B Pharm, 3rd Semester

PHARMACEUTICAL MICROBIOLOGY

BP 303 T

Unit-1

Isolation & Growth Curve of Bacteria

Dr. Meenakshi Gupta

Senior Assistant Professor

University Institute of Pharmacy

C. S. J. M. University Kanpur U.P

Contents

- Isolation for Pure Culture
- Isolation techniques
  - Streak plate method
  - Pour plate method
  - Spread plate method
  - Roll tube method
  - Micromanipulator method
- Maintenance & Preservation for Pure cultures
- Preservation methods for pure cultures,
- Growth Curve
- Quantitative measurement of bacterial growth (total & viable count).

Isolation
for
Pure cultures

Isolation of Bacteria

- Microorganisms are generally found in nature (air, soil and water) as **mixed populations**.
- To study the characteristics and role played by a specific microorganism in its environment, one must isolate the same in pure culture.
- **Mixed Culture**-clinical and environmental samples contain several different types of microbial species
- **Pure Culture**: is a population of cells growing in the absence of other species or Contains a single microbial species. A pure culture may originate from a single cell.

Isolation of Bacteria

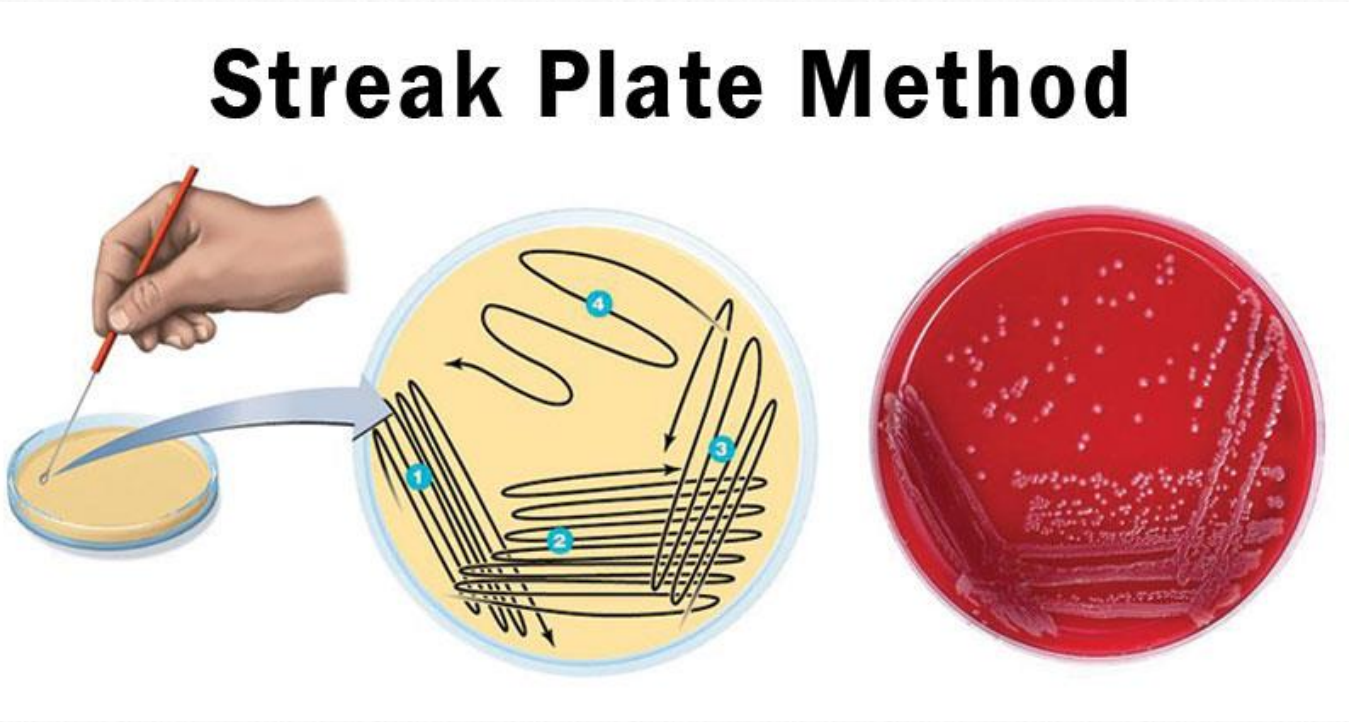
- A pure culture is usually obtained from a mixed culture by transferring a small sample into new, sterile culture medium in such a way as to disperse the individual cells into the medium surface or by diluting the sample many times before inoculating the new medium
- To obtain a **pure culture**, individual organisms must be **isolated**. The most common method of isolation is the streak plate , in which a sterile loop is inserted into a sample and streaked onto a plate in a pattern, to obtain individual colonies
- Colony: A group of descendants of an original cell.

Isolation techniques

- Streak plate method
- Pour plate method
- Spread plate method
- Roll tube method
- Micromanipulator method

Streak plate method

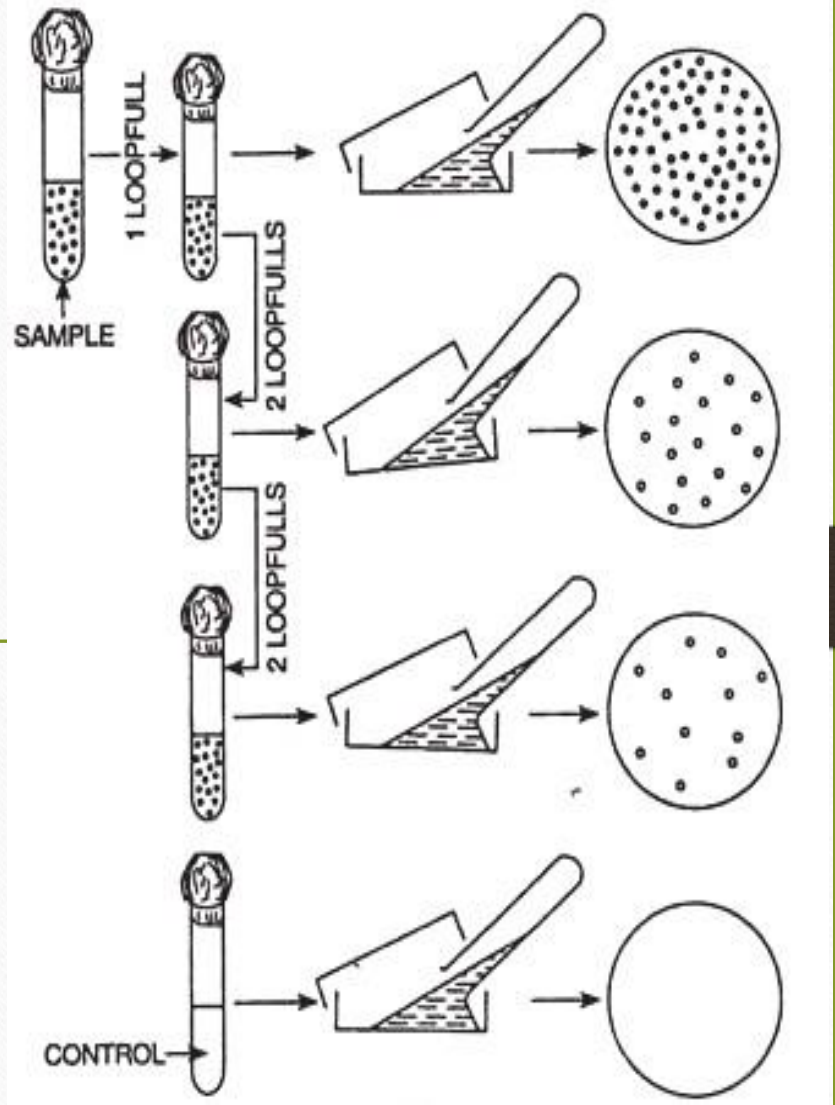
Streak Plate Method



- First the loop/needle is sterilize by flame to make red hot and allow it to cool for 30 seconds.
- small amount of mixed culture is placed on the tip of an sterile inoculation loop/needle and is streaked across the surface of the agar medium in such a away to provide series of dilution
- The purpose is to thin out inoculum to get separate colonies
- Further subculturing can be done by streaking well isolated colonies from streak plate to new plate

https://microbenotes.com/streak-plate-method-principle-methods-significance-limitations/

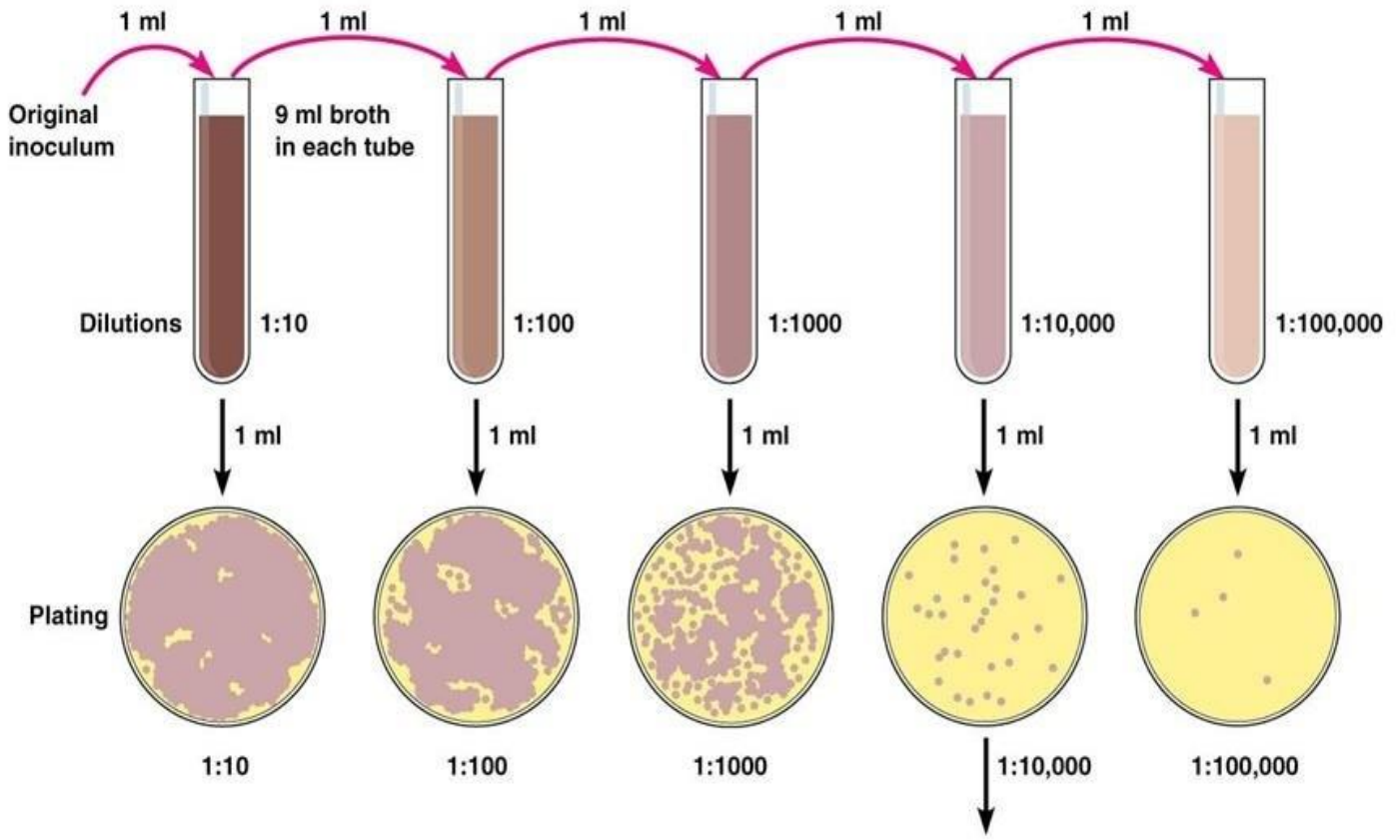
Pour plate method



https://www.biologydiscussion.com/organism/culture-organism/obtaining-pure-culture-of-microorganisms-6-methods/55042

Pour plate method

- The bacterial culture(inoculum) and liquid agar medium are mixed together.
- After mixing, the inoculated culture media is poured into sterilized petri dishes and allowed to solidifying.
- Then it is incubated
- After incubation colonies appear on the surface.



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

Pour plate method

- **DISADVANTAGES-**
- **Tedious and time consuming method**
- **Microorganism trapped beneath the surface of medium hence difficult to isolate subsurface colonies**
- **microbes are subjected to heat shock because liquid medium maintained at 45℃**
- **Unsuitable- Psychrophile**

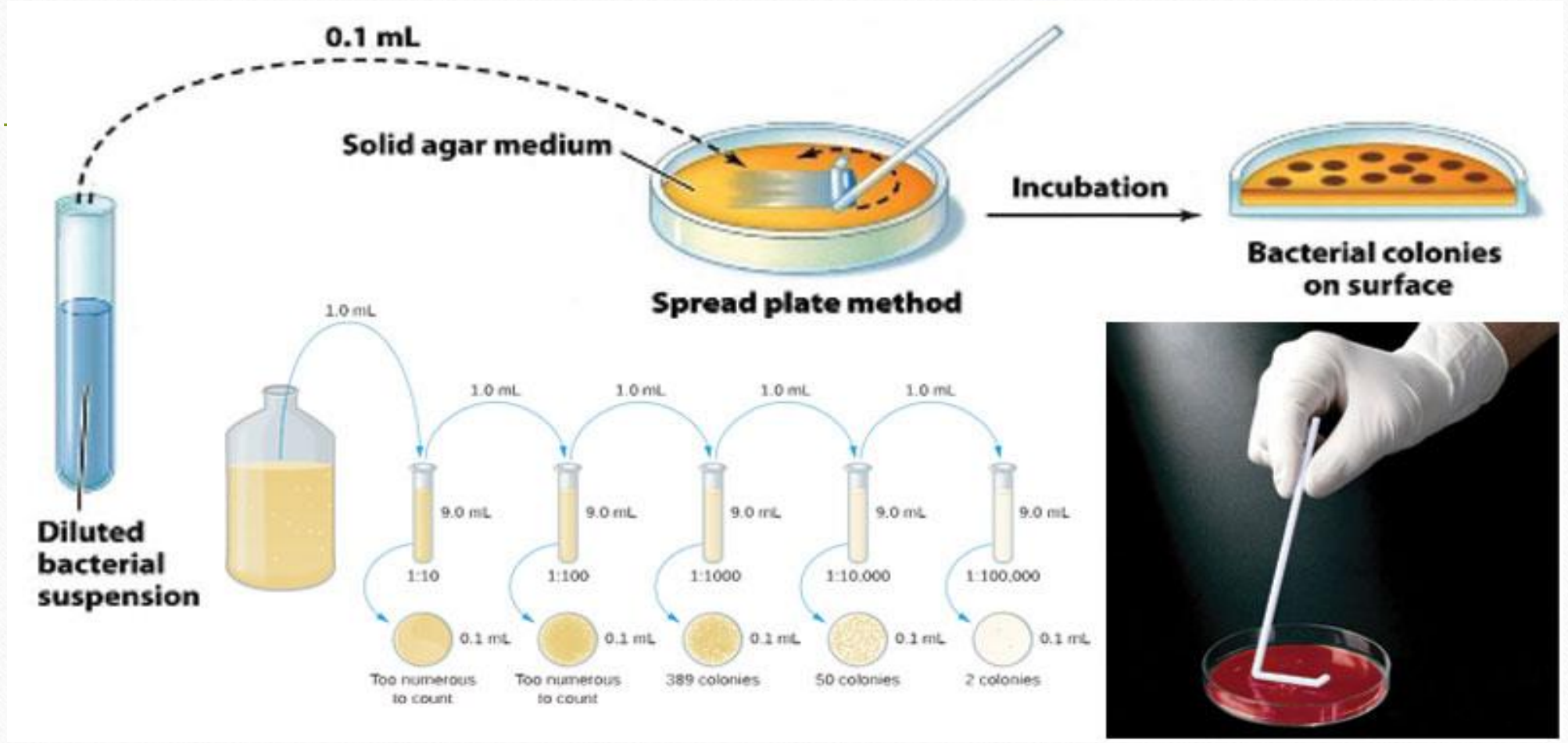
Spread plate method

- It is simple & best method to isolate the pure colonies
- Bacterial inoculum is mixed with normal saline and serially diluted.
- 0.1 ml of diluted inoculum is taken and is placed on the surface of the agar plate.
- Inoculum is spreaded evenly over the surface by using L-shaped spreaded and Incubate the plates
- After incubation, colonies are observed on the agar surface

ADVANTAGES

only surface colonies are formed & micro-organisms are not exposed to higher temperature

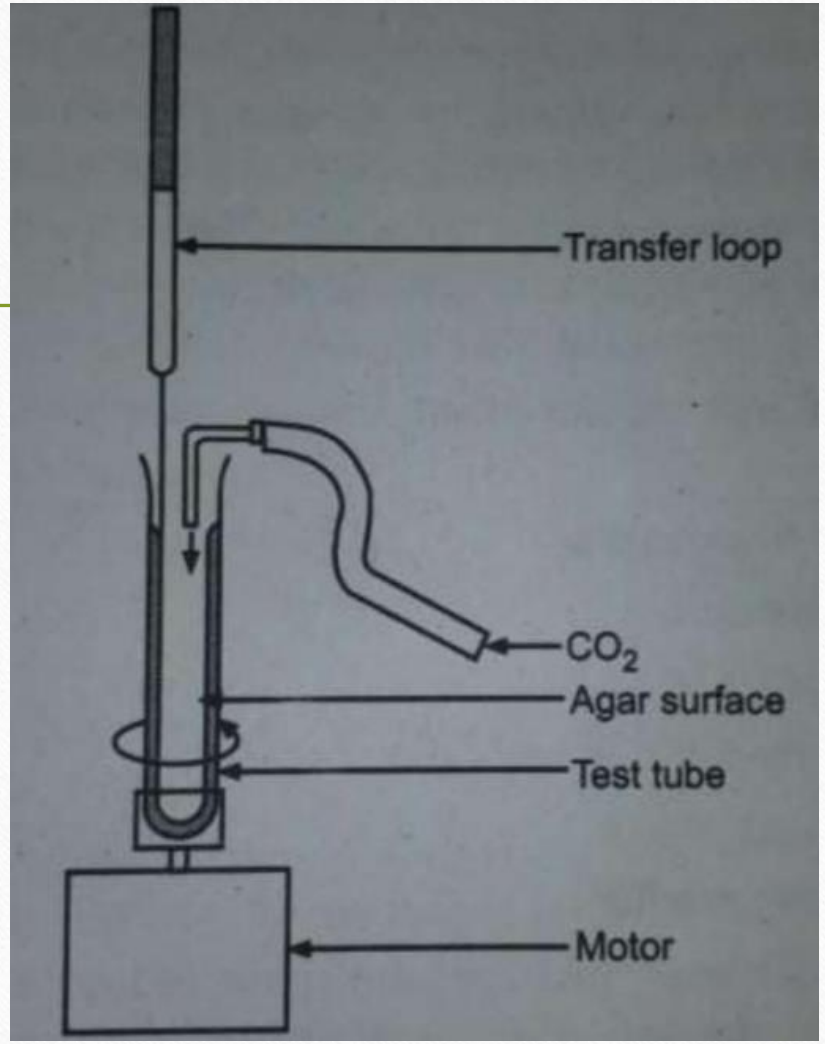
Spread plate method



https://microbenotes.com/spread-plate-technique/

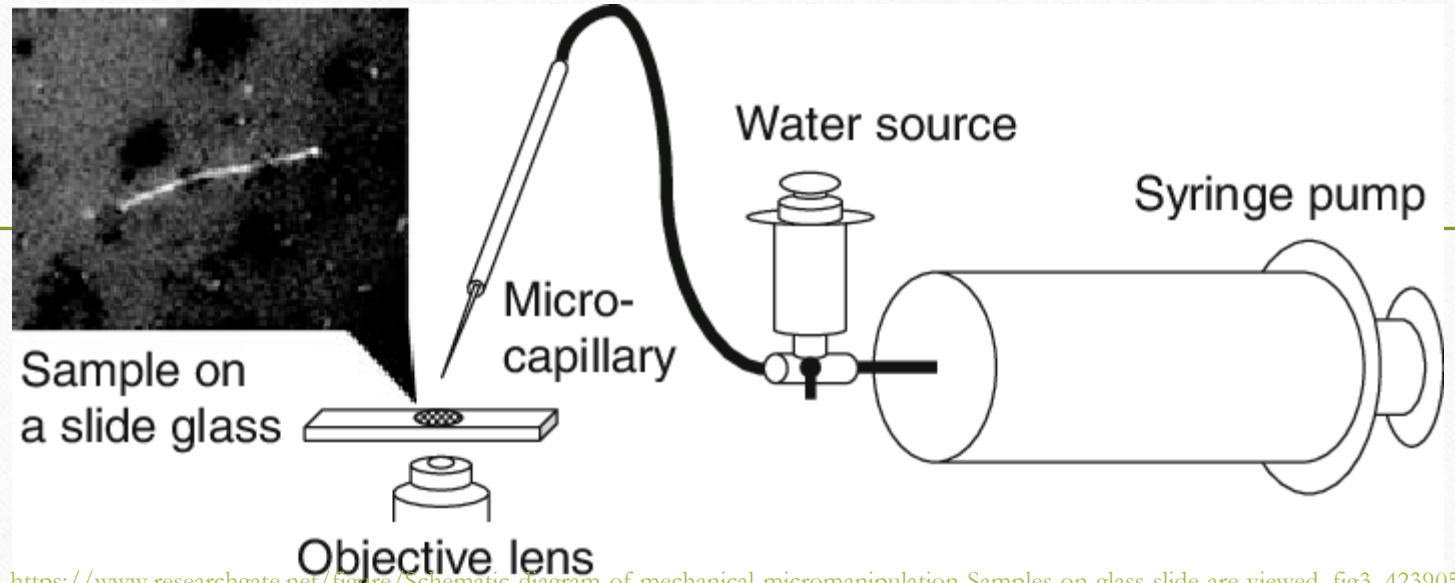
Roll tube method

- This method utilize thin layer agar medium distributed over the internal surface of test tubes along with an anaerobic atmosphere for the isolation of obligately anaerobic bacteria
- By displacing the air in the culture tube with an oxygen-free gas, such as CO2, H2, N2, exposure of bacteria and culture medium to air is avoided
- CO2 is the gas of choice because it is heavier than air, relatively cheap



https://www.slideshare.net/kruti495/isolation-and-preservation-of-pure-baterial-culture

Micromanipulator method



https://www.researchgate.net/figure/Schematic-diagram-of-mechanical-micromanipulation-Samples-on-glass-slide-are-viewed_fig3_42390514

- **This method permit to pick out a single cell from a mixed culture.**
- **This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation.**
- **the single cell of microbe sucked into micropipette and transfered to large amount of sterile medium.**

Enrichment Culture Method

- This method is used to isolate microorganisms which are present in relatively small numbers or that have slow growth rates compared to the other species present in the mixed culture
- It utilize specially designed cultural environment by incorporating a specific nutrient in the medium and by modifying the physical conditions of the incubation.
- The medium of known composition and, specific condition of incubation favors the growth of desired microorganisms but, is unsuitable for the growth of other types of microorganisms

Maintenance & Preservation for Pure cultures

Maintenance & Preservation for Pure cultures

- Once a microorganism has been isolated in the pure culture form, it is important to maintain the
  - **viability** (ability to survive or live)and
  - **purity** of the microorganism (keeping the pure culture free from contamination)
- **Sub-culturing**- is the periodic transfer of pure cultures into a fresh medium to allow continuous growth and viability of microorganisms.
- The transfer is always subject to **aseptic conditions** to avoid contamination
- repeated sub-culturing is time consuming and it becomes difficult to maintain a large number of pure cultures

Preservation for Pure cultures

- **Preservation**-refers to maintaining pure culture for extended periods in viable condition without any genetic change
- **Objectives of Preservation** –
  - To maintain isolated pure culture for extended periods in a viable conditions
  - To avoid contamination
  - To restrict Genetic Mutation

Preservation for Pure cultures

- **Importance of Preservation-**
- **99% of bacterial populations are of economic importance for human beings and plants**
- **Involved in maintaining soil fertility, food industry, in sewage treatment, in medical industry etc.**
- **So the preservation of bacteria is one of the most profitable practice economically**
- **Academic purpose**
- **Research Purpose**
- **Biotechnology field**
- **Fermentation Industry**

Preservation methods

- Periodic transfer to fresh medium
- Storage at low temperature
- Storage in sterile soil
- Preservation by overlaying culture with mineral oil
- Lyophillization or freeze drying

Preservation methods:
Periodic transfer to fresh medium

- To maintain the pure culture,sub-culturing is done in freshly prepared culture media periodically
- The culture medium, storage temperature, and time interval at which the transfers are made vary with the species .
- The technique should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible.
- heterotrophs remain viable for several weeks to months on a medium like Nutrient Agar.

Preservation methods:
Storage at low temperature

1. **REFRIGERATION**
2. **CRYOPRESERVATION**

Preservation methods :Refrigeration

- Pure cultures can be stored at 0-4°C (in refrigerators or in cold-rooms).
- This technique can be used for short duration only(2-3 weeks for bacteria and 3-4 months for fungi) as the metabolic activities of the microorganisms are greatly slowed down but not stopped.
- Thus their growth continue slowly, nutrients are utilized and waste products released in medium.
- This results in finally the death of the micro

Preservation methods: Cryopreservation

- Cryopreservation means preservation (living cells and tissues) by subjecting to extremely low temperatures i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C
- Cryopreservation helps long time storage of pure cultures.
- In this method, the pure culture are rapidly frozen in liquid nitrogen at - 196°C in the presence of stabilizing agents such as glycerol or Dimethyl Sulfoxide (DMSO) that prevent the cell damage due to formation of ice crystals and promote cell survival.
- This technique is used for the species that cannot be preserved by lyophilization and that can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics
- This method is expensive.

Preservation methods: Storage in sterile soil

- Storing organisms in soil fall into two groups

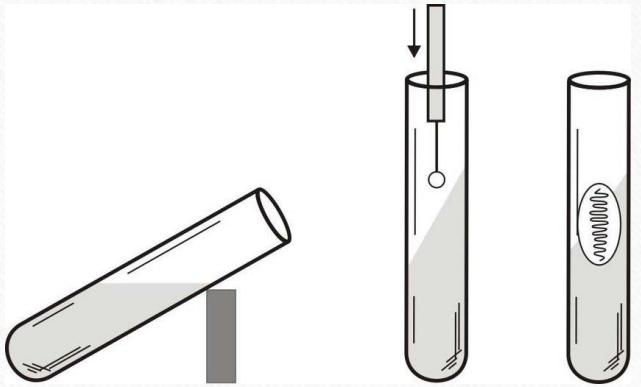
1 sterile soil infested with small amount of inoculum, immediately dried and stored in refrigerator.

2 Soil infested with the organism, than incubated allowing organism to grow thus the mycelium and propagative unit of second generation are preserved.

The soil preservation method is useful for fungi, and by this method actinomycetes are maintained in soil for 4 to 5 years, and there are several bacterial spp which are also maintained in soil for several years.

Preservation by overlaying culture with mineral oil

- This is a simple and economical method of maintaining pure cultures.
- In this technique, sterile liquid paraffin is poured over the slant of culture and stored upright at room temperature.
- The layer of paraffin ensures anaerobic conditions and prevents dehydration of the medium.
- This condition helps pure culture to remain in a dormant state and, therefore, the culture is preserved form months to years



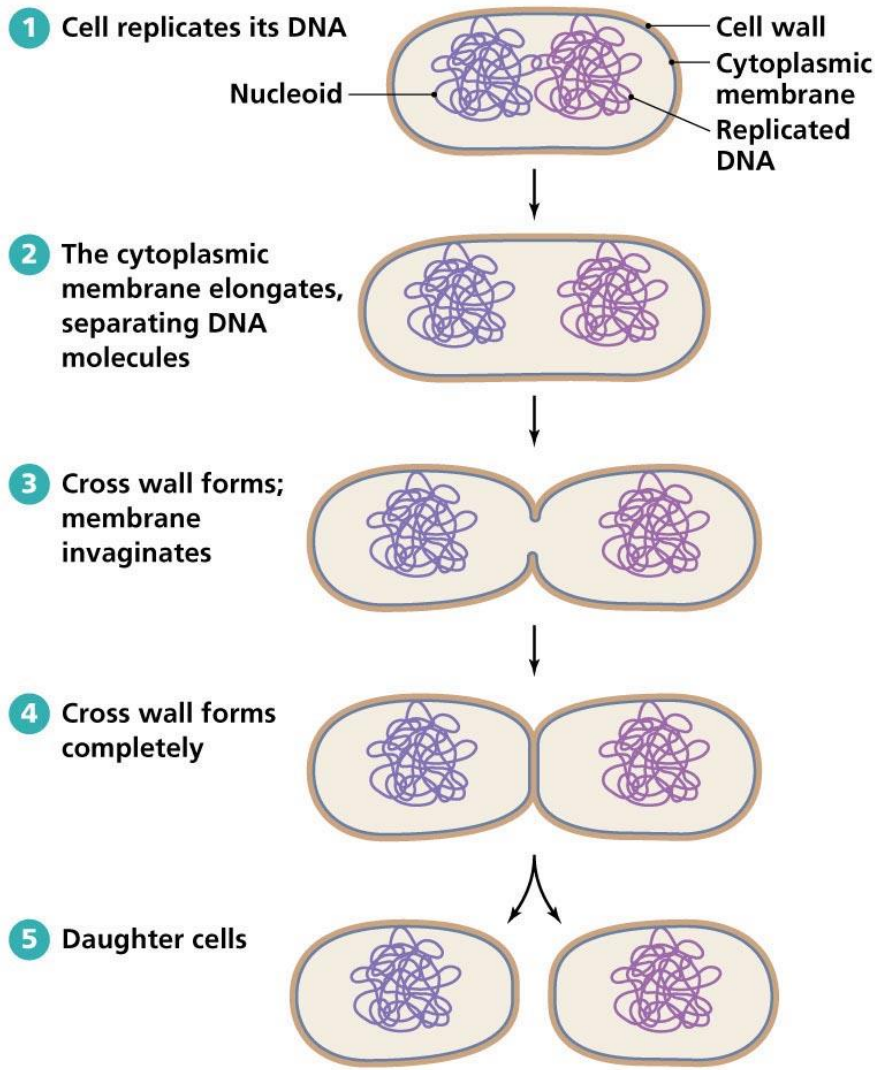
Preservation methods: Lyophilization or freeze drying

- Freeze drying is a process in which a substance is first frozen and then the quantity of the solvent is reduced, first by sublimation (primary drying stage) and then desorption (secondary drying stage)
- Better preservation occurs than with other methods because it reduces the risk of ice crystallization that affect viability
- Lyophilization can maintain viability as long as 30 years.

Bacterial Growth Curve

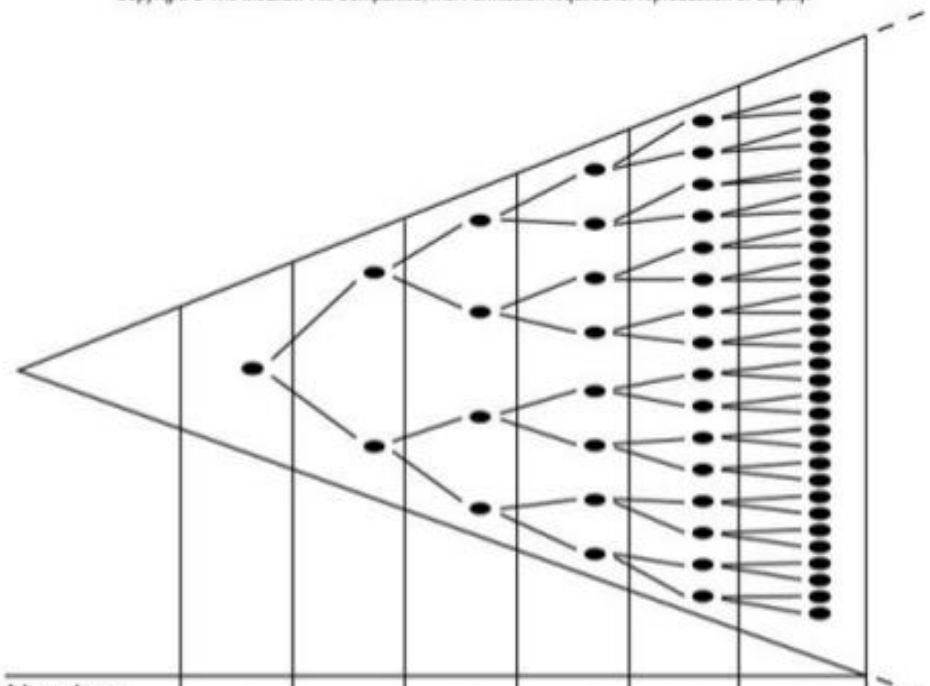
Growth Curve

- **Bacterial Division**- mainly by binary fission. A few bacterial species reproduce by budding or fragmentation.
- **Generation Time**- is the time required for a cell to divide, and double its population. Generation time varies. (E. coli 20 minutes, Most bacteria divide every 1 to 3 hours, Some bacteria require over 24 hours to divide.)



Bacterial Growth

- Growth is by geometric progression: 1, 2, 4, 8, etc. or 2^0, 2^1, 2^2, 2^3.....2^n (where n = the number of generations).
- This is called exponential growth.



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)

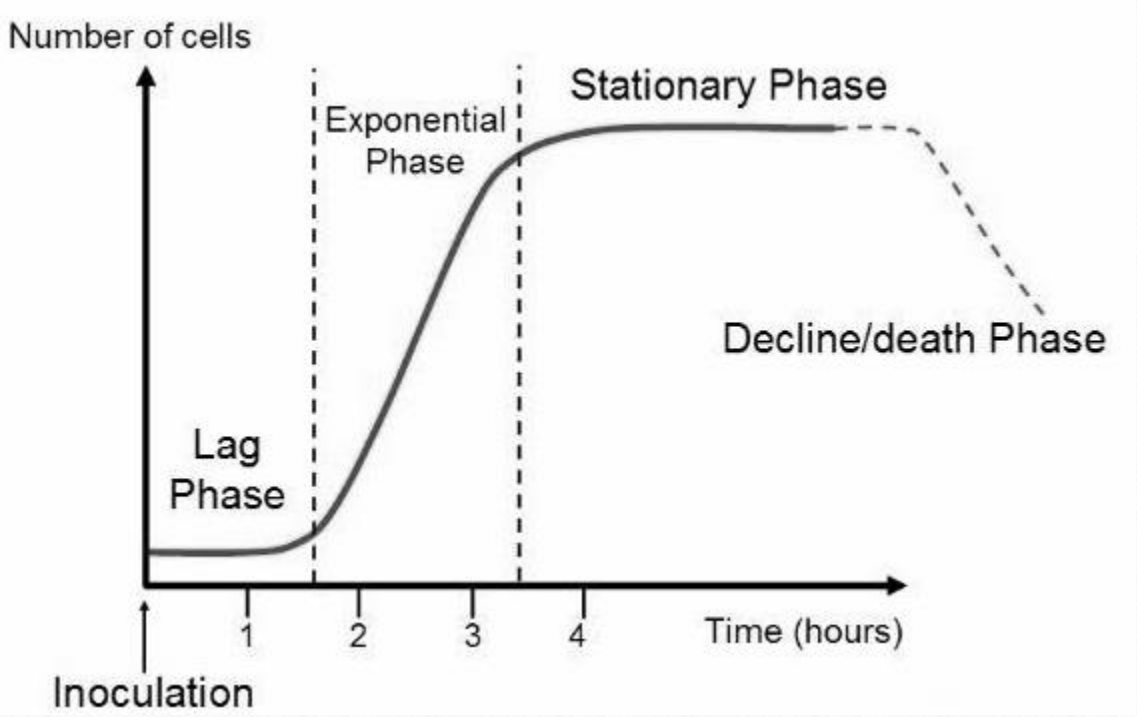
(a)

Bacterial Growth Curve

- **When bacteria are inoculated into a liquid growth medium, we can plot of the number of cells in the population over time.**

Four phases of Bacterial Growth:

- **Lag Phase**
- **Exponential Phase**
- **Stationary Phase**
- **Decline/Death phase**



https://orbitbiotech.com/bacterial-growth-curve-generation-time-lag-phase-log-phase-exponential-phase-decline-phase/

Bacterial Growth Curve

1. Lag Phase:

- There is no appreciable increase in number, though there may be an increase in the size of the cells.
- Little or no cell division occurs, population size doesn't increase.
- Phase of intense metabolic activity, in which individual organisms grow in size. May last from one hour to several days.
- The duration of the lag phase varies with the species, size of the inoculum, nature of the culture medium and environmental factors such as temperature.

Bacterial Growth Curve

2. Exponential Phase/Log Phase:

- **Following the lag phase, the cells start dividing and their numbers increase exponentially or by geometric progression with time.**
- **If the logarithm of the viable count is plotted against time, a straight line will be obtained.**
- **Number of cells produced > Number of cells dying. Cells are at highest metabolic activity.**
- **Cells are most susceptible to adverse environmental factors at this stage.**
  ● Radiation ● Antibiotics

Bacterial Growth Curve

3. Stationary Phase-

- Population size begins to stabilize.
- Number of cells produced = Number of cells dying
- Overall cell number does not increase.
- Cell division begins to slow down.
- Factors that slow down microbial growth: ● Accumulation of toxic waste materials ● Acidic pH of media ● Limited nutrients ● Insufficient oxygen supply

Bacterial Growth Curve

4. Decline/Death phase

- Population size begins to decrease.
- Number of cells dying > Number of cells produced
- Cell number decreases at a logarithmic rate.
- Cells lose their ability to divide.
- A few cells may remain alive for a long period of time.

Quantitative measurement of bacterial growth (total & viable count)

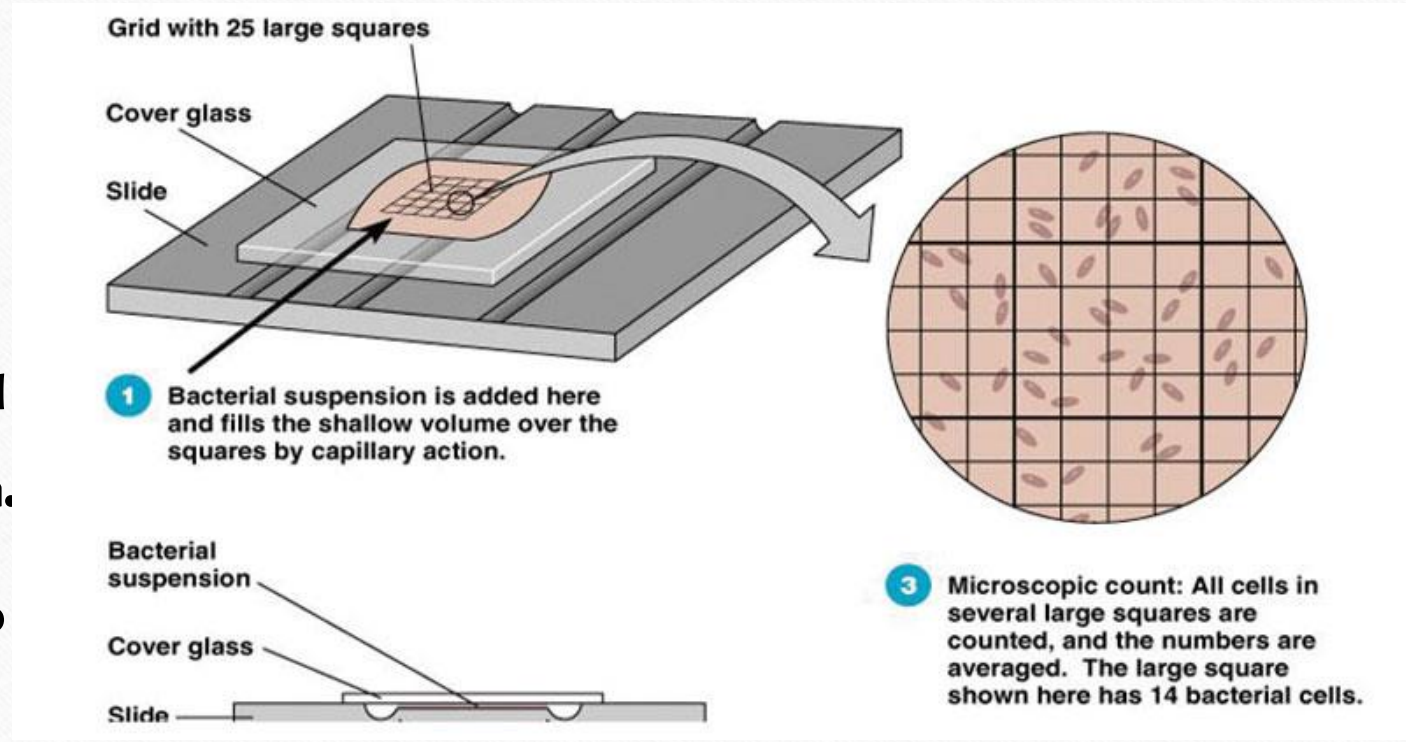
Quantitative measurement of bacterial growth

- The number of bacteria in a clinical sample serves as an indication of the extent of an infection.
- Quality control of drinking water, food, milk, medication, cosmetics and even air relies on estimates of bacterial counts to detect contamination and prevent the spread of disease.
- Two major approaches are used to measure cell number.
  - The direct methods involve counting cells, whereas
  - the indirect methods depend on the measurement of cell presence or activity without actually counting individual cells such as the turbidity (cloudiness) of the culture used to estimate and compare cell densities in a culture

Quantitative measurement of bacterial growth

Direct microscopic cell count

- Counting can be done by use of a slide and a cover slip.
- A drop of the diluted sample is put on the slide with a suitable agent for proper visualization of the sample.
- It is then covered wit a cover slip and put under a microscope and observed at a suitable magnification.
- The number of microbes in the grids is multiplied by the dilution factor to get the number in the original sample



https://microbenotes.com/direct-microscopic-counts/

Direct microscopic cell count

- **Direct cell counts may be divided into:**

  1. Total counts = which include both living cells and dead cells
  2. Viable counts = which count living cells only

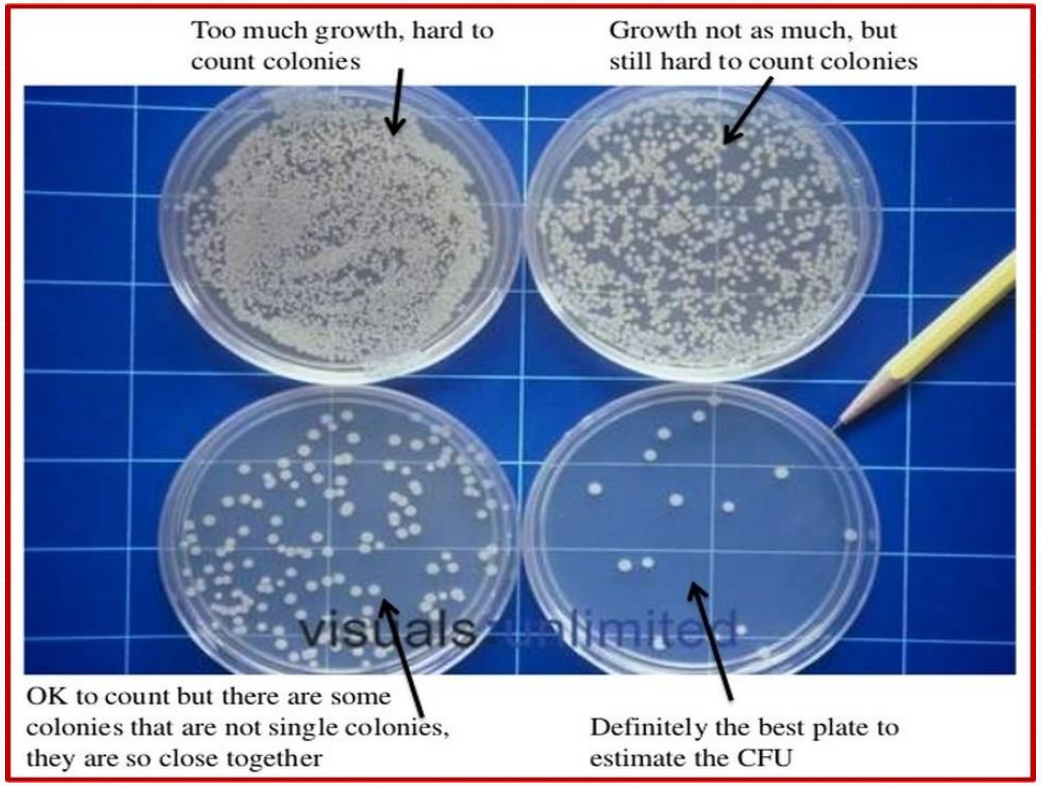
- **In practice, it is not possible to count whole populations of microorganisms.**

- **the cells in a very small sample of culture are counted, and the result multiplied up to give a population density in organisms per cm3 of culture**

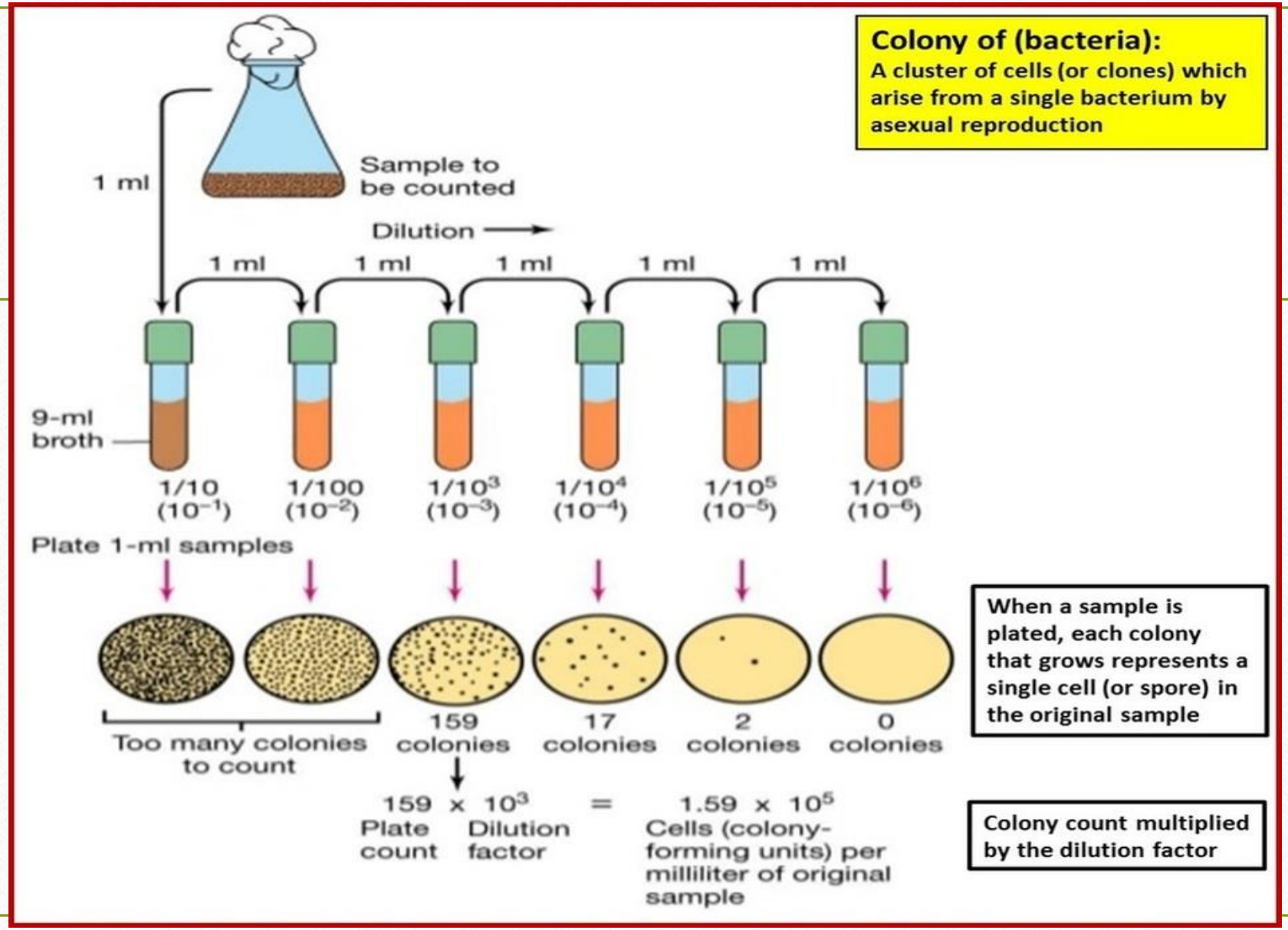
Quantitative measurement of bacterial growth

Petri dish count method

- the sample is diluted and inoculated into petri plates and allowed to incubate
- This method is time taking process one has to wait for the individual cells to grow into colonies
- Then after incubation the grown colonies are counted are multiplied by the dilution factor to get the number of count present in the original sample.
- The results are expressed in colony forming units per milliliter i.e. CFU/ML. The time taken for the cells to grow into individual colonies is called the incubation period.



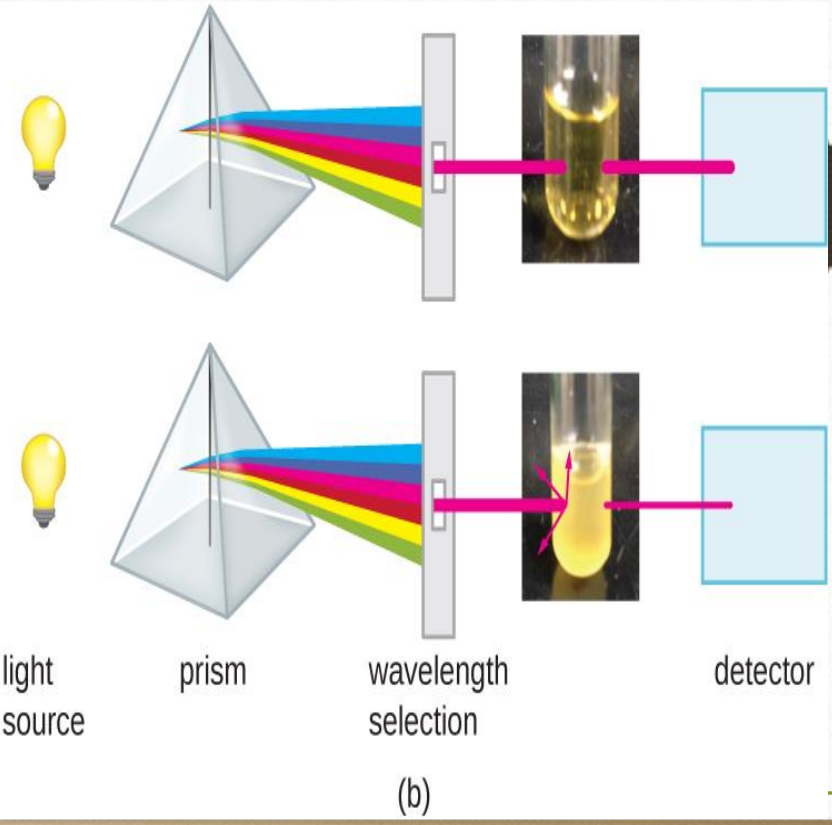
http://loretocollegebiology.weebly.com/measuring-bacterial-growth.html



http://loretocollegebiology.weebly.com/measuring-bacterial-growth.html

Quantitative measurement of bacterial growth :Indirect Method

- As the numbers of bacteria in a suspension increase, the turbidity also increases and causes less light to reach the detector.
- The decrease in light passing through the sample and reaching the detector is associated with a decrease in percent transmission and increase in absorbance measured by the spectrophotometer.



https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(OpenStax)/09%3A_Microbial_Growth/9.1%3A_How_Microbes_Grow#:~:text=Measurement%20of%20Bacterial%20Growth,-Estimating%20the%20number&text=Two%20major%20approaches%20are%20used,without%20actually%20counting%20individual%20cells.

References

- Pelczar, M. J., E. C. S. Chan, and N. R. Krieg. "Microbiology. International edition." 1996 ;Tata McGraw Hill Inc. Page No-133-148
- Kar, A; “Pharmaceutical Microbiology” New Age International Publisher,2008 Page no-149


THANK
YOU