Practical Microbiology

Cihan University
Medical Laboratory Analysis

Lab 4: Microbial growth requirements, & inoculation, incubation & isolation techniques

Lec: Karokh Ali Khdir

MSc: Microbiology

Second stage (1st semester)

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Objectives

❖**Students should be able to understand:**

✓Inoculation & microbial culture.

✓Microbial growth, requirements, incubation, pure, mixed & contaminated cultures.

✓Isolation & techniques in isolation.

Aseptic techniques

- Protect yourself from biohazards.
- Contamination during lab session is a constant problem (media & equipment) so aseptic techniques help ensure that only microbes that came from the sample are present (protect the target microbes).
- Another concern is the release of infectious agents from cultures into the environment (protect the society).

Inoculation & Culture

❖**Inoculation:** introduces a tiny microbial sample (inoculum) into a culture medium which provides an environment in which they multiply.

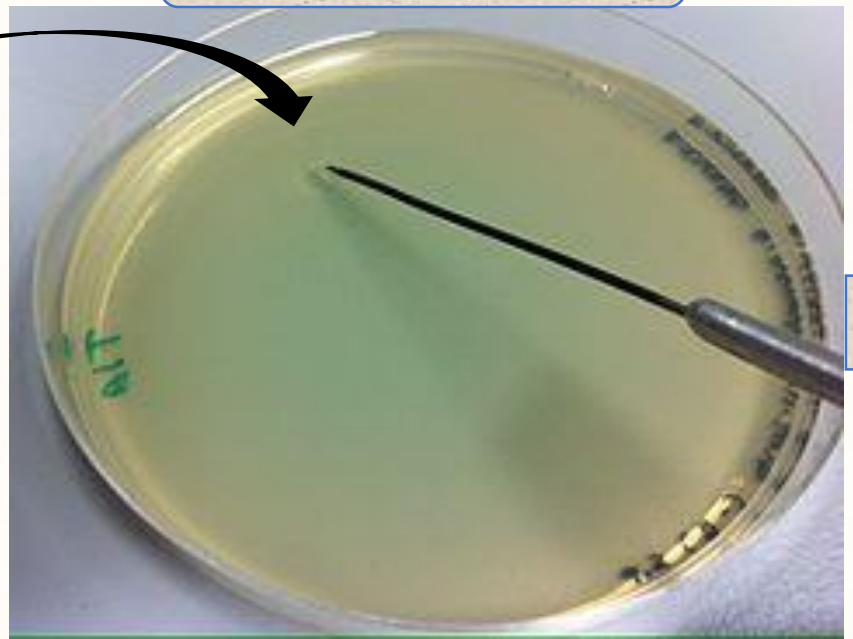
➢Loop, needle, swab, or pipette used for inoculation.

➢Inoculum sources: body fluids, soil, water, air, food, sewage, feces, inanimate objects.

The observable growth that later appears in or on the medium is called **culture.**

Urine

Inoculation



Incubation

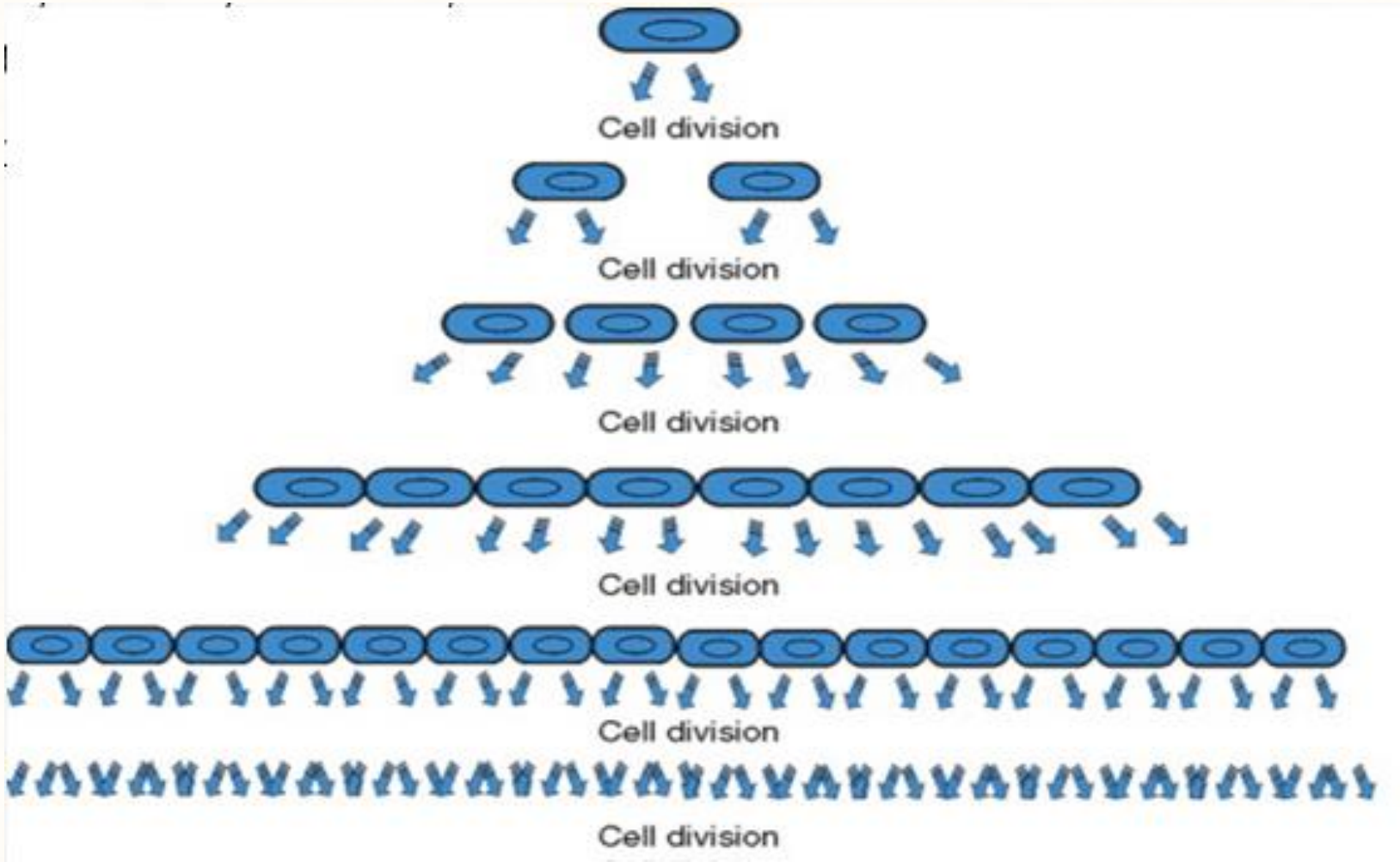
Culture

Incubation

- **Incubation:** put the microbes under optimal temperature conditions in order to grow properly.
- We usually use incubator.
- Duration of incubation is different from microbe to microbe:
- Bacteria (24-48 hours or may be 2 weeks at 35-37 °C).
- Fungi (at least 3 days to 1 month at 25-30 °C.
- Viruses (on animal cells need days to weeks).

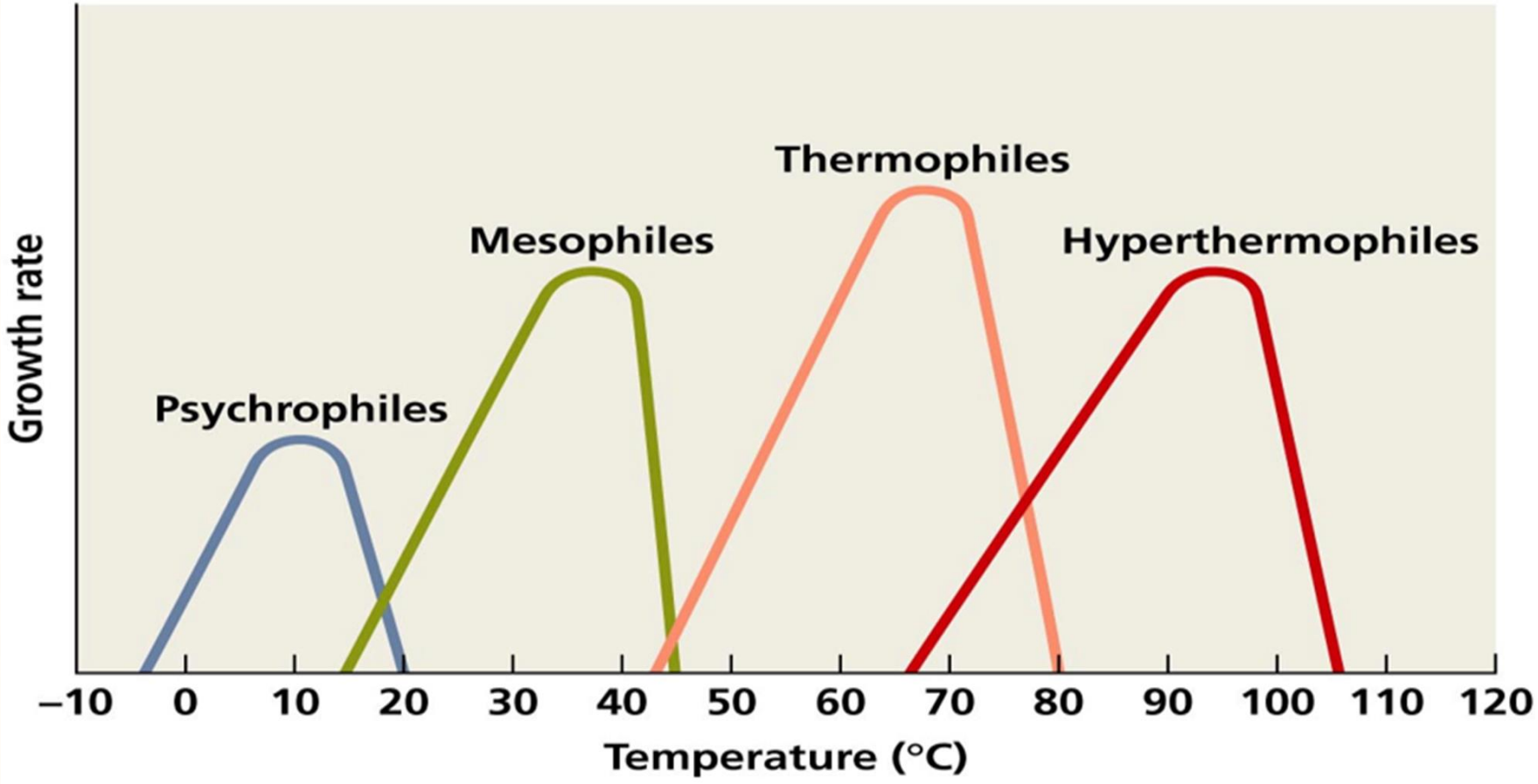
Growth and its requirements

- **Growth** defined as an increase in cell number as well as the size.
- Physical & chemical growth requirements:
- ✓Nutrients, pH & humidity (culture media).
- ✓Temperature.
- ✓Aeration.



Temperature

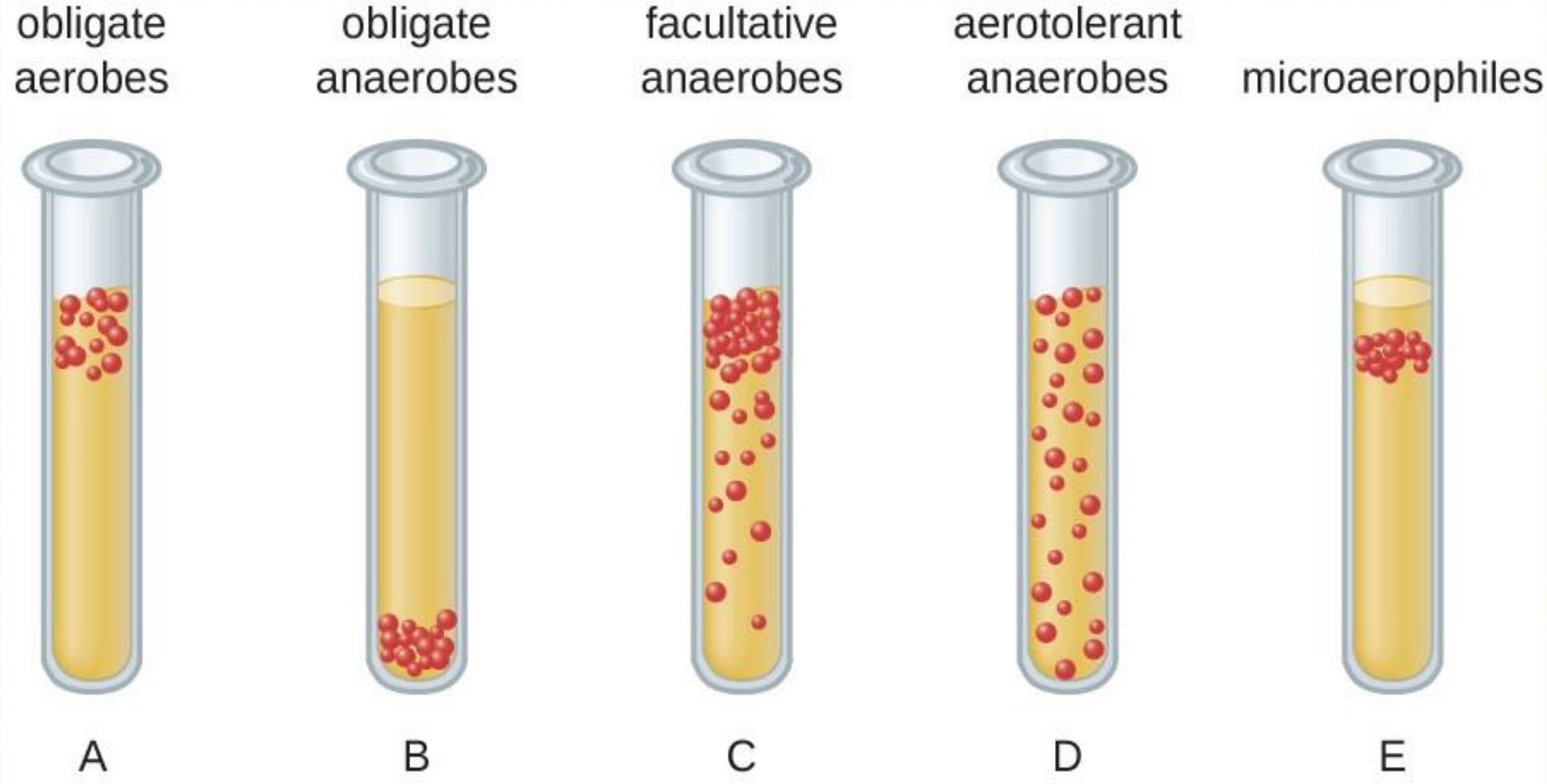
- **Psychrophiles:** grow between 0-20 °C.
- **Mesophiles:** grow between 15 °C and 45 °C with optimum around 37 °C.
- **Thermophiles:** grow between 55 °C to 80 °C.
- **Hyerthermophiles:** grow at 100 °C or more.



Oxygen

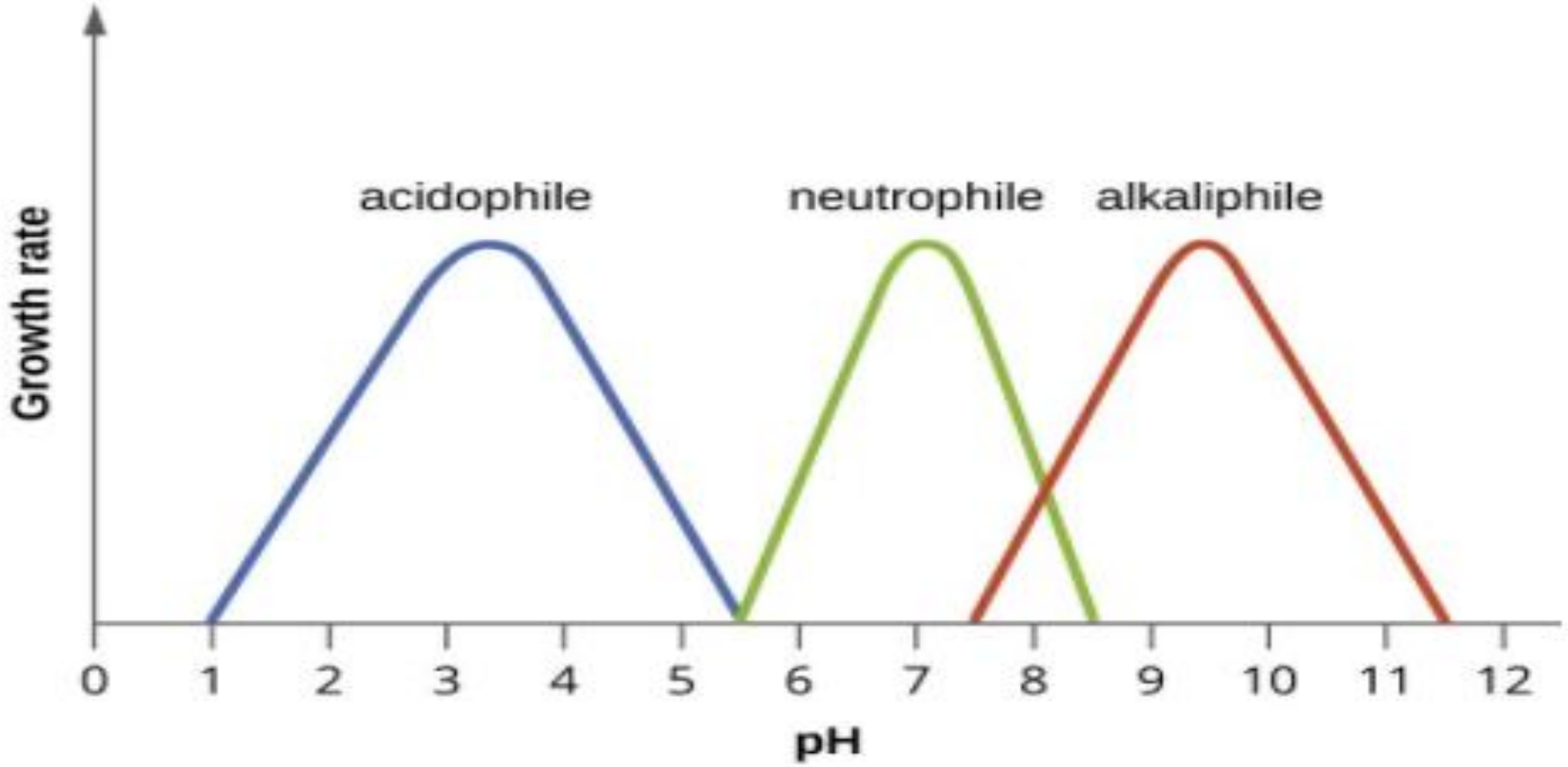
- **Obligate aerobes:** absolute requirement for oxygen.
- **Obligate anaerobes:** only grow in the absence of oxygen (O_2 is toxic).
- **Facultative anaerobes:** can grow either in the presence or absence of oxygen (note: grow better when O_2 is present).
- **Aerotolerant anaerobes:** anaerobic microbes capable of growing despite the presence of oxygen.
- **Microaerophilic:** do best in reduced amounts of oxygen usually lower than atmospheric tension (5-10% O_2) and prefer more CO_2.

Oxygen


obligate aerobes
obligate anaerobes
facultative anaerobes
aerotolerant anaerobes
microaerophiles
A
B
C
D
E

pH

- ✓**Neutrophiles:** grow at the neutral pH of 7.
- ✓**Acidophiles:** grow at pH less than 5.55.
- ✓**Alkaliphiles:** grow at pH between 8.0 to 10.5.



Types of culture

Pure culture: contains only a single known species or type of microorganism.

Mixed culture: contains two or more known or easily differentiated species of microorganisms.

Types of culture

Contaminated culture: contains unwanted microbes of unknown identity that introduced into culture through contamination.

Isolation

❖**Isolation:** process of separation of one microbe from another one or other microbes.

➢**The main goal in isolation is to obtain isolated colony.**

➢**Colony:** is a cluster of cells that grow on solid media, arises from a single cell.

➢So isolated colony represents only one microbial species.

Materials Required for isolation

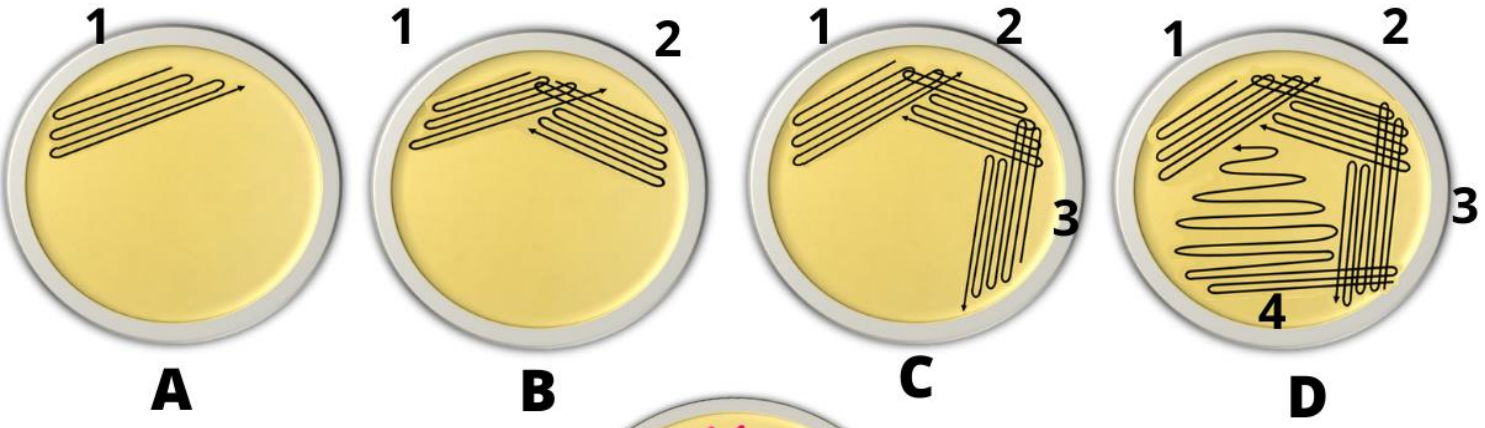
- Bunsen burner.
- Loop (metal or disposable).
- L-shape glass rod or sterile swab.
- Sterile nutrient agar plates.
- Mixed culture of bacteria (broth or plate).
- Melted, cooled agar ((45-48^{o}C).
- Micropipette (100-1000 ul).
- Ethanol.

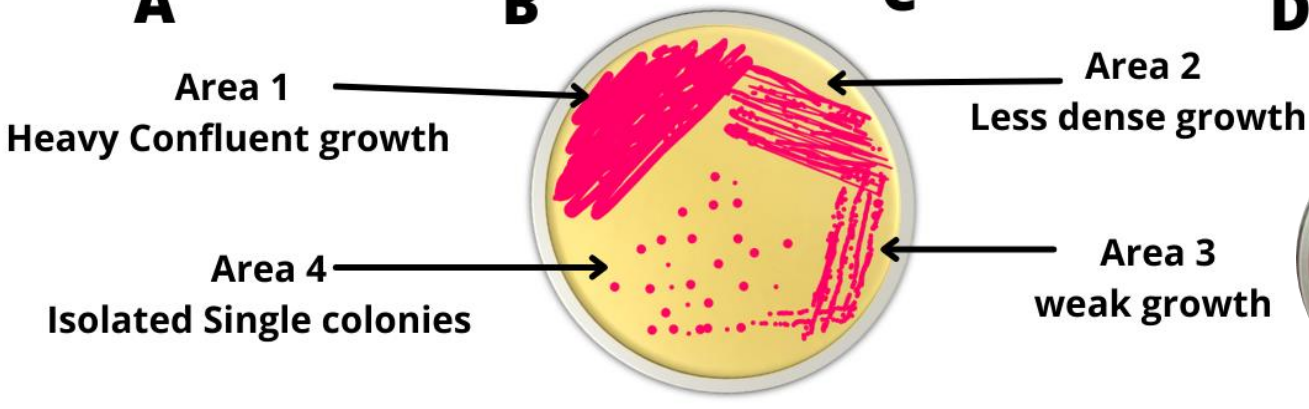
Isolation techniques

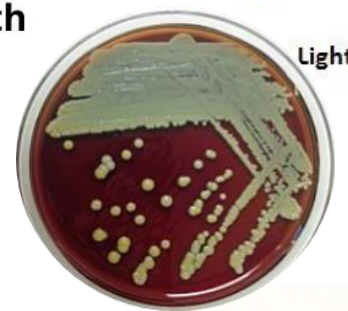
1) **Streak-Plate Technique:** the bacterial mixture is transferred to the edge of an agar plate with an inoculating loop and then streaked out over the surface in one of several patterns. Pure cultures can be obtained by picking well-isolated colonies and re-streaking these on fresh agar plates.

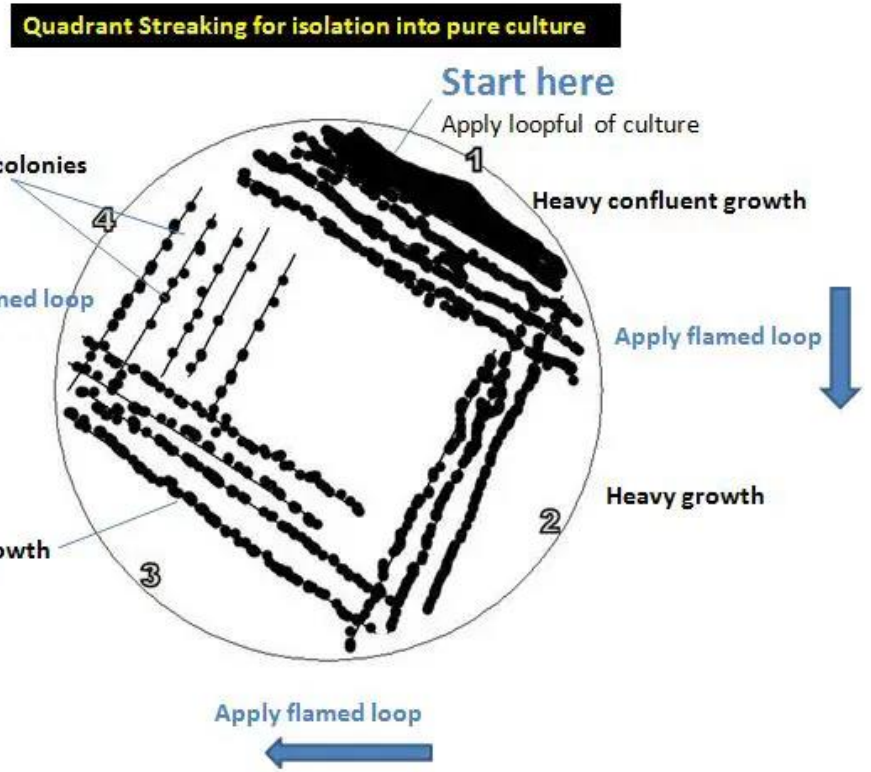
➢**Quadrants streaking:**

Don't forget to flame the inoculating loop at each step of streaking

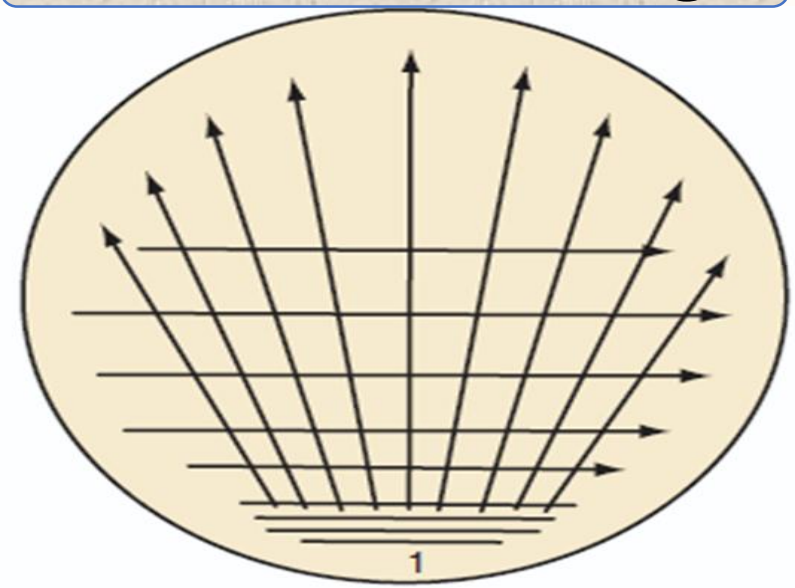


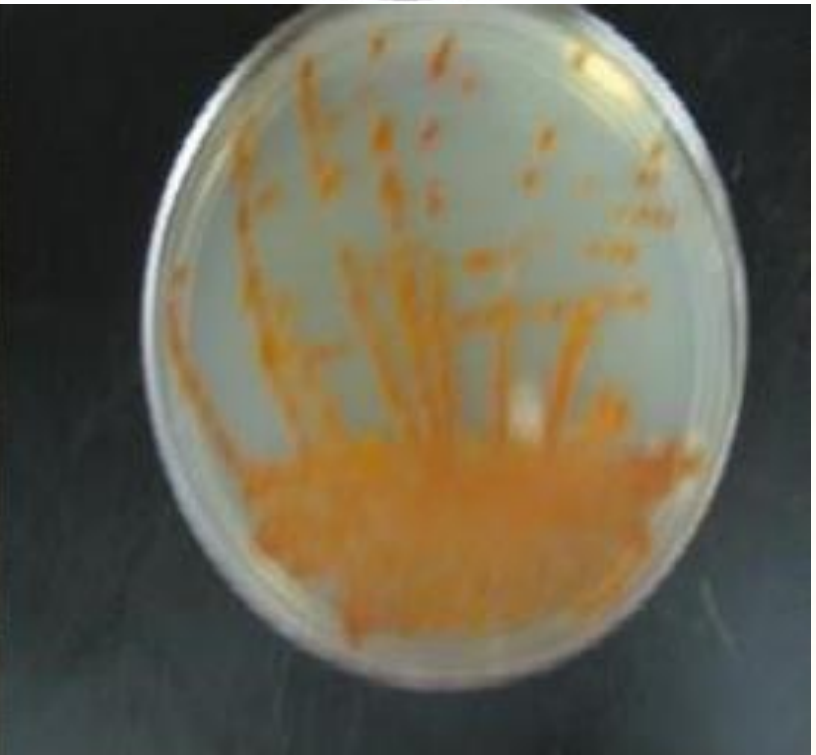




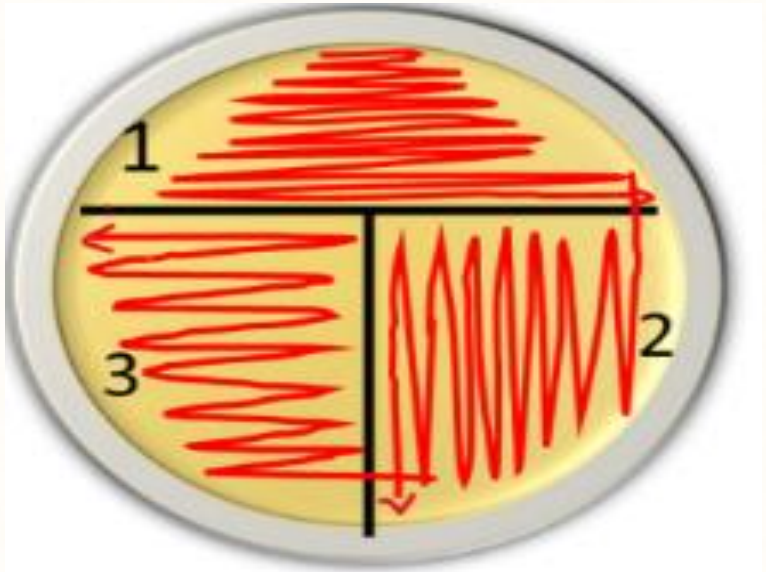


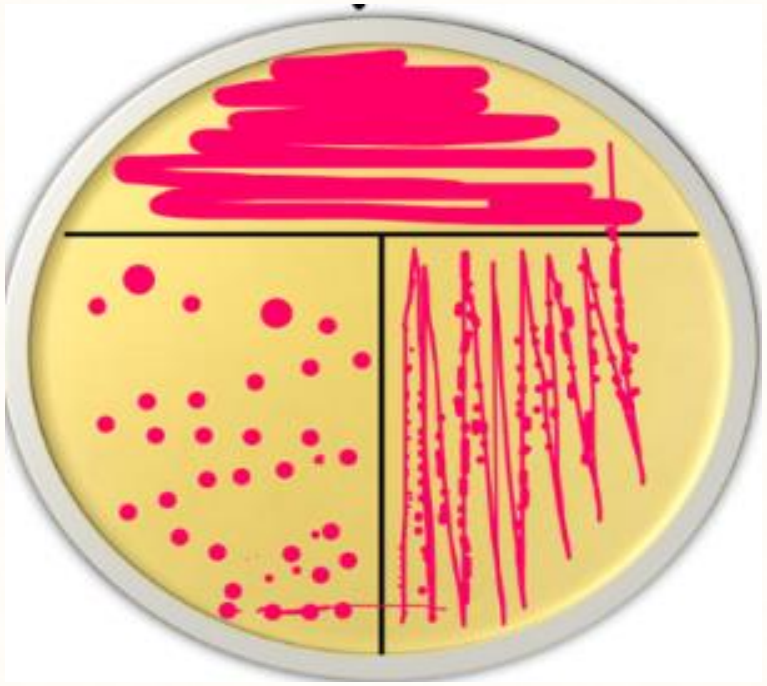
➢ Radiant Streaking



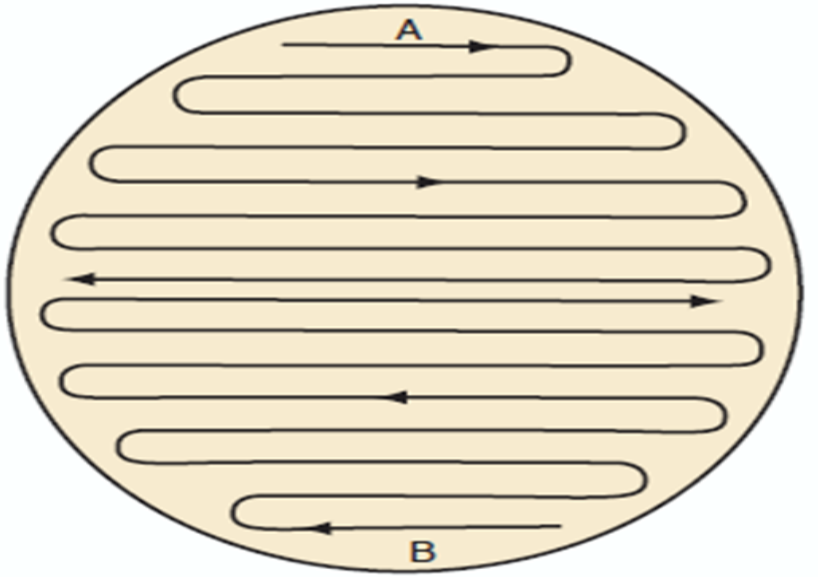


T-Streaking



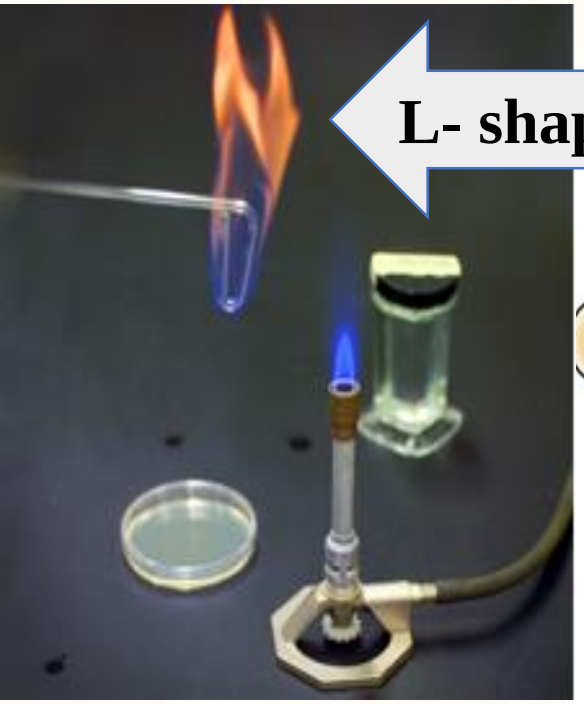


➢ Continuous Streaking

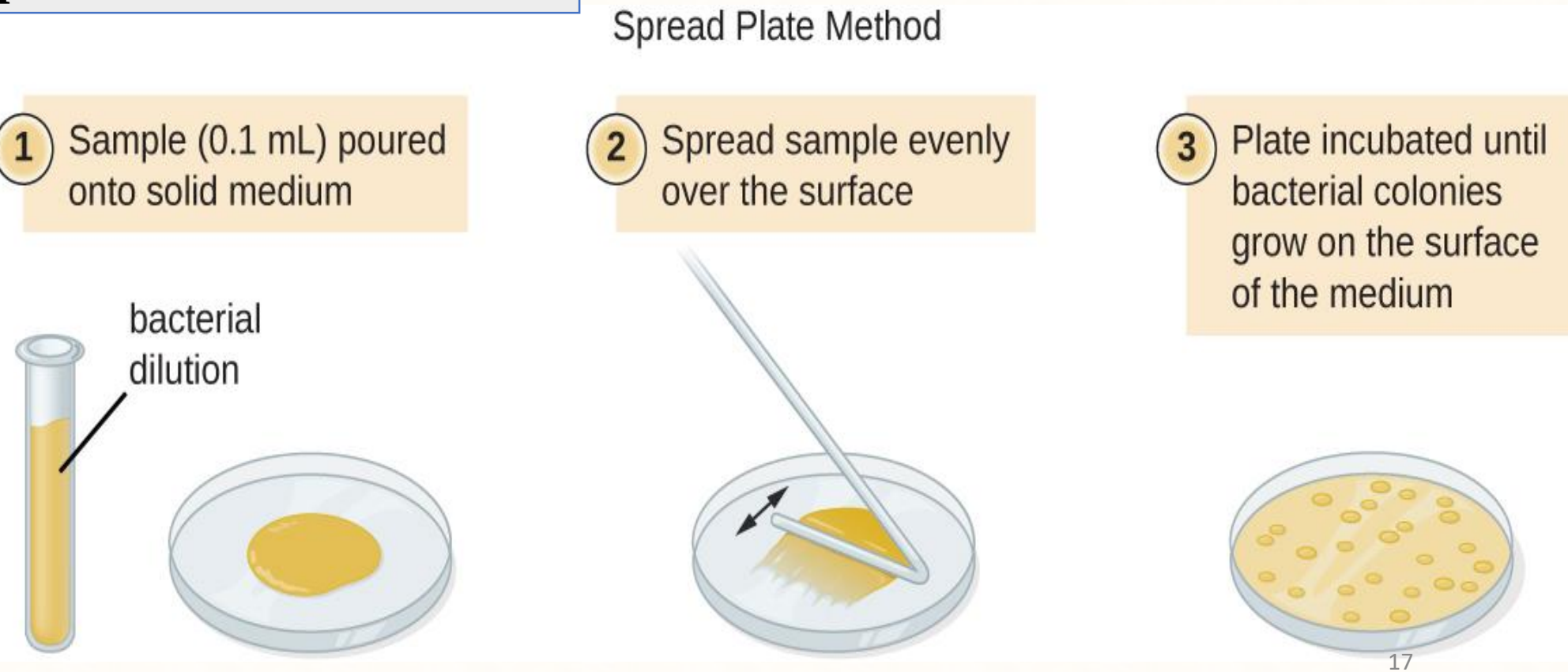


Isolation techniques

2) Spread-Plate Technique: in this technique, a small volume of dilute bacterial mixture (0.1 ml or 100 ul) is transferred to the center of an agar plate and is spread evenly over the surface with a sterile L shaped glass rod or sterile swab.



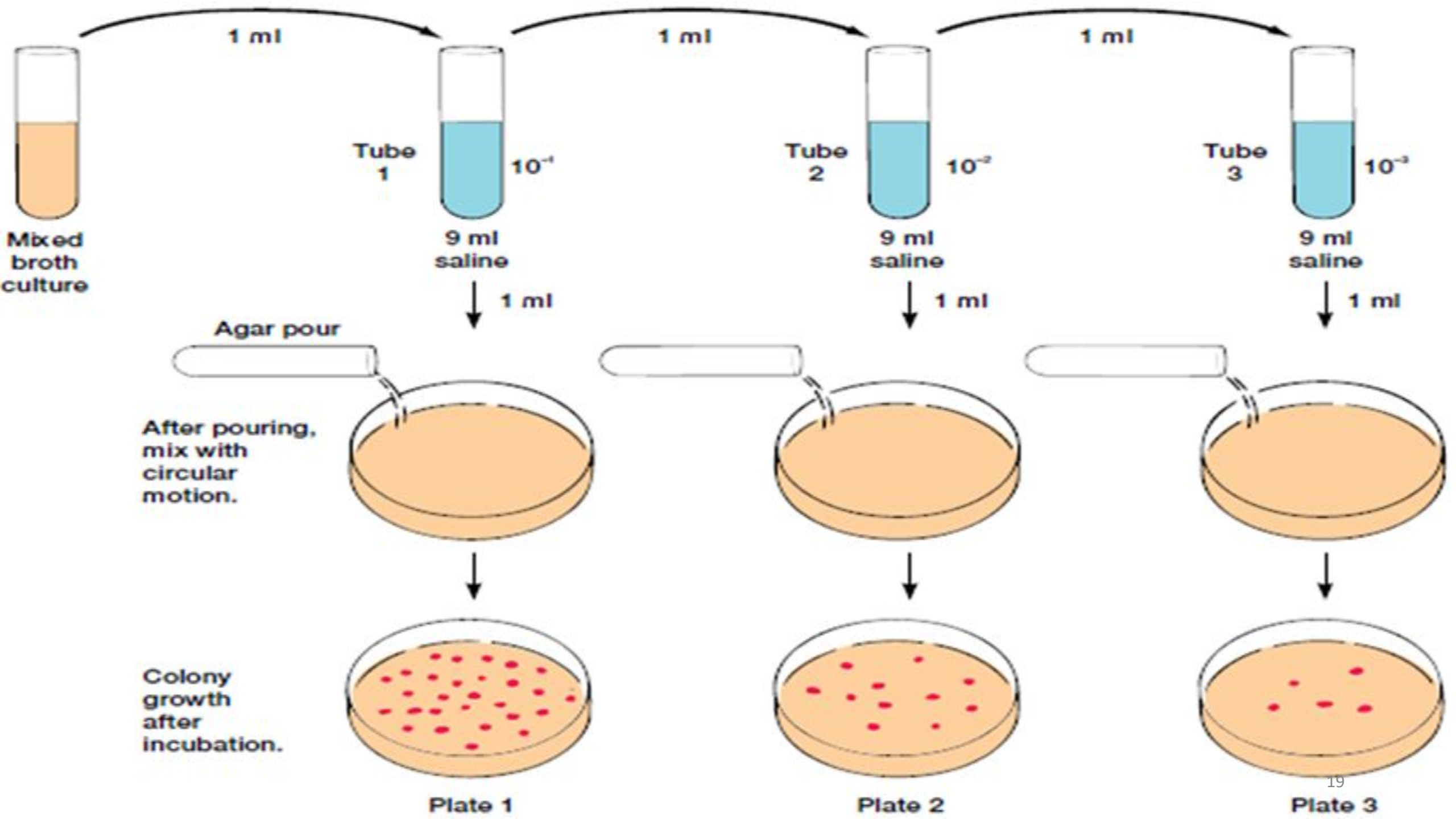
L- shape sterilized before use



Isolation techniques

3) Pour-Plate Technique:

- A suspension of the specimen (1 ml) to be cultured is placed in the bottom of an empty, sterile petri dish then melted, cooled agar (45-48^{o}C) is poured over it.
- Quickly, before the agar cools, the plate is gently rocked to disperse the inoculum.
- When the agar has solidified, the plate is incubated.
- Any microbes will grow either embedded within the agar layer or localized on its surface.

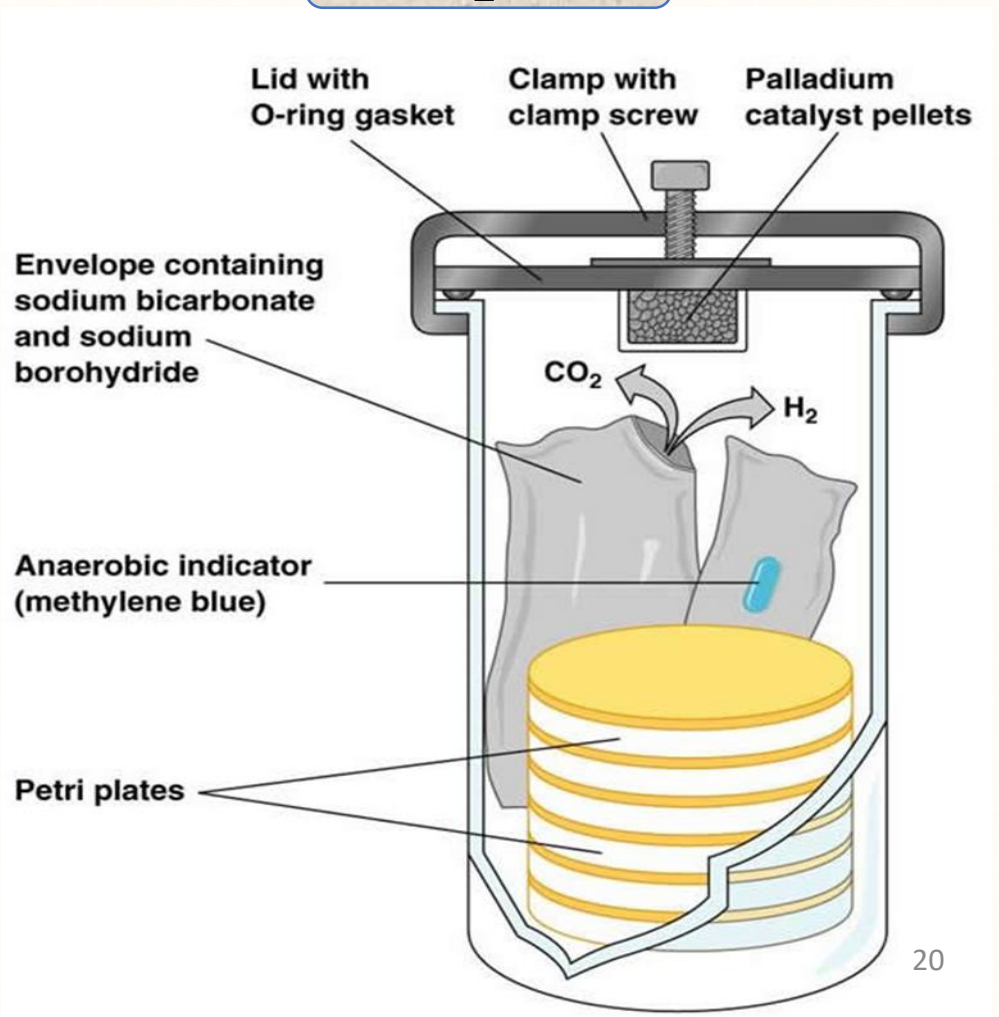

1 ml
1 ml
1 ml
Mixed broth culture
Tube 1
10^{-1}
Tube 2
10^{-2}
Tube 3
10^{-3}
9 ml saline
9 ml saline
9 ml saline
1 ml
1 ml
1 ml
Agar pour
After pouring, mix with circular motion.
Colony growth after incubation.
Plate 1
Plate 2
Plate 3

Isolation techniques

4) Anaerobic technique: used for isolation of anaerobic microbes from aerobics.

Candle jar

Gas pack



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