Cell Culture Systems

Course 11

Bacterial Cultivation

Cultivation of bacteria

The process of growing microorganisms in culture by:

- Taking bacteria from an infection site by specimen collection - *in vivo*
- Growing bacteria in the artificial environment of the laboratory - *in vitro*

Why cultivate bacteria?

- Obtain definitive identification and characterization
- Grow and isolate all bacteria present in an infection
- Determine which bacteria is most likely causing infection
- Determine which bacteria is likely a contaminant or colonizer

Why cultivate bacteria?

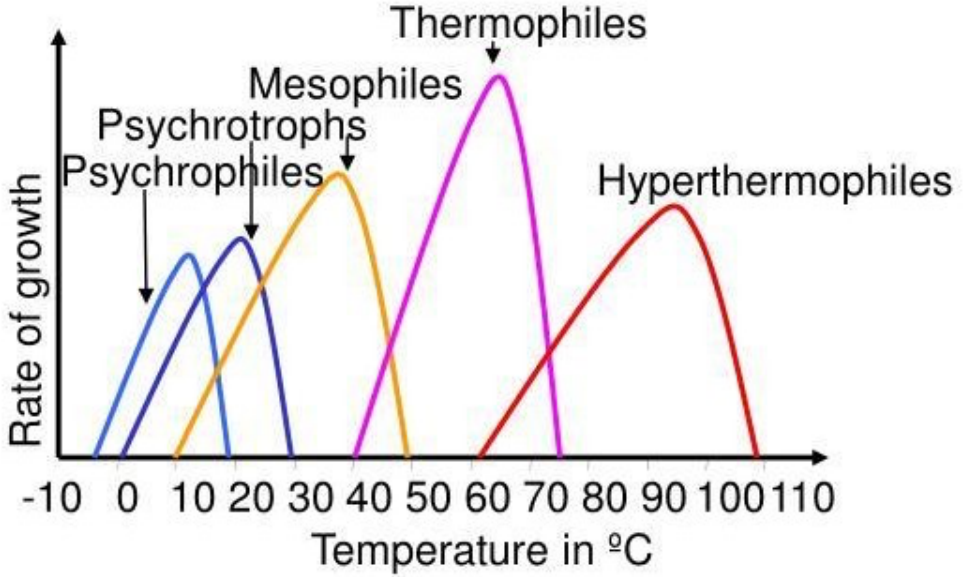
Obtain sufficient growth of clinically relevant bacteria to:

- Test antimicrobial susceptibility
- Measure response to treatment
- Characterize the agent
- Bank strain for future use including vaccine development

Growth requirements

Physical

- Temperature
- pH
- Osmotic pressure
- Moisture & desiccation



Chemical

- Carbon source
- Nitrogen, sulfur phosphorus
- Oxygen

Physical growth factors:

1-Acidity (pH requirements):

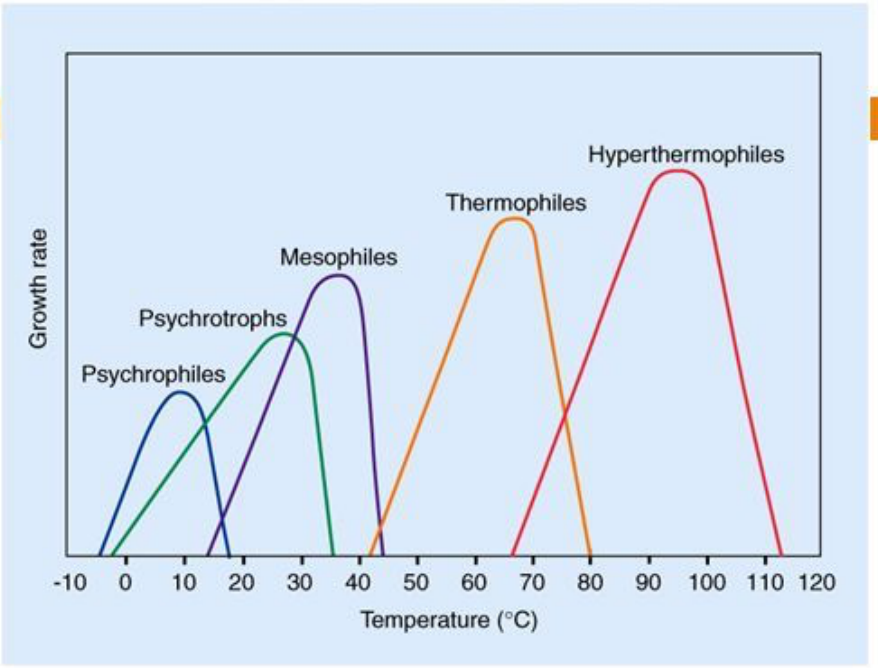
Pathogenic bacteria grow best at **neutral or biological pH** which is typically between pH 6.8 to pH 7.4.

Fungi such as yeasts and molds prefer slightly more acidic conditions and grow best between pH 5 to pH 6.

The pathogen ***Helicobacter pylori*** is able to survive with pH of the stomach by producing urease. So it is **acidophilic** bacterium.

On the other extreme, bacteria that prefer alkaline (basic) conditions are known as **alkaliphiles**. Example: ***Vibrio cholerae*** (prefers pH 9).

Temperature



- Psychrophile
  - 0° to 18° C
- Psychrotroph
  - 20°C to 30°C
    - Important in food spoilage
- Mesophile
  - 25°C to 45°C
    - More common
    - Disease causing
- Thermophiles
  - 45°C to 70°C
    - Common in hot springs and hot water heaters
- Hyperthermophiles
  - 70°C to 110°C
    - Live at very high temperatures, high enough where water threatens to become a gas
    - Usually members of *Archaea*
    - Found in hydrothermal vents

Chemical Requirements

- **Carbon**
  - **Structural organic molecules, energy source**
  - **Chemoheterotrophs use organic carbon sources**
  - **Autotrophs use CO_2**

Oxygen

Obligate aerobes

- Only aerobic growth, oxygen required

Facultative anaerobes (most human pathogens)

- Greater growth in presence of oxygen

Obligate anaerobes

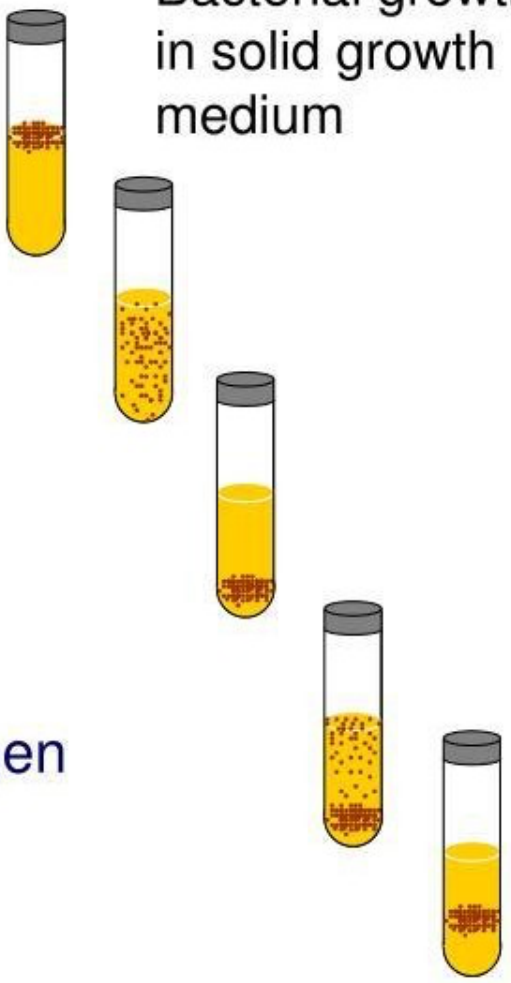
- Only anaerobic growth, cease with oxygen

Aerotolerant anaerobes (e.g., *C. perfringens*)

- Only anaerobic growth, continues with oxygen

Microaerophiles (e.g., *M. tuberculosis*)

- Only aerobic growth with little oxygen



Bacterial growth in solid growth medium

Chemical Requirements

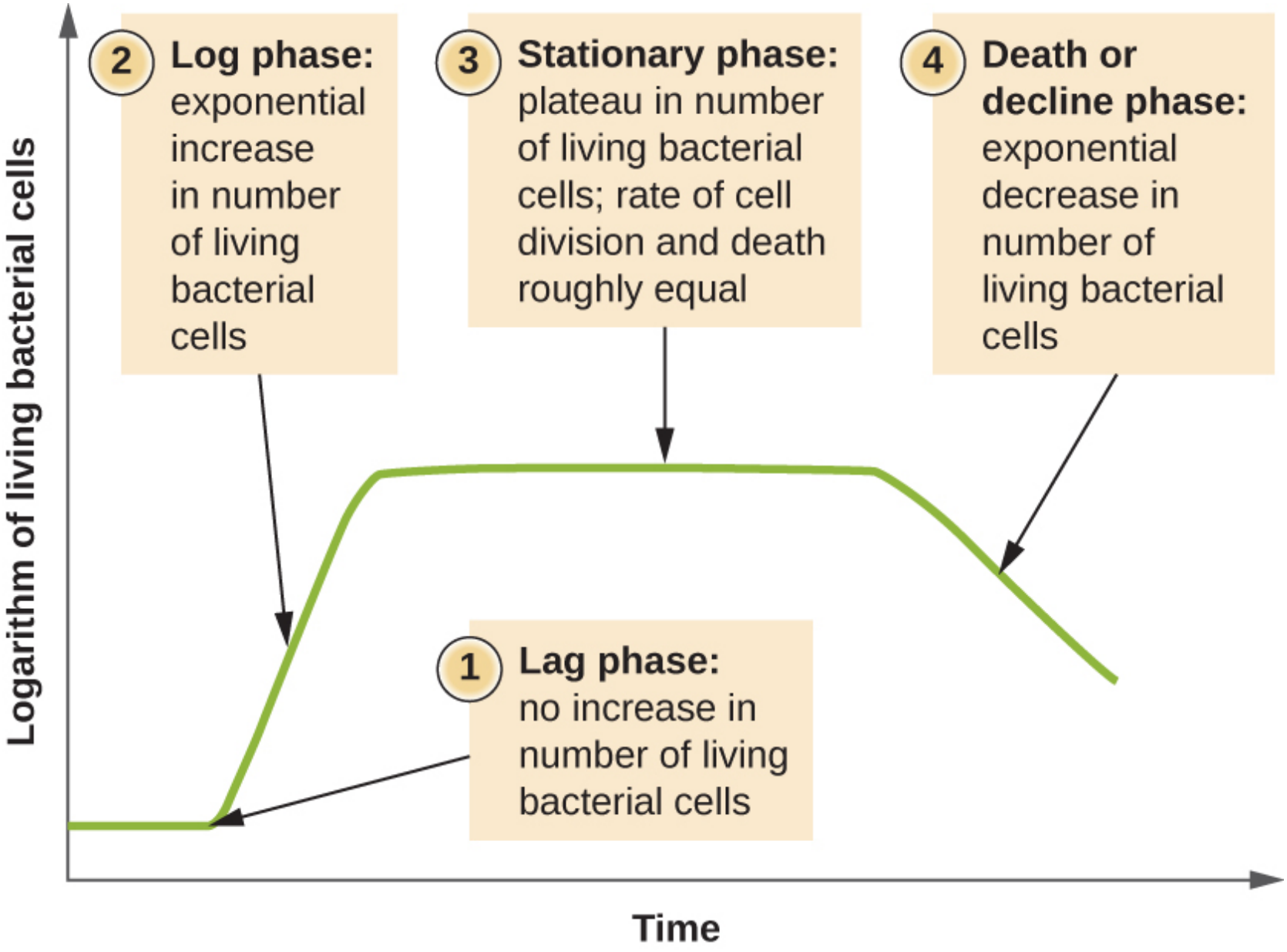
- Microorganisms use a variety of chemicals (**nutrients**) as a source of energy to build organic molecules and cell structures

- ***Several core chemicals are required for bacterial growth***

Chemical	Function
Carbon, oxygen, hydrogen	Required for cell structures
Nitrogen	Required for making bacterial amino acids and nucleic acids
Sulfur	Required for making some bacterial amino acids
Phosphorus	Required for making bacterial nucleic acids, membrane phospholipid bilayer, and ATP
Potassium, magnesium, calcium	Required for functioning of certain bacterial enzymes
Iron	Required for bacterial metabolism

Table 10.2 Microbiology: A Clinical Approach (© Garland Science)

- **Chemoheterotrophs**, which include pathogenic bacteria, use organic molecules as a source of carbon and energy


Logarithm of living bacterial cells
Time
1 Lag phase: no increase in number of living bacterial cells
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

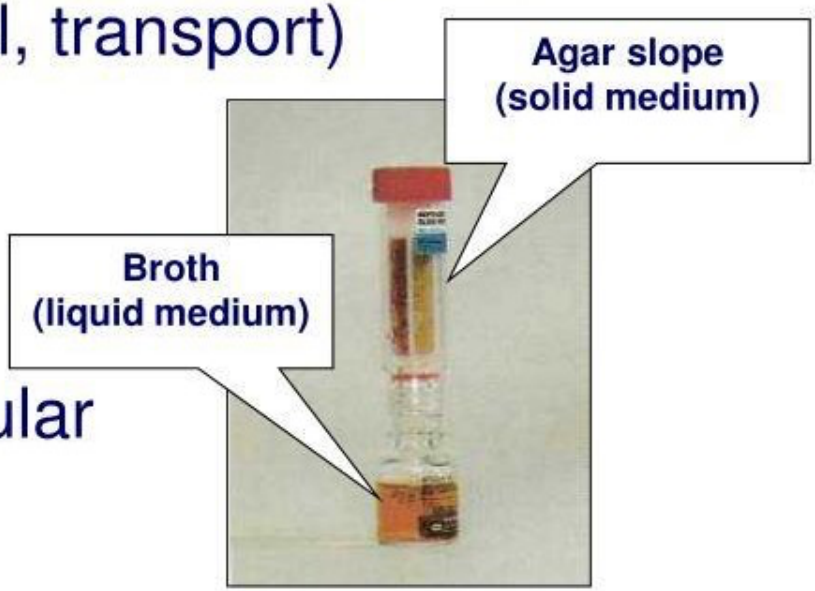
Types of bacterial culture media

Solid, semisolid, liquid, biphasic

Simple media, special media (enriched, selective, enrichment, indicator/ differential, transport) synthetic media

Aerobic and anaerobic media

Cell culture for obligate intracellular bacteria (e.g., *Chlamydia spp*)



Biphasic culture medium

Selective & differential media

Selective

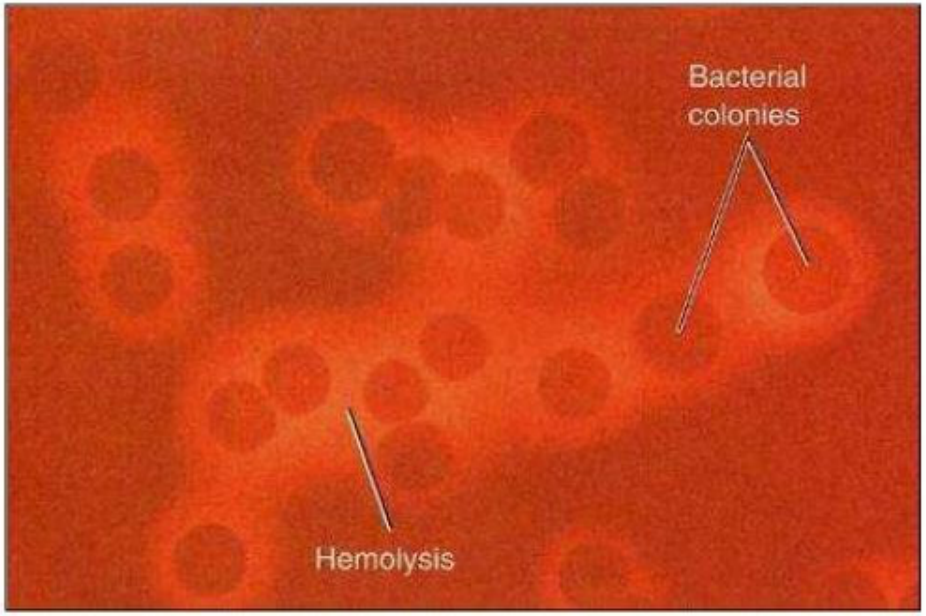
- Bismuth sulfite for *Salmonella typhi* (inhibits gram-positive and most gram-negative intestinal bacteria)

Differential

- Blood agar plates for *Streptococcus pyogenes*

Selective & differential

- Mannitol salt agar for *Staphylococcus aureus*



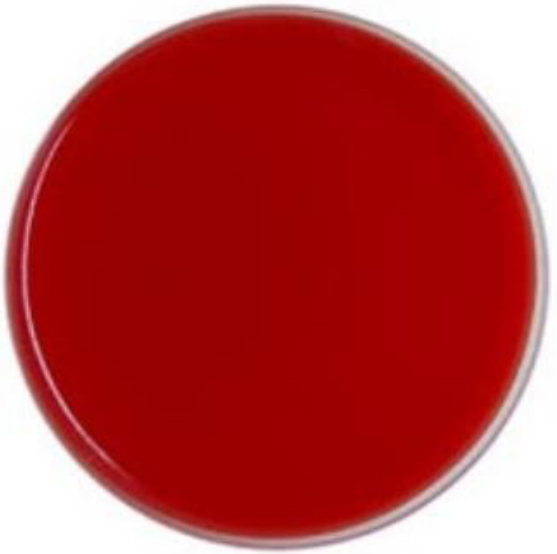
Type of hemolysis reaction aids identification of *S. pyogenes*

Simple media

- These media support growth of most bacteria.
- They **don't** have inhibitors or pH indicators.
- **Examples:**
  - Trypticase Soy broth.
  - Nutrient broth.

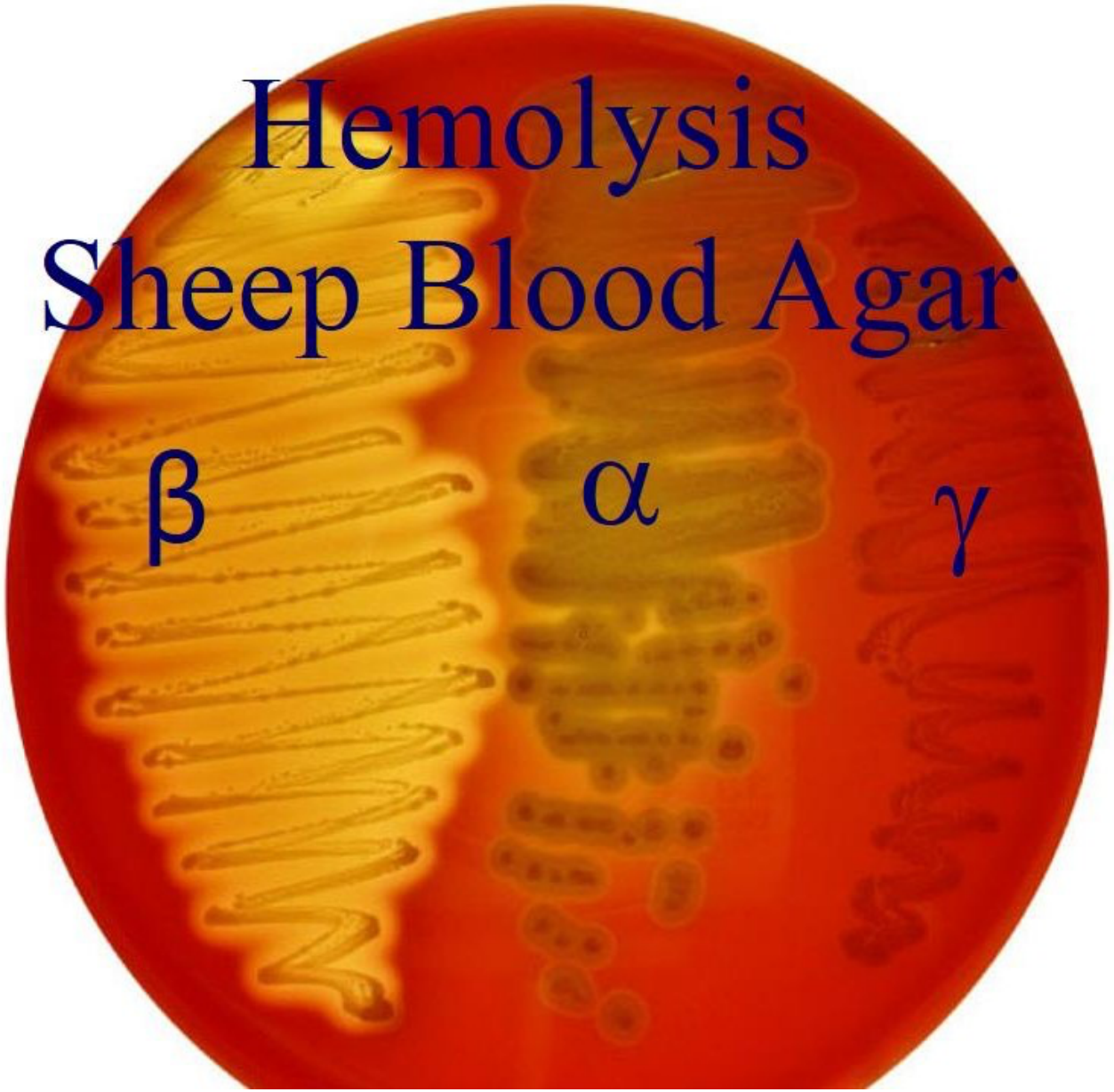
Enriched media

- These media contain:
  - The base growth medium.
  - Special supplements.
- Examples:
  - Blood Agar (also differential).
  - Chocolate Agar.



Differential media

- More than one type of MOs grow on it.
- Separation is based on color and other characteristic differences.
- Example:
  - Blood Agar.
    - It separates bacteria according to the type of hemolysis.


Hemolysis
Sheep Blood Agar
β
α
γ

Selective media

- The inhibitory substance is added to a solid media to inhibit commensal or contaminating bacteria such as :

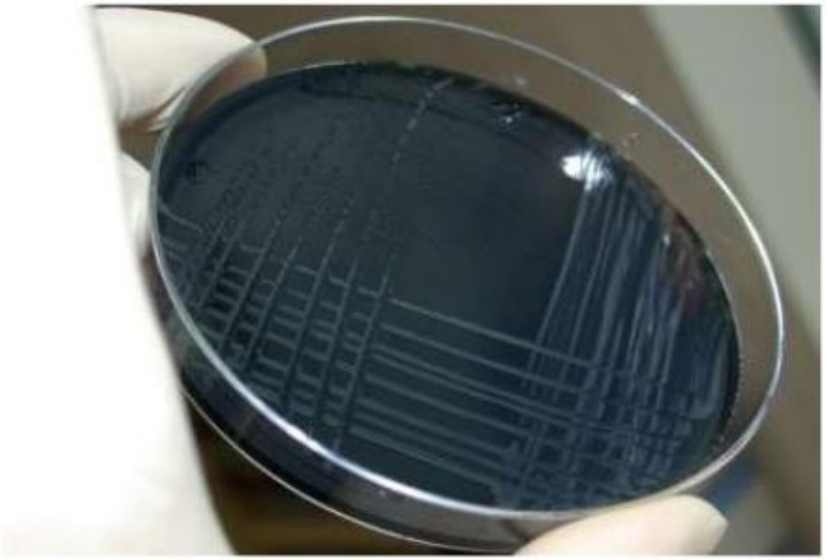
- Antibiotics
- Dyes
- Chemicals
- Alteration of pH

Eosin methylene blue

- selective for gram negative bacteria
- The dye methylene blue in the medium inhibit the growth of gram positive bacteria.

Campylobacter agar

- Is used for isolation of *Campylobacter jejuni* from fecal or rectal swab.
- Contain Bacteriological **charcoal** , **Cefoperazone** and **Amphotericin B**.



Selective-Differential media

- Containing inhibitors (Selective) and pH indicators (Differential).
- Examples:
  - MacConkey agar.
  - Mannitol Salt agar.

MacConkey agar

- Selective;
  - It contains **Bile salts** and **Crystal violet**.
  - Prevent the growth of Gram +ve bacteria.
- Differential;
  - Lactose fermenter Vs Non-lactose fermenter.
  - It contains **lactose & Neutral red** (a dye).
  - If bacteria ferment lactose, the medium will acidify and the dye turns **red**.
  - If bacteria don't ferment lactose, no color change occurs.

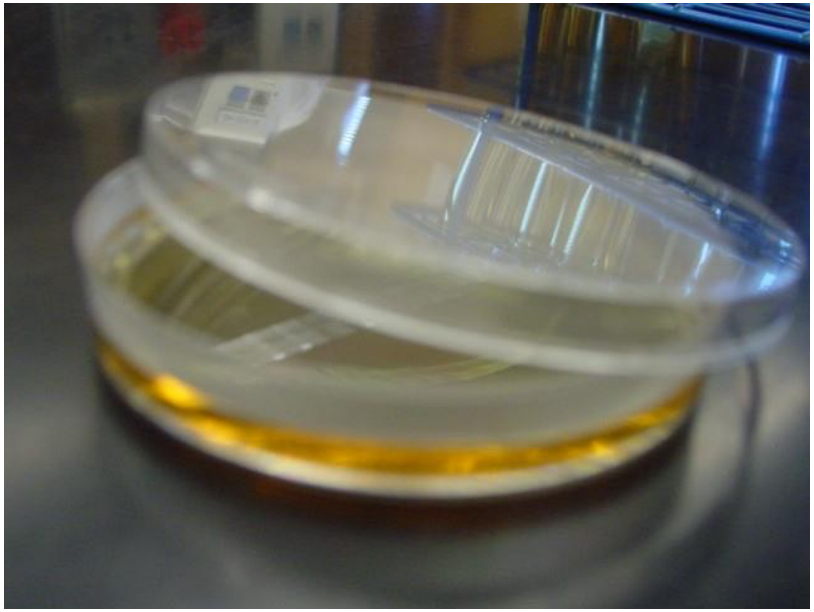
MacConkey agar

- Examples of Lactose fermenters:
  - *Escherichia coli*, *Enterobacter* and *Klebsiella*
- Examples of Non-Lactose fermenters:
  - *Salmonella* , *Proteus species* , *Yersinia* , *Pseudomonas aeruginosa* and *Shigella*

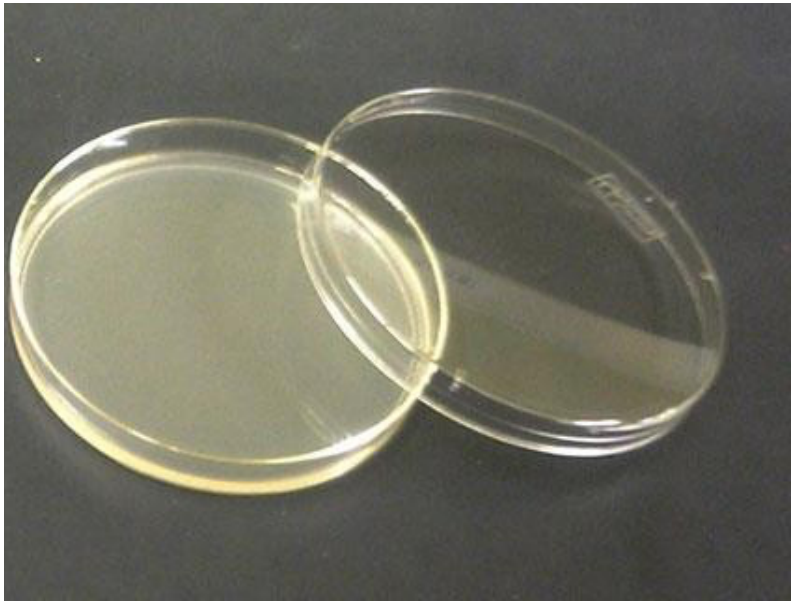
Pouring plates

- Autoclaved media (nutrients + agar) is poured on petri dishes. Agar is a matrix that solidifies on cooling.

Pouring plates

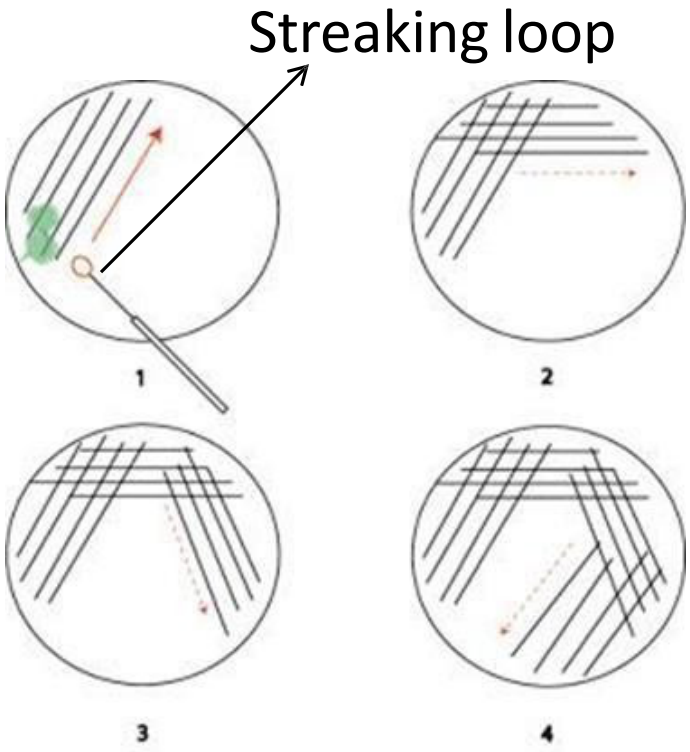


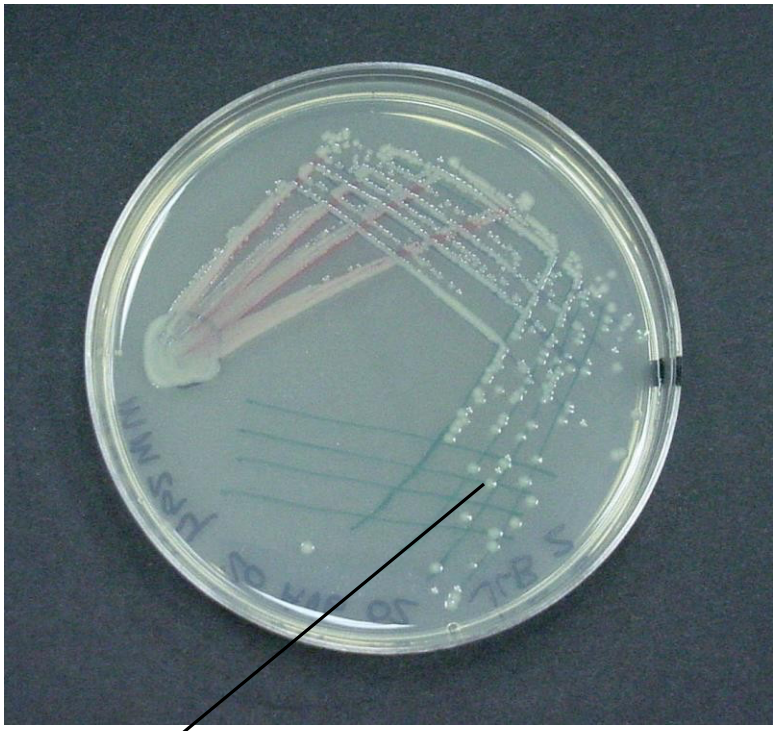
Freshly poured



After cooling

“Streaking” bacteria on media plates





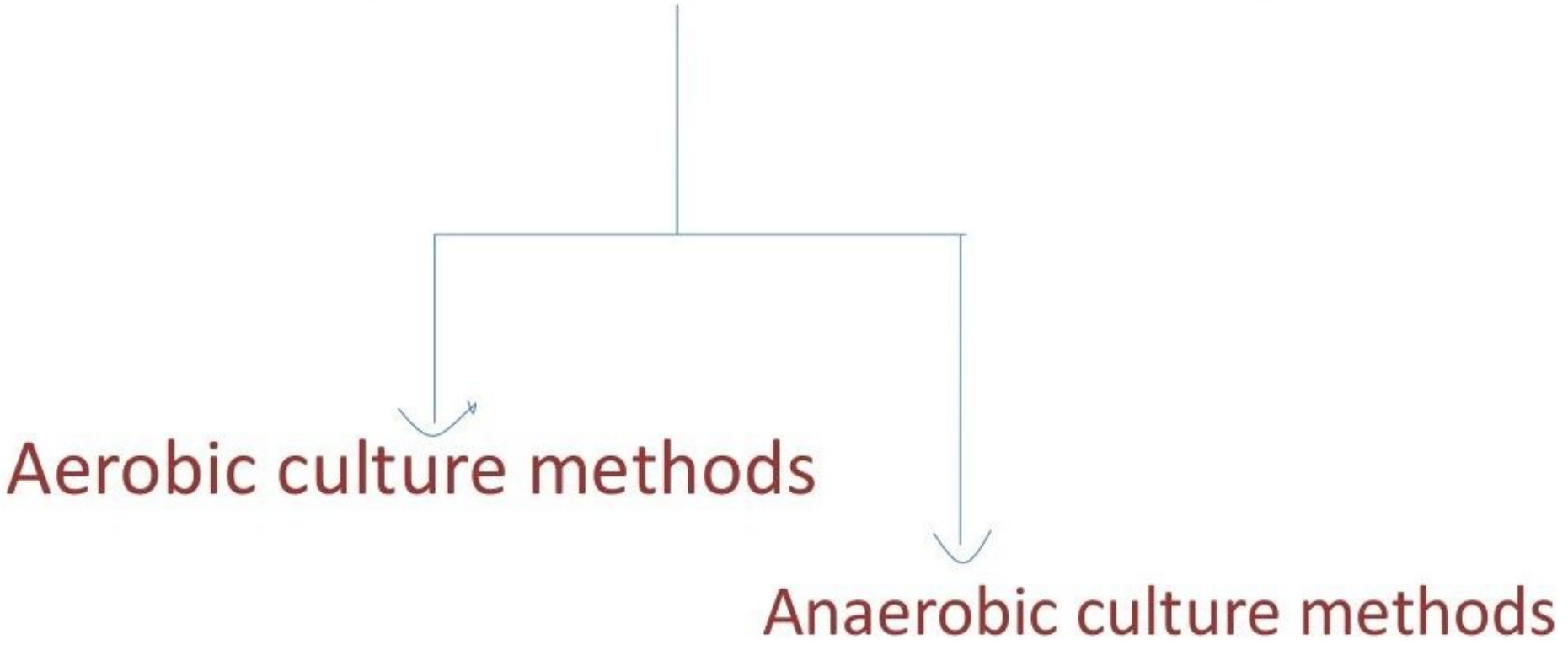
Colonies (various sizes, shapes, colours)

Picking a colony and inoculating liquid culture

Picking a colony

Put the picked colony in liquid media to grow bacteria

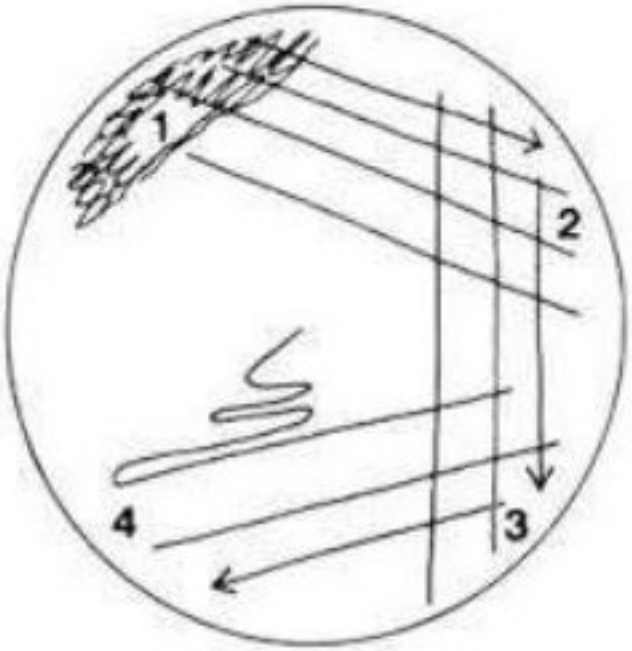
Culture method are of two types

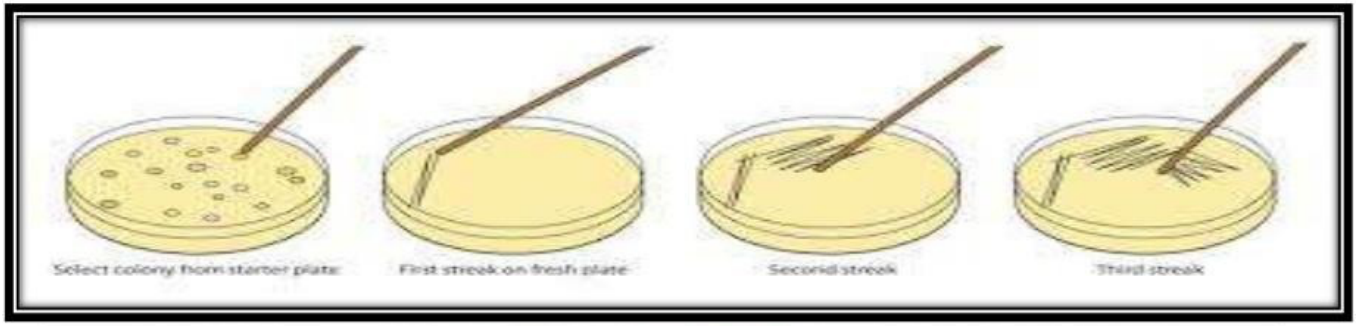


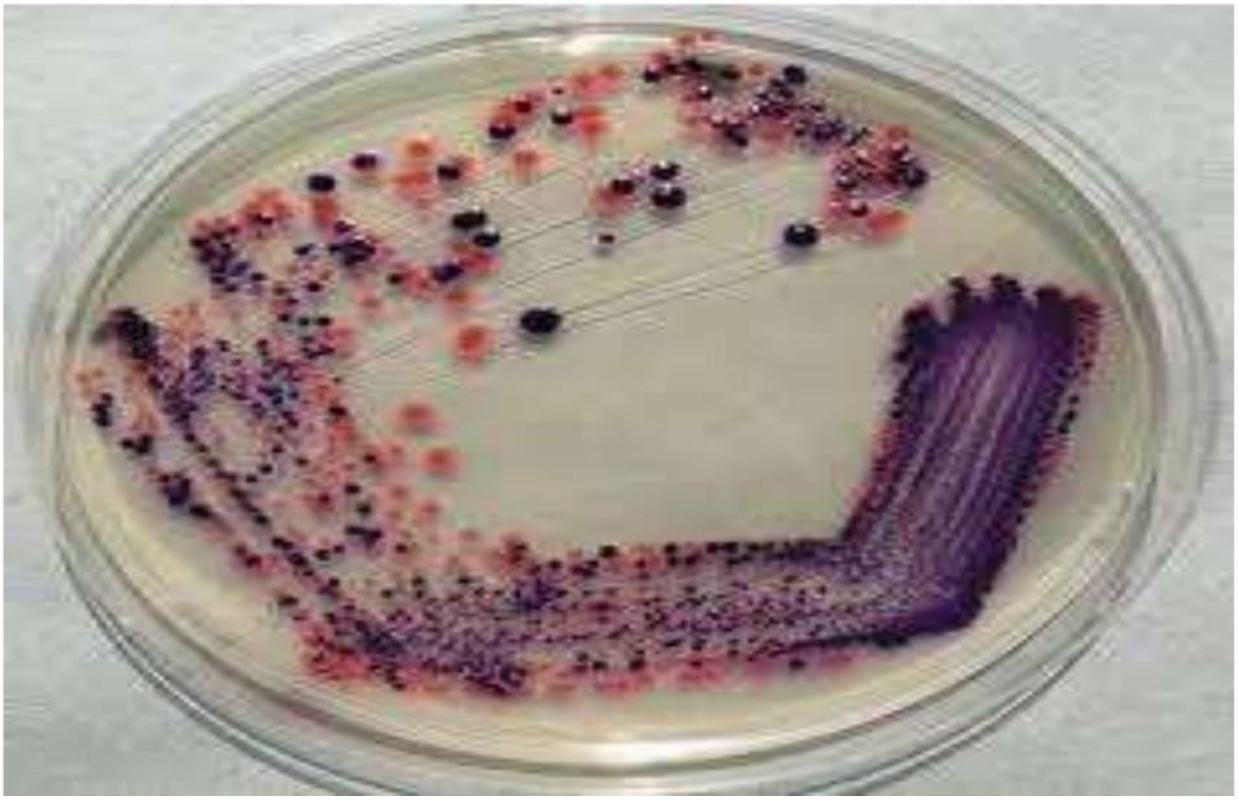
AEROBIC CULTURE METHODS

- 1. Streak plate

The streak culture (surface plating) method
Is routinely used for the isolation of bacteria in Pure culture from clinical specimens




Select colony from starter plate
First streak on fresh plate
Second streak
Third streak



Lawn Culture

- Provides a uniform surface growth of the bacterium.
- Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium
- Uses

– For bacteriophage typing.

– Antibiotic sensitivity testing.

– In the preparation of bacterial antigens and vaccines.

2.Lawn culture

- The lawn or carpet culture provides uniform surface growth of the bacterium and is useful for bacteriophage typing and antibiotic sensitivity testing

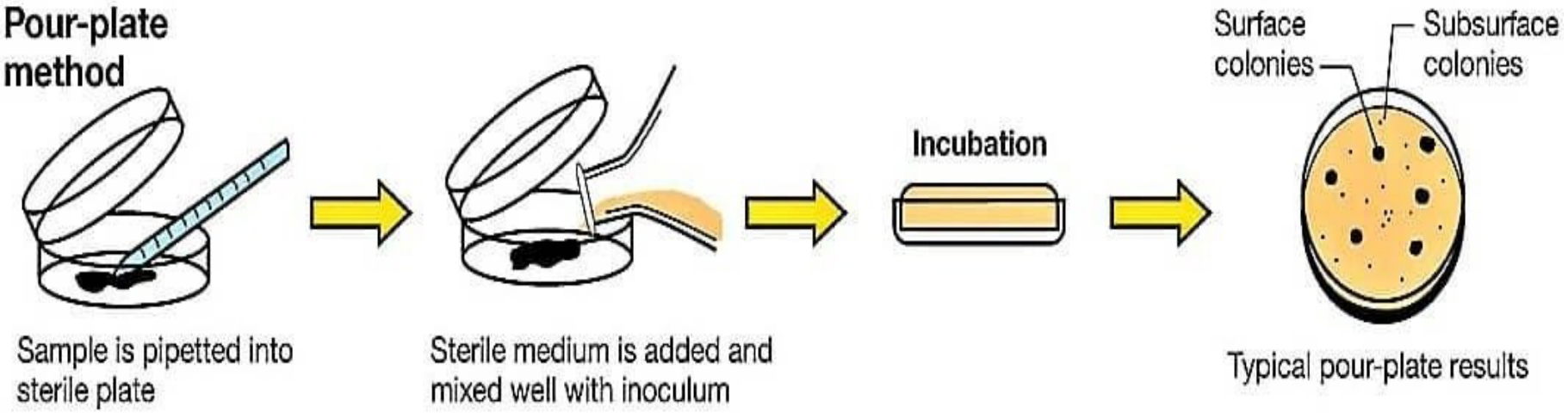
Pour Plate Culture

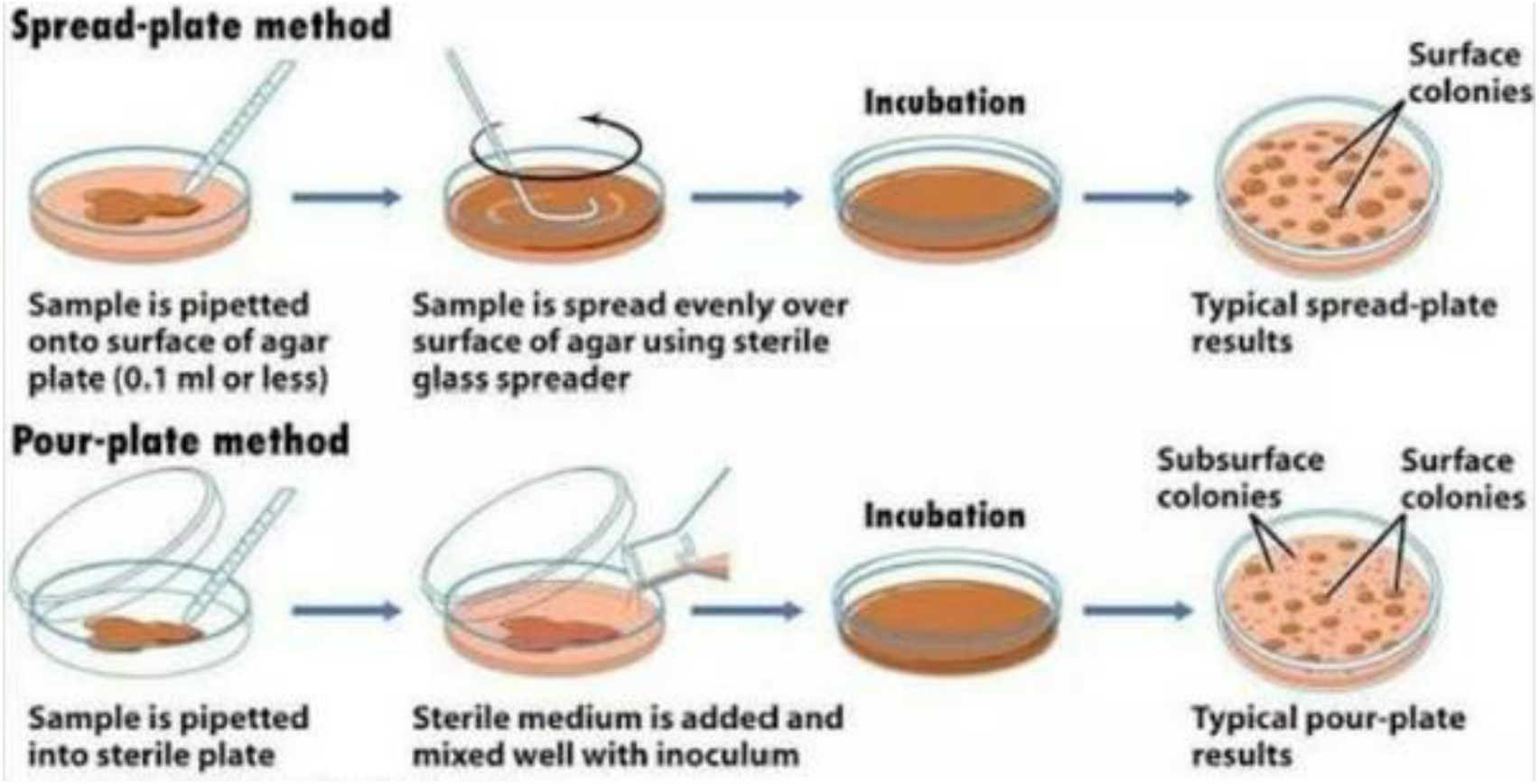
- 1 ml of the innoculum is added to the molten agar.
- Mix well and pour to a sterile Petri dish.
- Allow it to set.
-

  depth of the medium.

Uses:

- Gives an estimate of the viable bacterial count in a suspension.
- For the quantitative urine cultures.


Pour-plate method
Sample is pipetted into sterile plate
Sterile medium is added and mixed well with inoculum
Incubation
Surface colonies
Subsurface colonies
Typical pour-plate results


Spread-plate method
Incubation
Surface colonies
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
Typical spread-plate results
Pour-plate method
Incubation
Subsurface colonies
Surface colonies
Sample is pipetted into sterile plate
Sterile medium is added and mixed well with inoculum
Typical pour-plate results

Anaerobic growth

Reducing media containing thioglycolate to deplete oxygen; cooked meat broth

Anaerobic jar, anaerobic chamber, anaerobic bags/ pouch