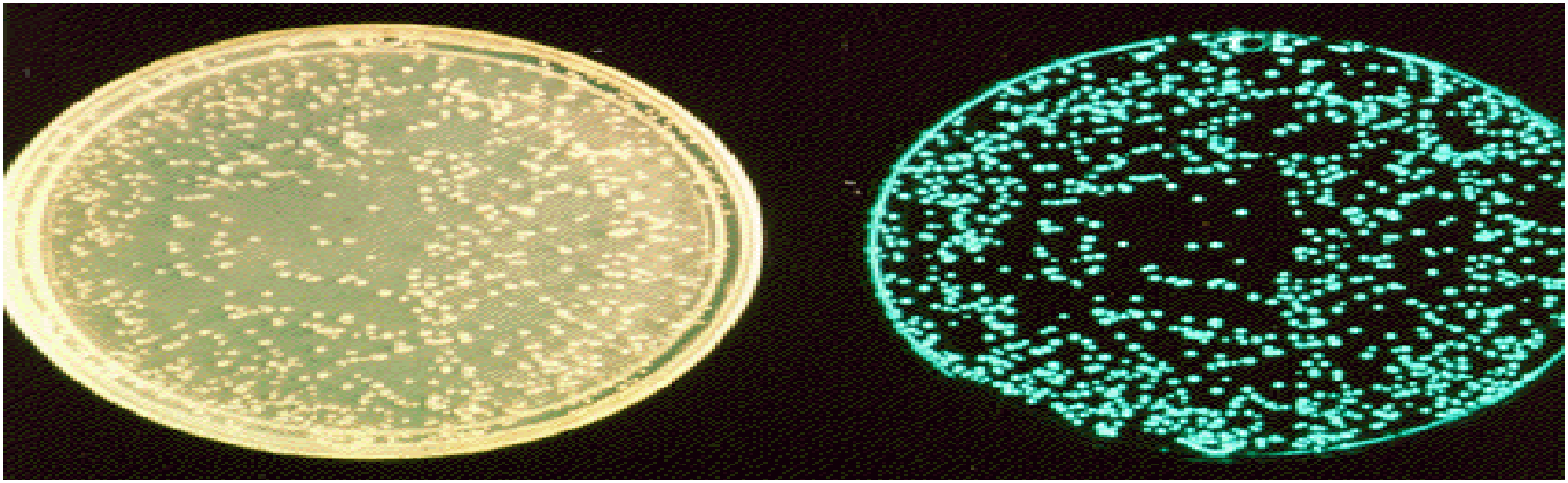
It is Very fast , easy to use but;

Very EXPENSIVE.

# 3- Viable count assays (Colony Counting) :

Colony counting after plating dilutions of the sample onto growth medium.

This is the method we will be using to quantify our samples.



Count only those cells capable of growing

Viable counts can be accomplished by such techniques as pour plating. Assumption each viable cell gives rise to a colony.

**tow viable count assays:**

1 - Spread plates 2- Pour plates.

**The advantages** of these methods are that

- The colonies stay small and compact.

- The plates with a lot higher concentration because the colonies will not be touching one another.

The surface count plate method gives reliable and consistent results in spread plate

**The main disadvantage :**

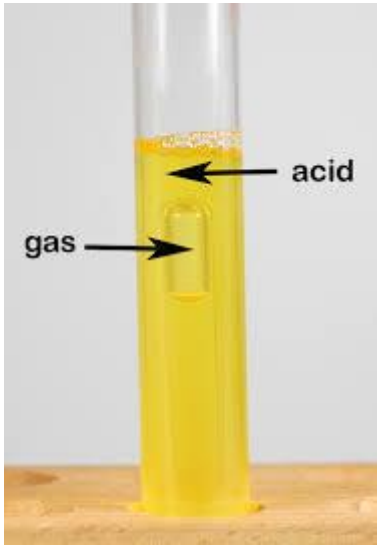
**-I**s the difficulty in keeping the agar hot enough to keep it from setting up until pour it and cool enough to not heat shock or kill the bacteria.

-Count require at least a few hours, usually overnight, for incubation

# Indirect Method :

Indirect methods often rely on the results of metabolic tests or other growth characteristics. And it's to:

- Measurement of metabolic activity.
  - -Gas or Acid Production.




**- Turbidity using a spectrophotometer.**

spectrophotometry, using a spectrophotometer .

**These Indirect counts depend on:**

- The effects of the organisms to estimate their numbers.

- As organisms grow they make the nutrient broth turbid.

- This turbidity can be measured with a colorimeter

# Assessing Microbial Metabolism Using a Simple Oxygen Consumption Assay

information

Assay Format:

The assay is a simple ‘mix and measure’ test:

1. Microbes are dispensed into the wells of a 96 well plate in 100 µl volumes in the appropriate growth medium.
2. 10 µl of MitoXpress(-Xtra probe is added to each well).
3. 100 µl of mineral oil is added to exclude ambient O2
4. The plate is measured kinetically at the required temperature.
5. Oxygen profiles are then related to metabolic activity.

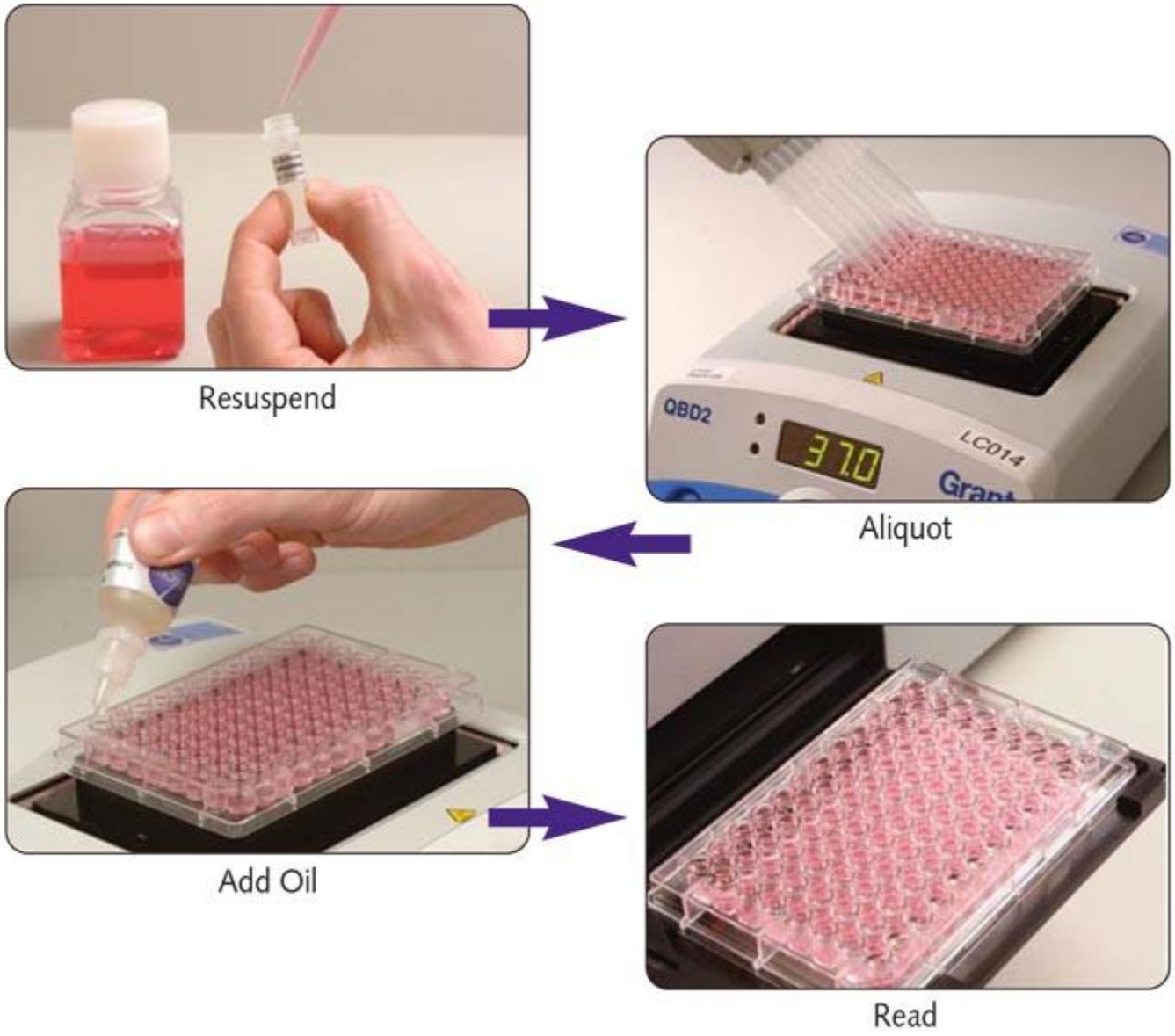



**Figure 1:** Flow diagram showing preparation and use of MitoXpress® Xtra - Oxygen Consumption Assay (HS Method)