

Ministry of Higher Education
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Isolation and culturing bacteria Grade 3rd (2018-2019) lab.5

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Growth of bacterial population

Growth of bacterial cultures is defined as an increase in the number of bacteria in a population rather than in the size of individual cells

The growth of a bacterial population occurs in a geometric or exponential manner: with each division cycle (generation), one cell gives rise to 2 cells, then 4 cells, then 8 cells, then 16, then 32, and so forth

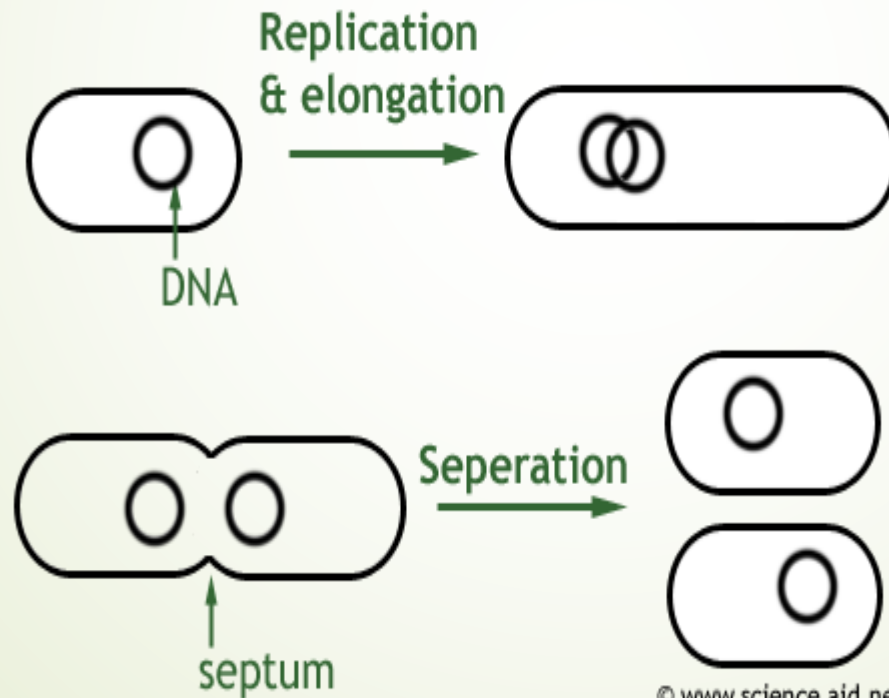
The generation time, which varies among bacteria, is controlled by many environmental conditions and by the nature of the bacterial species. For example

Mycobacterium tuberculosis 12-16 hours

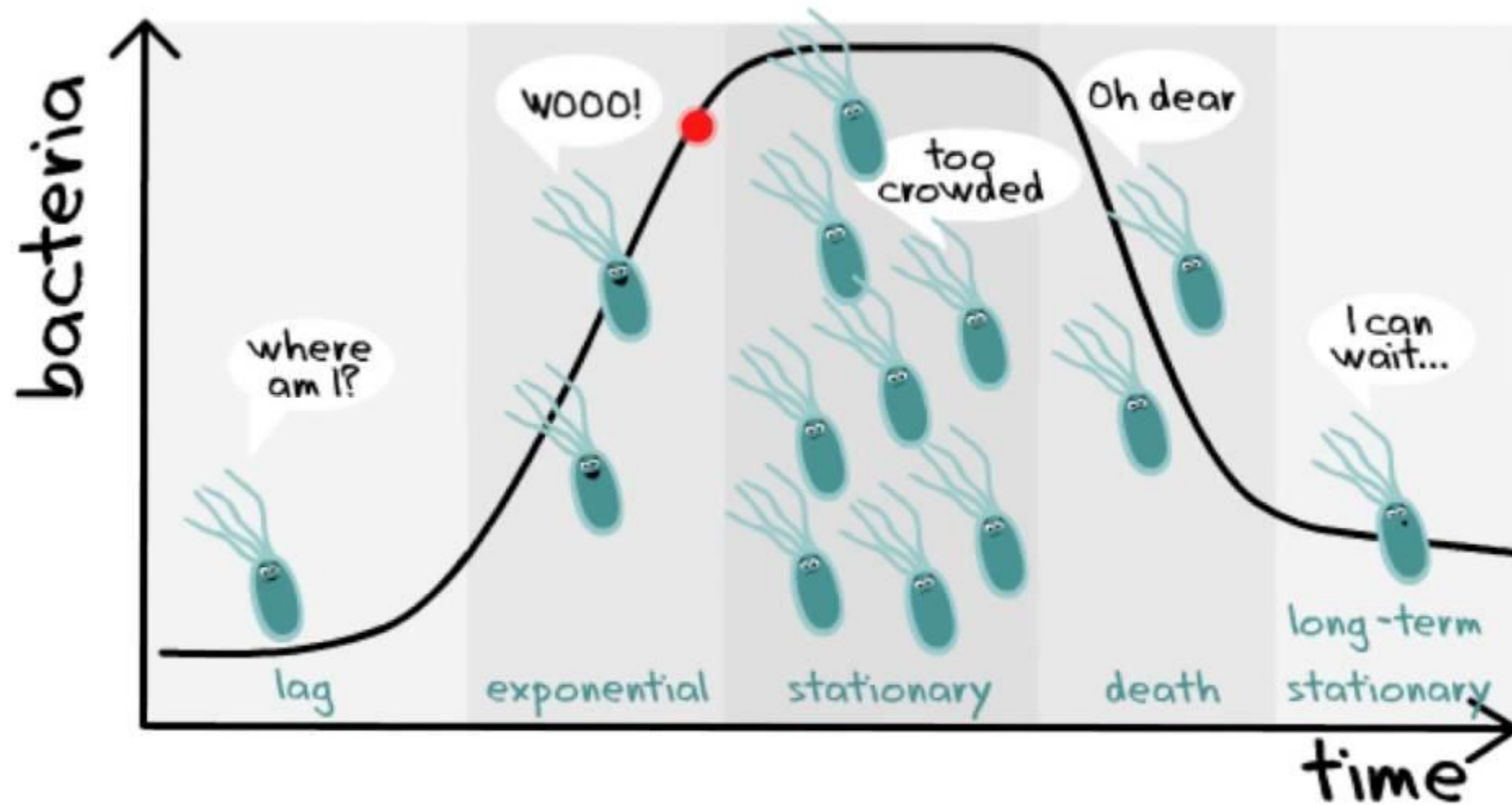
Clostridium perfringens, 10 minutes

Escherichia coli 20 minutes

Bacteria reproduce by an asexual process called binary fission. First, the DNA replicates and the cell elongates. In the middle of the elongated cell, a septum forms and this develops into a cell wall that divides two separate cells.



Bacterial Growth Phase

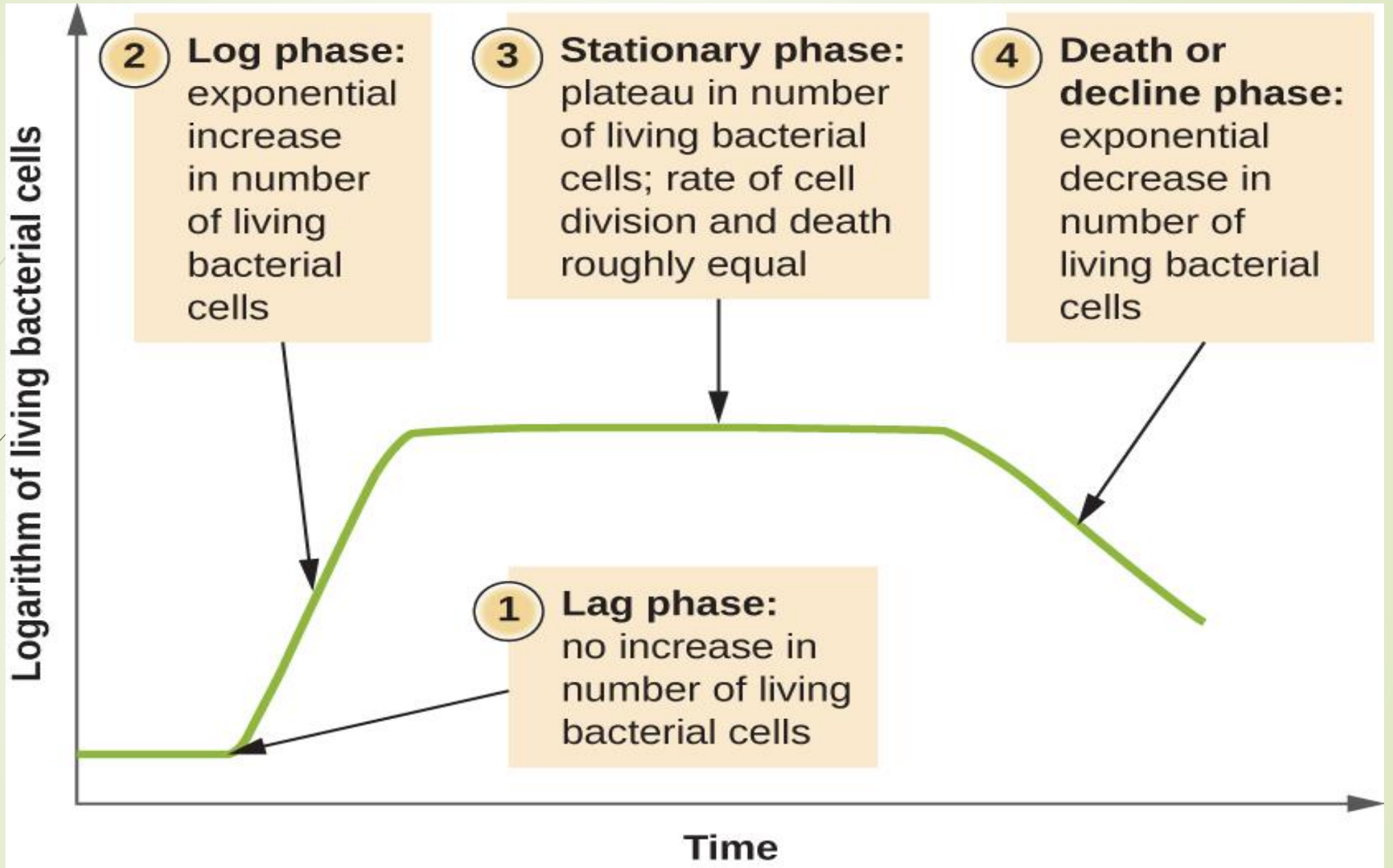


During the lag phase the bacteria adapt themselves to growing conditions and synthesize RNA, enzymes as well as other molecules

The log phase is when the bacteria grow very rapidly

The stationary phase occurs when a nutrient is depleted in the environment so death and growth is equal

The death phase is when the bacteria die due to lack of nutrients



Classes of Microbes based on Oxygen Requirements ...

- **Obligate aerobes** (Organisms that have an absolute requirement for oxygen.)
- **Obligate anaerobes** (Organisms that cannot grow in the presence of oxygen.)
- **Facultative aerobes** (Organisms that can grow either in the presence or absence of oxygen.)
- **Microaerophilic** (which do best in reduced amounts of oxygen, and organisms that prefer more CO₂ than the amount normally found in the atmosphere)
- **Aerotolerant anaerobes** (do not require oxygen but does not have an oxygen sensitive biomolecules so it can grow in an oxygen rich environment. Produce energy by fermentation ?

According to grown in ranges of temperature bacteria can divided into:-

1. Psychrophilic: (cold loving)

They can grow between -10°C and 20°C with optimum growth around 10°C .

2. Mesophiles :

they can grow between 15°C and 45°C with optimum around 37°C .

3. Thermophiles (heat loving)

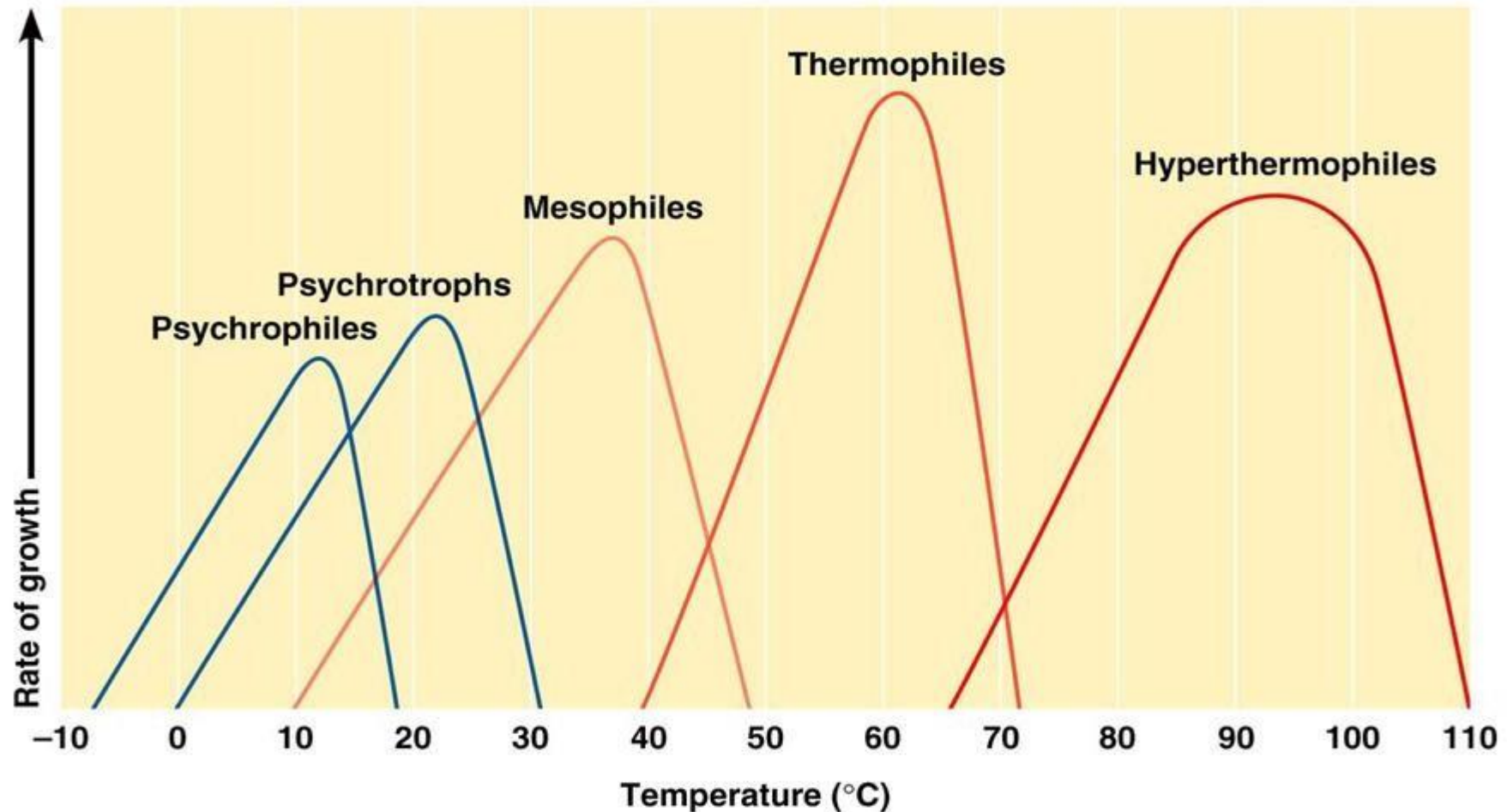
they can grow between 40°C and 75°C with optimum around 55°C .

4. Hyerthermophiles:

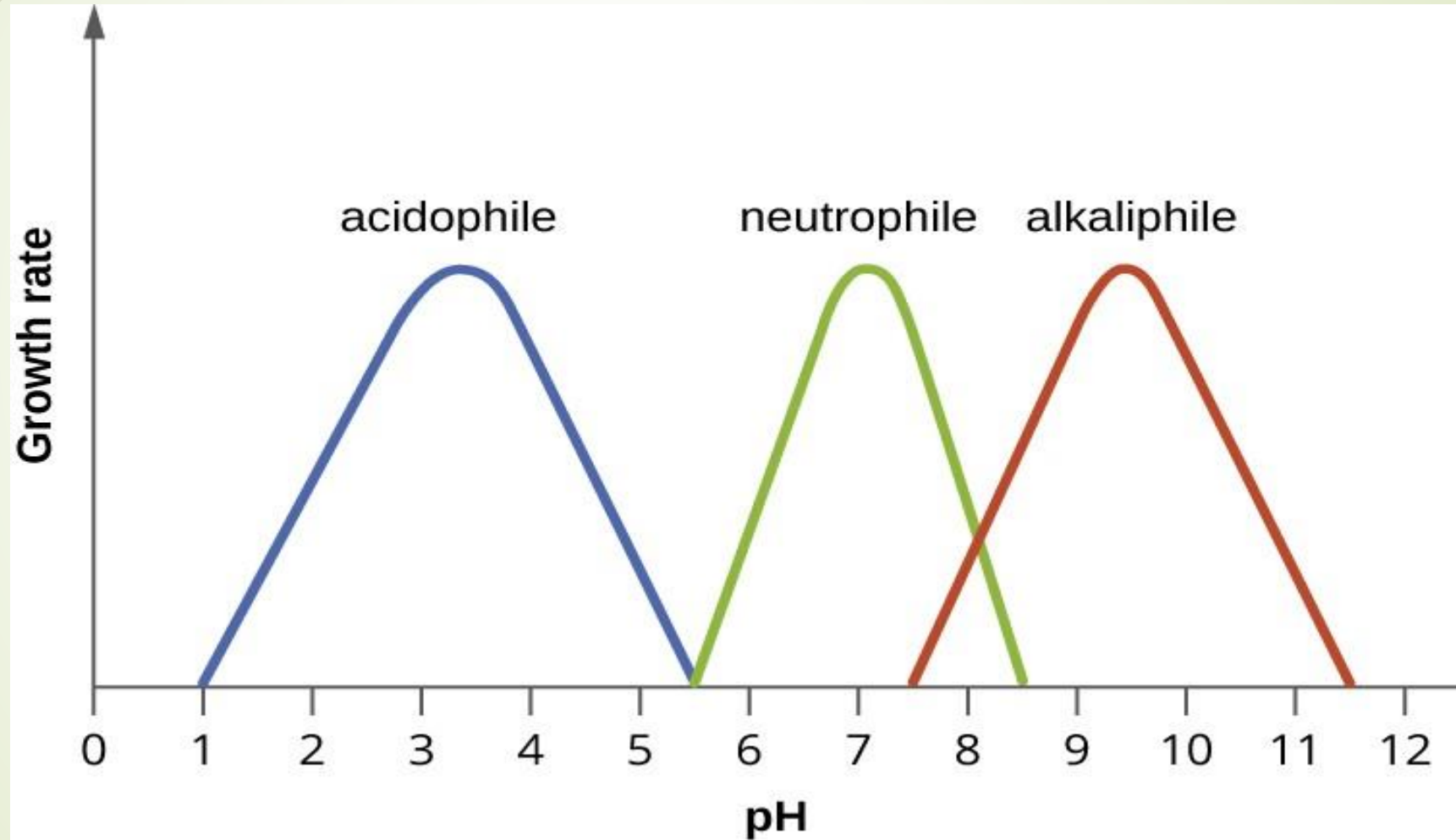
they can grow in greater than 100°C

Bacterial growth

Physical requirements – Temperature



According to growth in ranges of pH, bacteria can be divided into:-





Steps of identifying Bacteria

- Inoculate
 - Incubate
 - Isolation
 - Inspection
 - Identification
- 

Microbial culture

A **microbiological culture**, or **microbial culture**, is a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions

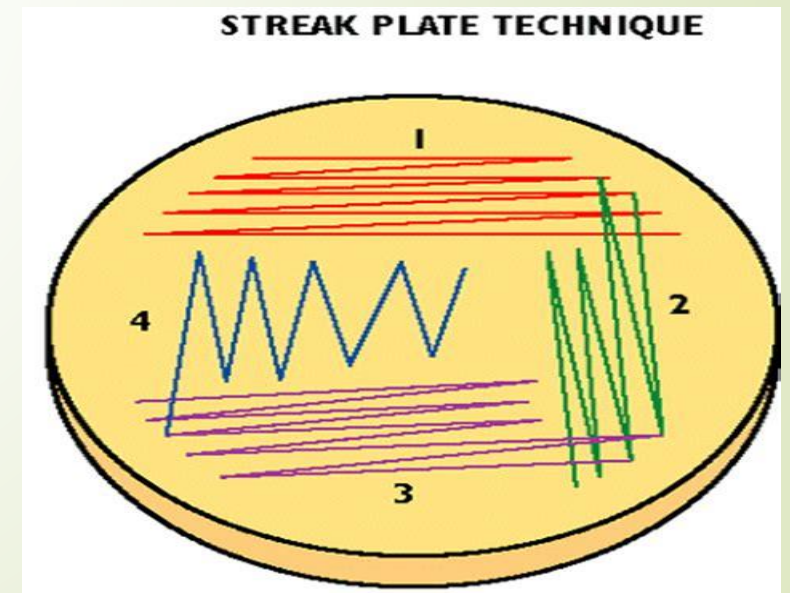
Methods of culturing of microorganism

- Streak plate technique
- Spread-Plate Technique.
- Pour-Plate Technique
- Stabbing technique

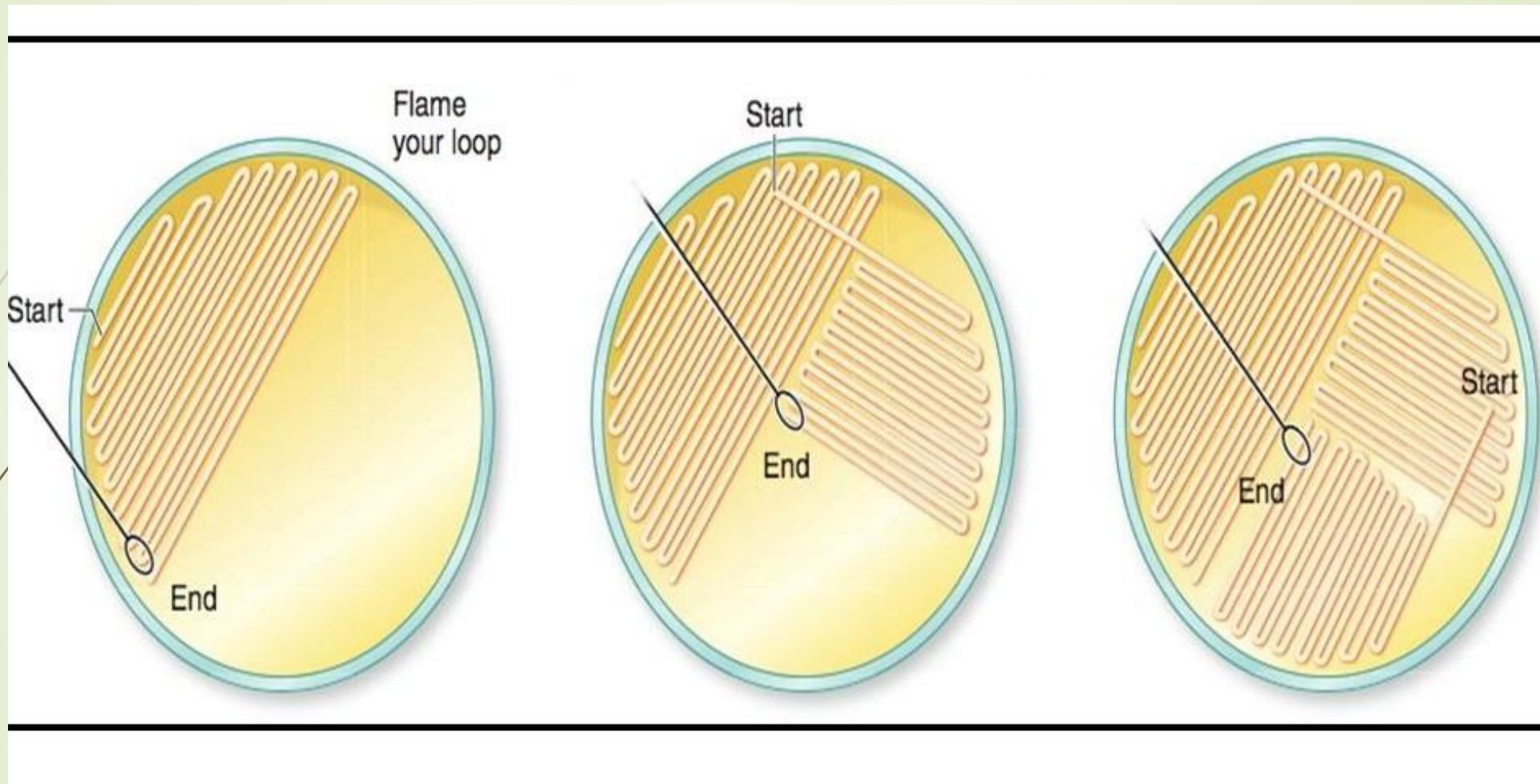
1-Streak Plate Technique

The medium is inoculated by drawing an infected loop across the agar. In this technique, the bacterial mixture is transferred to the edge of an agar plate with an inoculating loop and then streaked out over the surface in one of several patterns.

The purpose of streak plating is to spread out a clinical sample on solid growth media, so that individual, isolated bacterial colonies will grow.

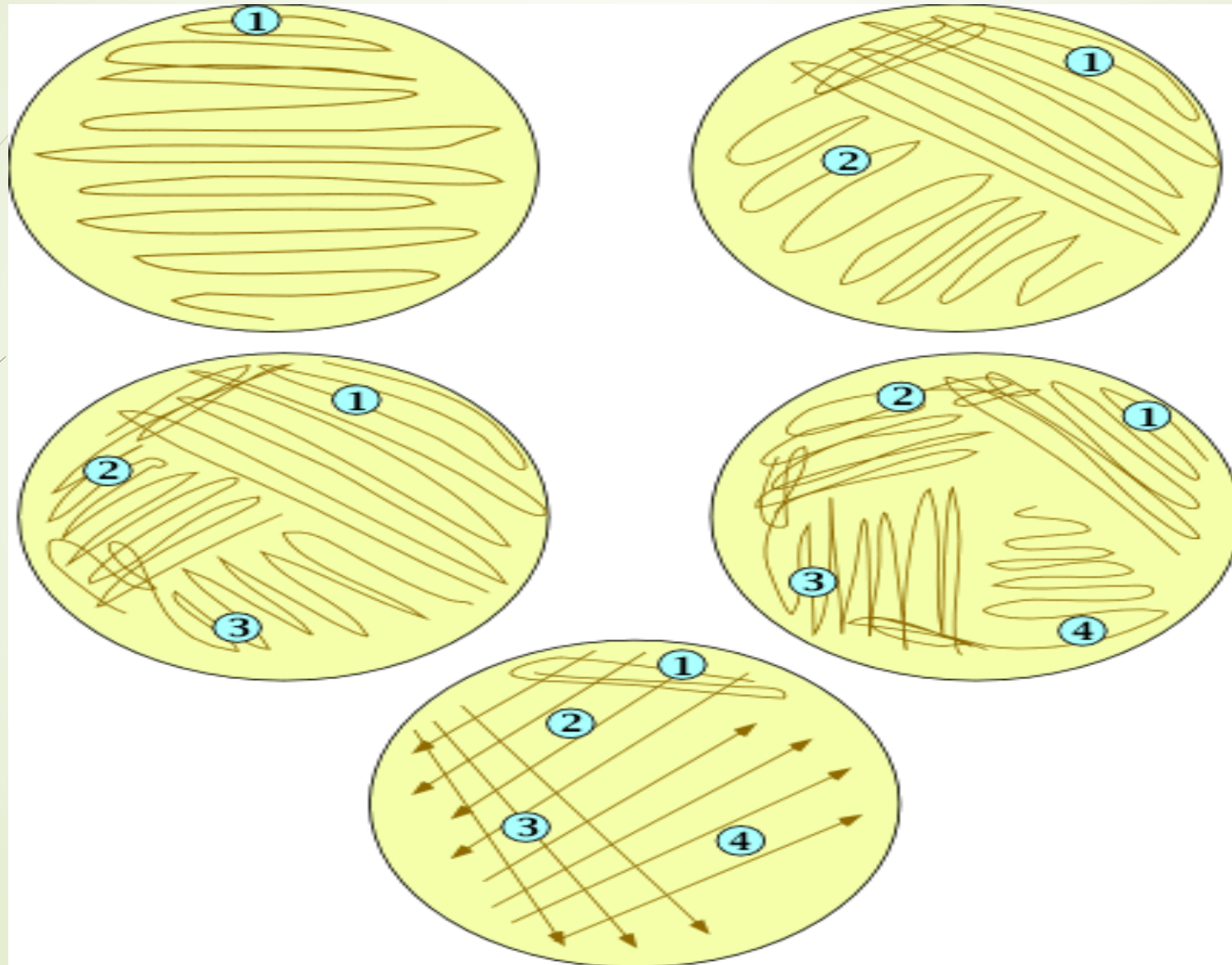


Streak Plate Technique



https://www.youtube.com/watch?v=_1KP9zOtjXk

Streak Plate Technique



Some time swab can be used to inoculate of bacteria into a plate



2-The spread plate technique

The spread plate technique is a method for transferring bacteria to an agar plate and distributing it evenly. The technique makes it easier to quantify bacteria in a solution

The spread plate technique involves using a sterilized spreader with a smooth surface made of metal or glass to apply a small amount of bacteria suspended in a solution over a plate

<https://www.youtube.com/watch?v=Rye6DfMv66Q>

3-Pour-Plate Technique

Pour plate method is usually the method of choice for counting the number of colony-forming bacteria present in a liquid specimen.

In this method, fixed amount of inoculum (generally 1 ml) from a broth/sample is placed in the center of sterile Petri dish using a sterile pipette. Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate is inverted and incubated at 37°C for 24-48 hours.

<https://www.youtube.com/watch?v=ey19jM6y7-c>

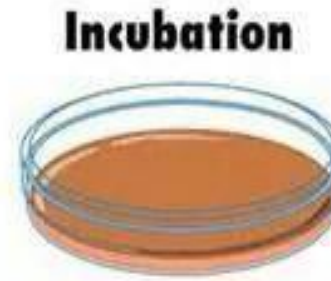
Spread-plate method



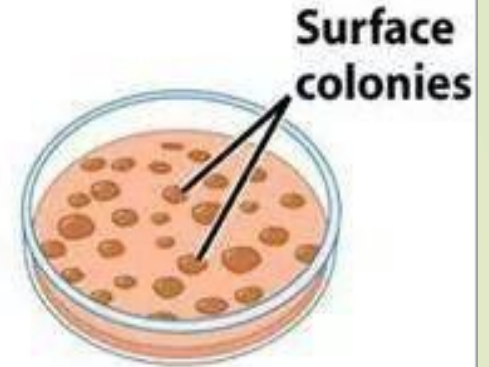
Sample is pipetted onto surface of agar plate (0.1 ml or less)



Sample is spread evenly over surface of agar using sterile glass spreader

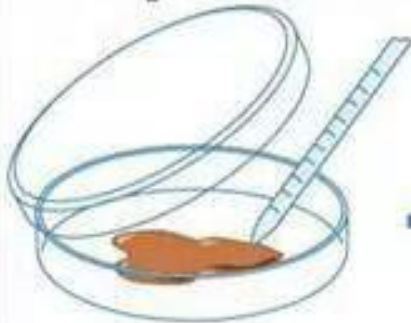


Incubation



Typical spread-plate results

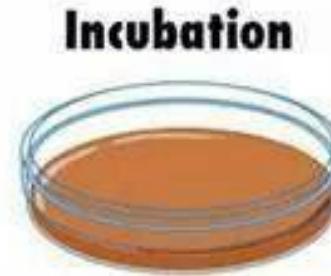
Pour-plate method



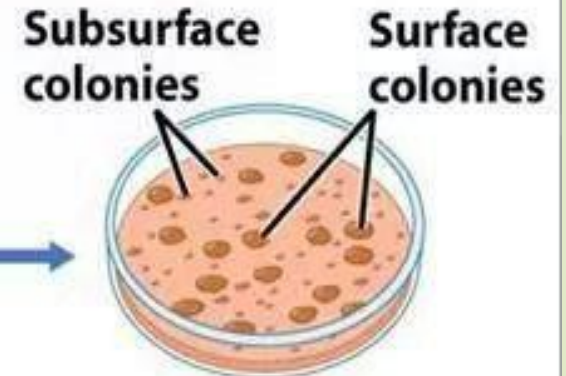
Sample is pipetted into sterile plate



Sterile medium is added and mixed well with inoculum



Incubation

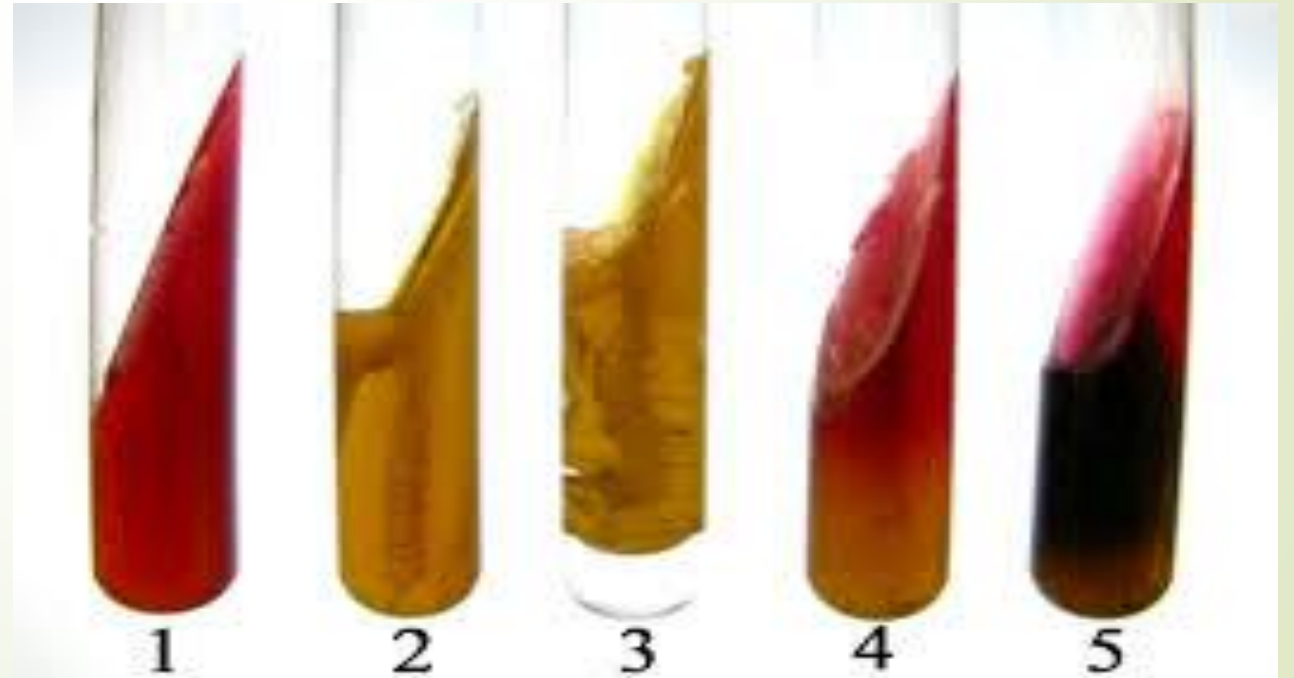



Typical pour-plate results

Figure 6-10 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

4- Stabbing technique

Stab cultures are similar to agar plates, but are formed by solid agar in a test tube. Bacteria is introduced via an inoculation needle or a pipette tip being stabbed into the center of the agar. Bacteria grow in the punctured area. Stab cultures are most commonly used for short-term storage.





-When inoculation is finished, the inoculated media should be placed in a suitable place for growth; that is **incubator**.
Choice of incubator depends on the bacteria you are looking for! Such as:

-Aerobic, obligate anaerobic, facultative anaerobic and microaerophilic.

-Psychophiles, Mesophiles, Thermophiles and Hyperthermophiles



THANK YOU FOR YOUR ATTENTION 😊😊😊

ANY QUESTIONS ????????