# MICROBIOLOGY

## FOR THE STUDENTS OF DIPLOMA IN PHARMACY PART-I

Prepared and presented by

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# MICRO BIOLOGY

**Definition:** *Microbiology is the study of living organisms of microscopic size, which include bacteria, fungi, algae, protozoa, and the infectious agents at the border line of life that are called viruses.*

- *It is concerned with their form ,structure, reproduction, physiology, metabolism and classification.*

- *It includes the study of their distribution in nature, their relationship to each other and to other living organisms, their effects on human beings and on other animals and plants, de abilities to make physical and chemical changes in our environment, and their reactions to physical and chemical agents.*

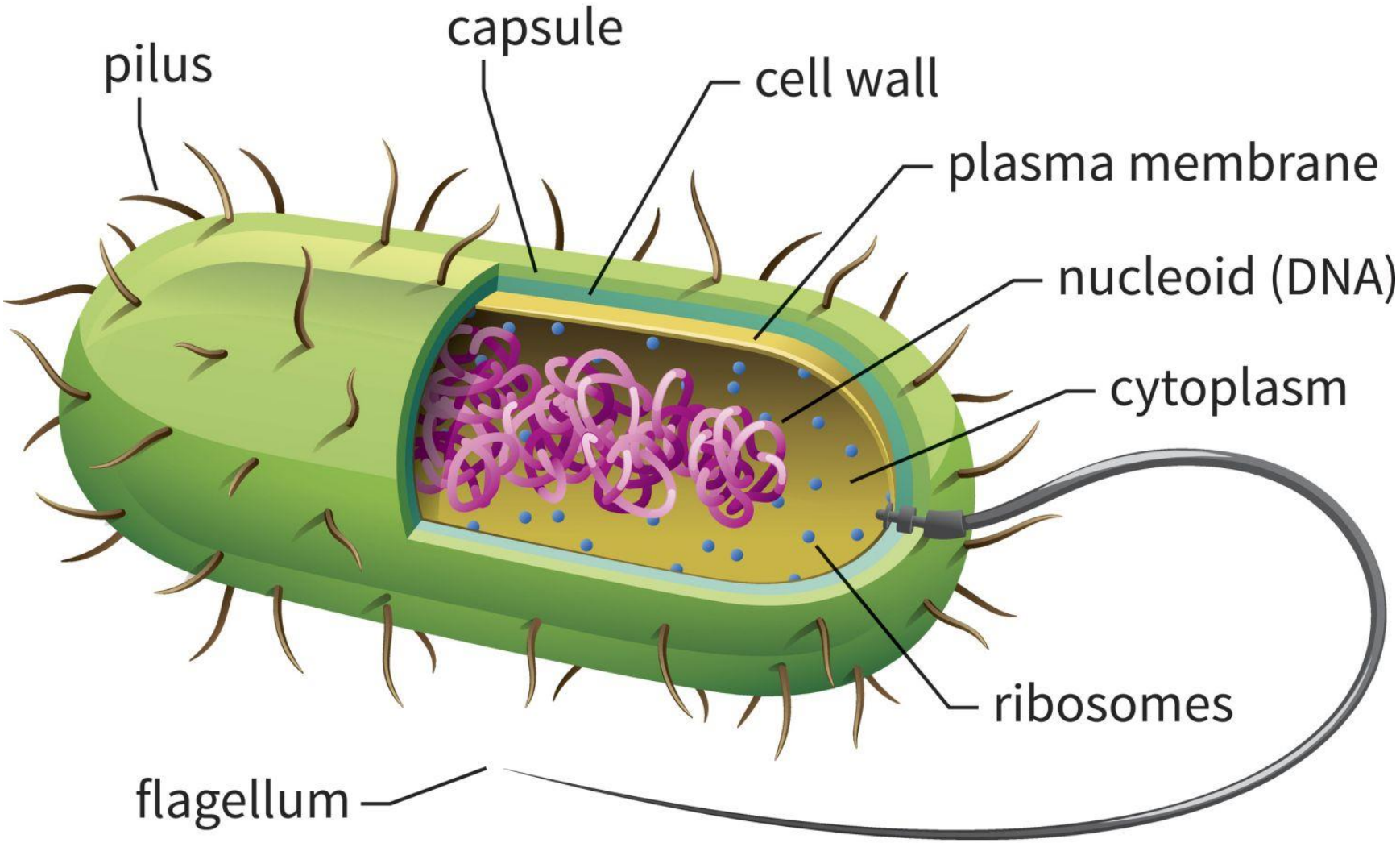
# Major groups of microorganisms

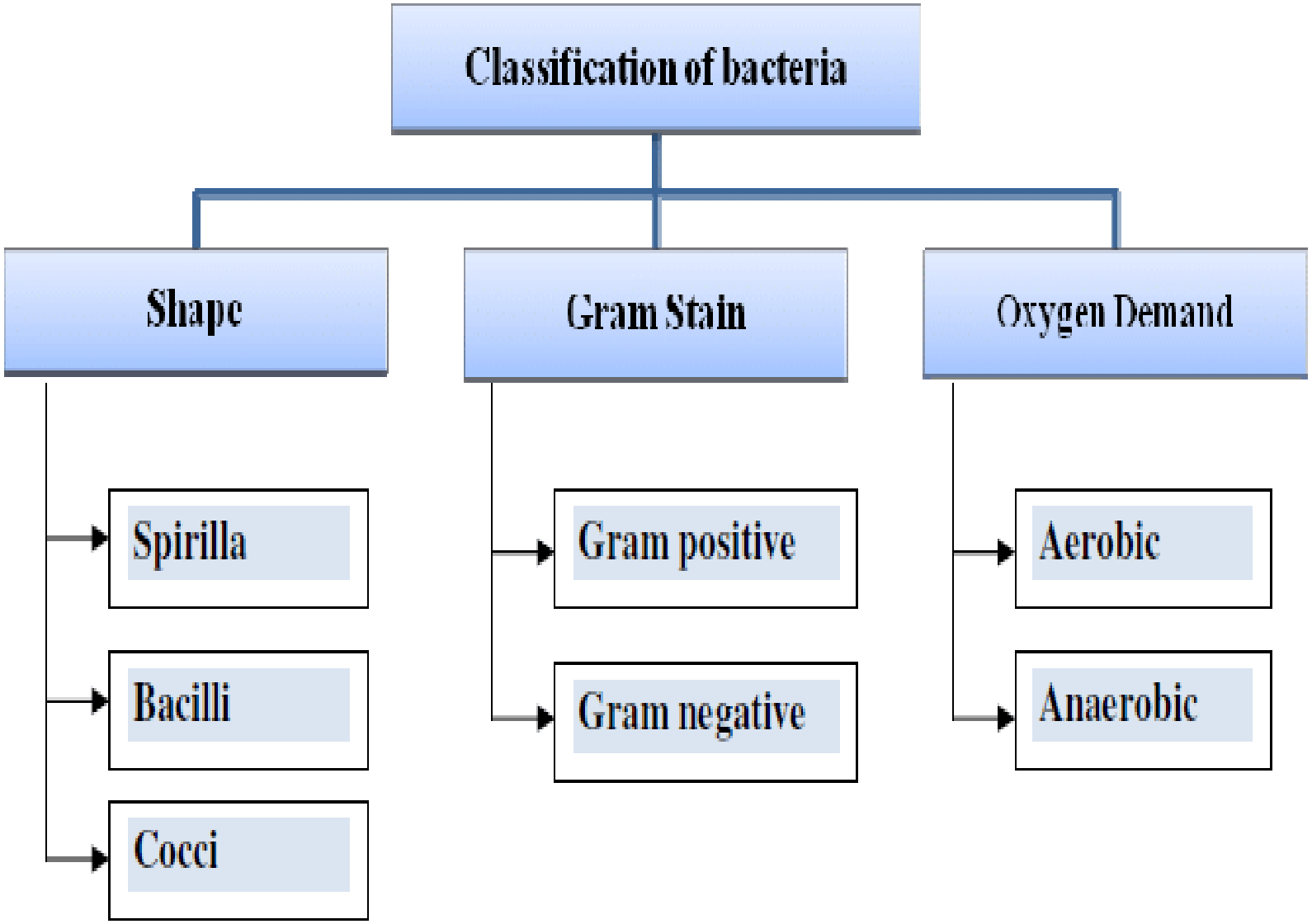
- ***Algae:*** *They are relatively simple organisms. The most primitive types are unicellular. Others are aggregations of similar cells with little or no differentiation in structure of function. Regardless of size or complexity, all algal cells contain chlorophyll and are capable of photosynthesis. Algae are found most commonly in aquatic environments or in damp soil.*
- ***Virus:*** *They are very small non-cellular parasites or pathogens of plants, animals, and bacteria and other microorganisms. They are so small that they can be visualised only by the electron microscope. Viruses can be cultivated only in living cells.*
- ***Bacteria:*** *they are unicellular prokaryotic organisms or simple associations of similar cells. They grow on artificial laboratory media, reproduce asexually by simple cell division.*
- ***Protozoa:*** *they are unicellular eukaryotic organisms. They are differentiated on the basis of morphological, nutritional, and physiological characteristics. Some of the protozoa are cultivated in the laboratory while some are intracellular parasites. They reproduce by asexual and sexual processes. Some protozoa are food for aquatic animals, Some cause diseases.*
- ***Fungi:*** *They are eukaryotic lower plants devoid of chlorophyll. They are usually multi cellular but are not differentiated into roots, stems and leaves. They range in size from single celled microscopic yeasts two giant multi cellular mushrooms. True fungi are composed of filaments and masses of cells which make up the body of the organism known as mycelium. Fungi reproduce by fission, by budding or by means of spores.*

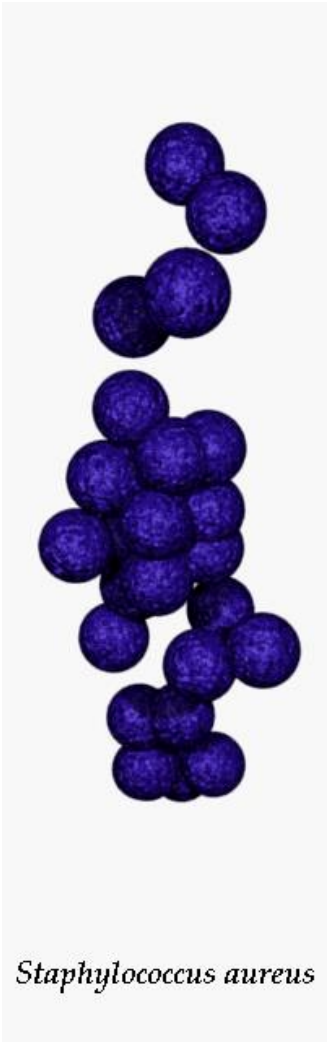
## Features distinguishing Prokaryotic from Eukaryotic cells

| Features | Prokaryotic Cells | Eukaryotic Cells |
|---|---|---|
| Groups where found | Bacteria | Algae,Fungi,Protozoa,Plants & Animals |
| Size range of organism | 1-2 by 1-4μm or less | Greater than 5μm in width or diameter |
| Genetic system location | Nucleoid,Chromatin body,or nuclear material | Nucleus,mitochondria,chloroplasts |
| Structure of nucleus | Not bound by nuclear membrane:<br>One circular chromosome<br>Chromosome does not contain histones;<br>no mitotic division<br>Nucleolus absent | Bounded by nuclear membrane;more than one chromosome<br>Chromosomes have histones: mitotic nuclear division<br>Nucleolus present |
| **Cytoplasmic nature and structures** | | |
| Cytoplasmic streaming | Absent | Present |
| Pinocytosis | Absent | Present |
| Mesosome | Present | Absent |
| Ribosomes | 70S,distributed in the cytoplasm | 80S on endoplasmic reticulum;70S in mitochondria and chloroplasts |
| Mitochondria | Absent | Present |
| Chloroplasts | Absent | May be present |
| Golgi structures | Absent | Present |
| Endoplasmic Reticulum | Absent | Present |
| Membrane bound(true)vacuoles | Absent | Present |
| **Other cell structures** | | |
| Cell Wall | Peptidoglycan (murein or mucopolypeptide) as component | Absence of peptidoglycan |
| Locomotor Organs | Simple fibril | Multifibrilled with “9+2” microtubules |
| Pseudopodia | Absent | Present in some |
| Metabolic mechanisms | Wide variety,particularly that of anaerobic energy-yielding reactions;some fix nitrogen gas;some accumulate poly-β-hydroxybutyrate as reserve material | Glucolysis is the pathway for anaerobic energy-yielding mechanism |

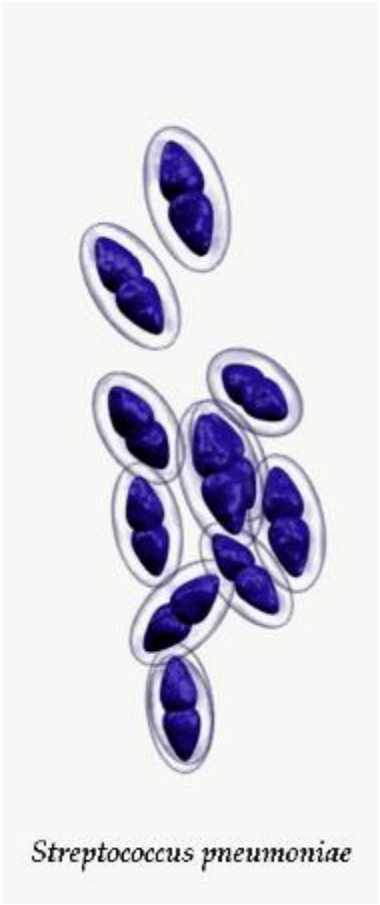
# Bacterial Cell structure



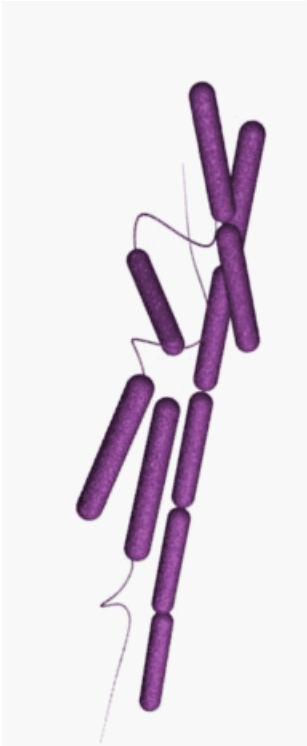

Classification of bacteria
Shape
Gram Stain
Oxygen Demand
Spirilla
Bacilli
Cocci
Gram positive
Gram negative
Aerobic
Anaerobic



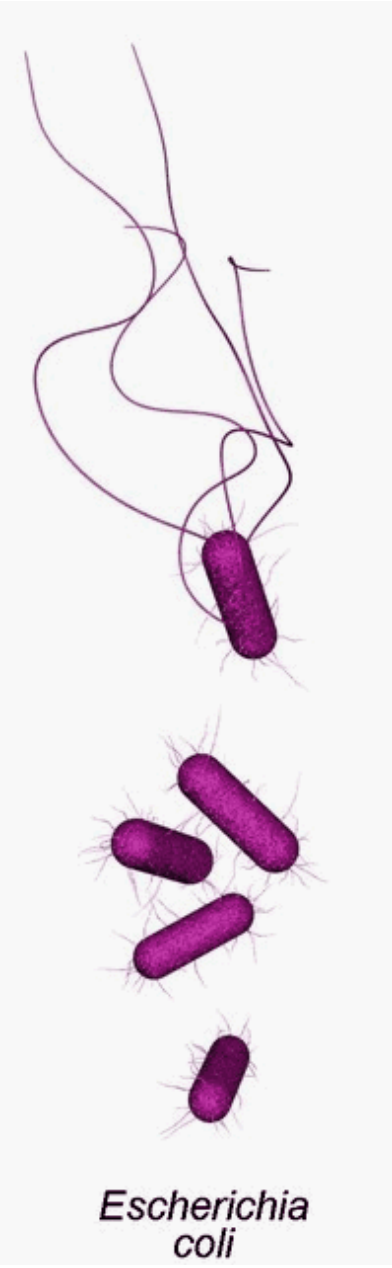
*Staphylococcus aureus*



*Streptococcus pneumoniae*



*Pseudomonas aeruginosa*



*Escherichia coli*

# CLASSIFICATION OF BACTERIA

## RODS (BACILLI)

**Chain of bacilli**
*(Bacillus anthracis)*

**Flagellate rods**
*(Salmonella typhi)*

**Spore-former**
*(Clostridium botulinum)*

## SPIRALS

**Vibrios**
*(Vibrio cholerae)*

**Spirilla**
*(Helicobacter pylori)*

**Spirochaetes**
*(Treponema pallidum)*

## SPHERES (COCCI)

**Diplococci**
*(Streptococcus pneumoniae)*

**Streptococci**
*(Streptococcus pyogenes)*

**Tetrad**

**Staphylococci**
*(Staphylococcus aureus)*

**Sarcina**
*(Sarcina ventriculi)*

**Oxygen requirements:** The categorization of bacteria by their oxygen requirements provides information on their metabolic pathways and requirements for culture:

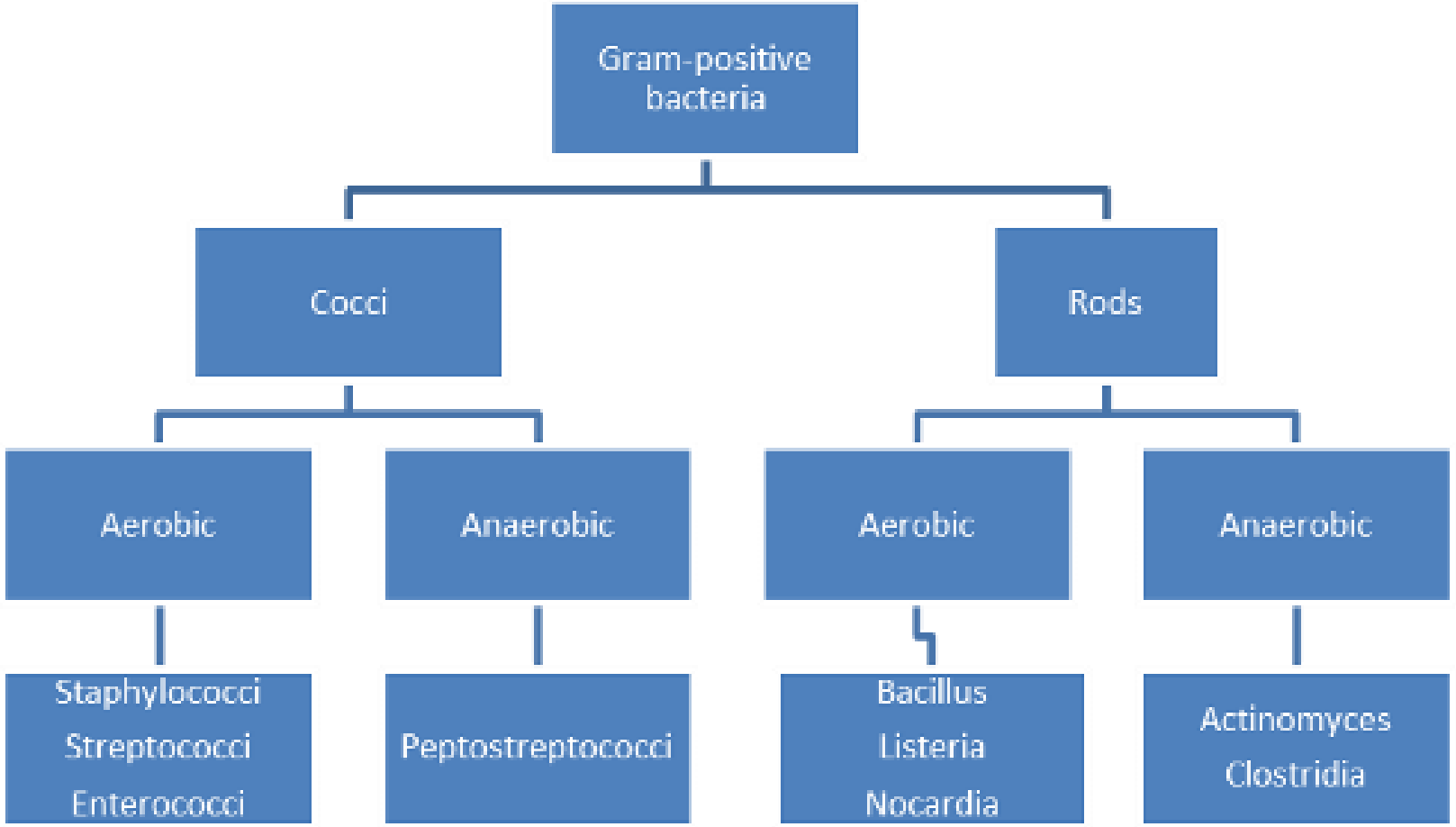
Obligate aerobes – must have oxygen for growth

Obligate anaerobes – can only grow in the absence of oxygen

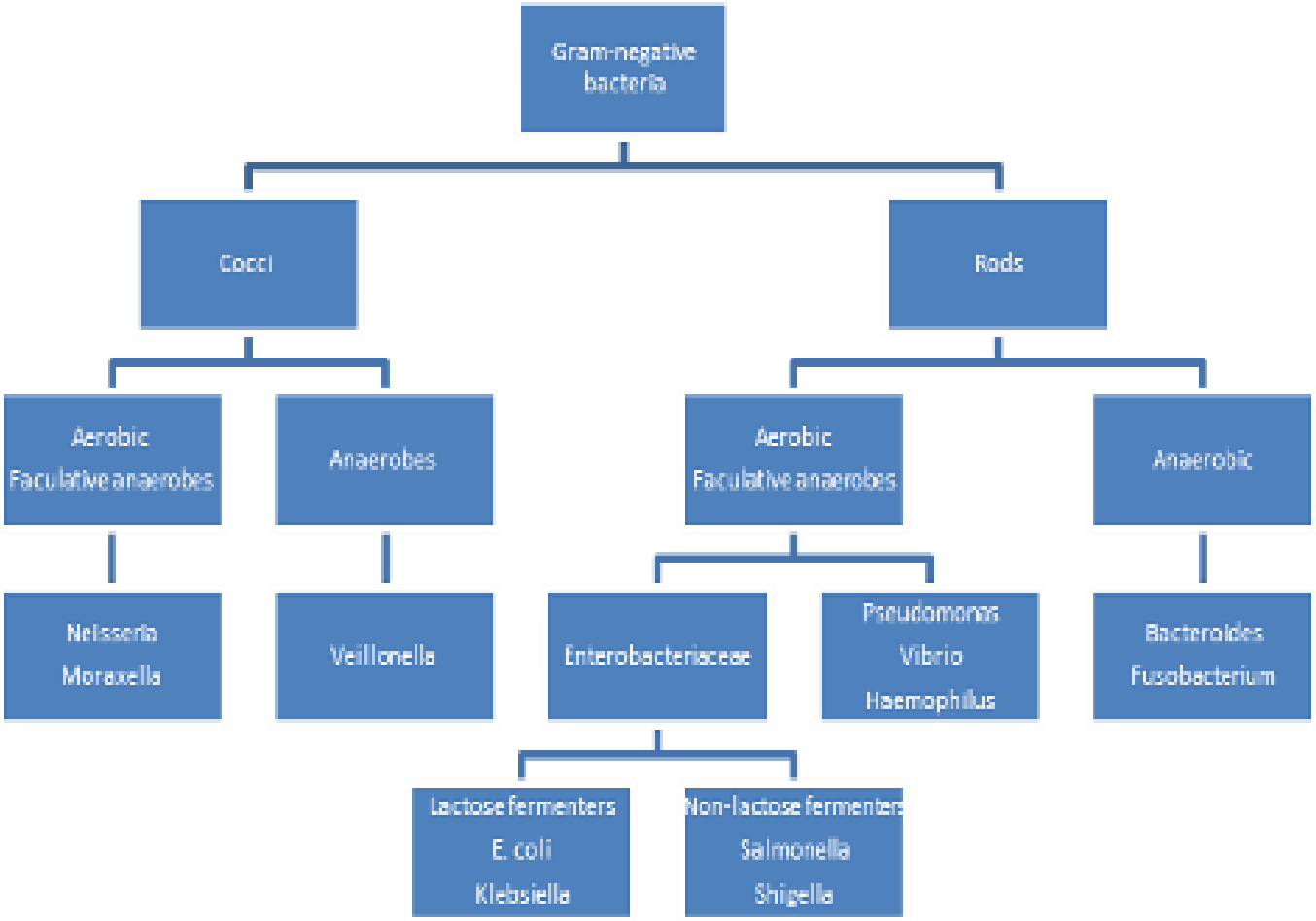
Facultative anaerobes – grow aerobically when oxygen is present but can also function in the absence of oxygen

Microaerophiles – grow best when concentration of oxygen is lower than in atmospheric air

# Gram Positive Bacteria classification



# Gram Negative bacteria classification



## Isolation of Microorganisms:

Microorganisms occur in natural environment like soil. They are mixed with several other forms of life. Many microbes are pathogenic. They cause a number of diseases with a variety of symptoms, depending on how they interact with the patient. The isolation and growth of suspected microbe in pure culture is essential for the identification and control the infectious agent.

The primary culture from natural source will normally be a mixed culture containing microbes of different kinds. But in laboratory, the various species may be isolated from one another. A culture which contains just one species of microorganism is called a pure culture. The process of obtaining a pure culture by separating one species of microbe from a mixture of other species, is known as isolation of the organisms.

## Enrichment Media Method:

This procedure involves the use of media and conditions of cultivation which favour the growth of the desired species. For example, when a man suffers with typhoid, the intestinal discharge posses small number of *Salmonella typhi* when compared with *E. coli* and other forms. It is almost impossible to isolate the typhoid organisms because they represent only a fraction of a per cent of the total microorganisms present. The media are therefore derived, which allow the rapid growth of the desired organisms, at the same time inhibiting the growth of other microorganisms.

## What is Streak Plate Method ?

Streaking is a method that isolates a pure strain from a species of bacteria. A sample is taken from a colony and a microbiological culture is grown on the new plate in order for the organism to be identified properly.

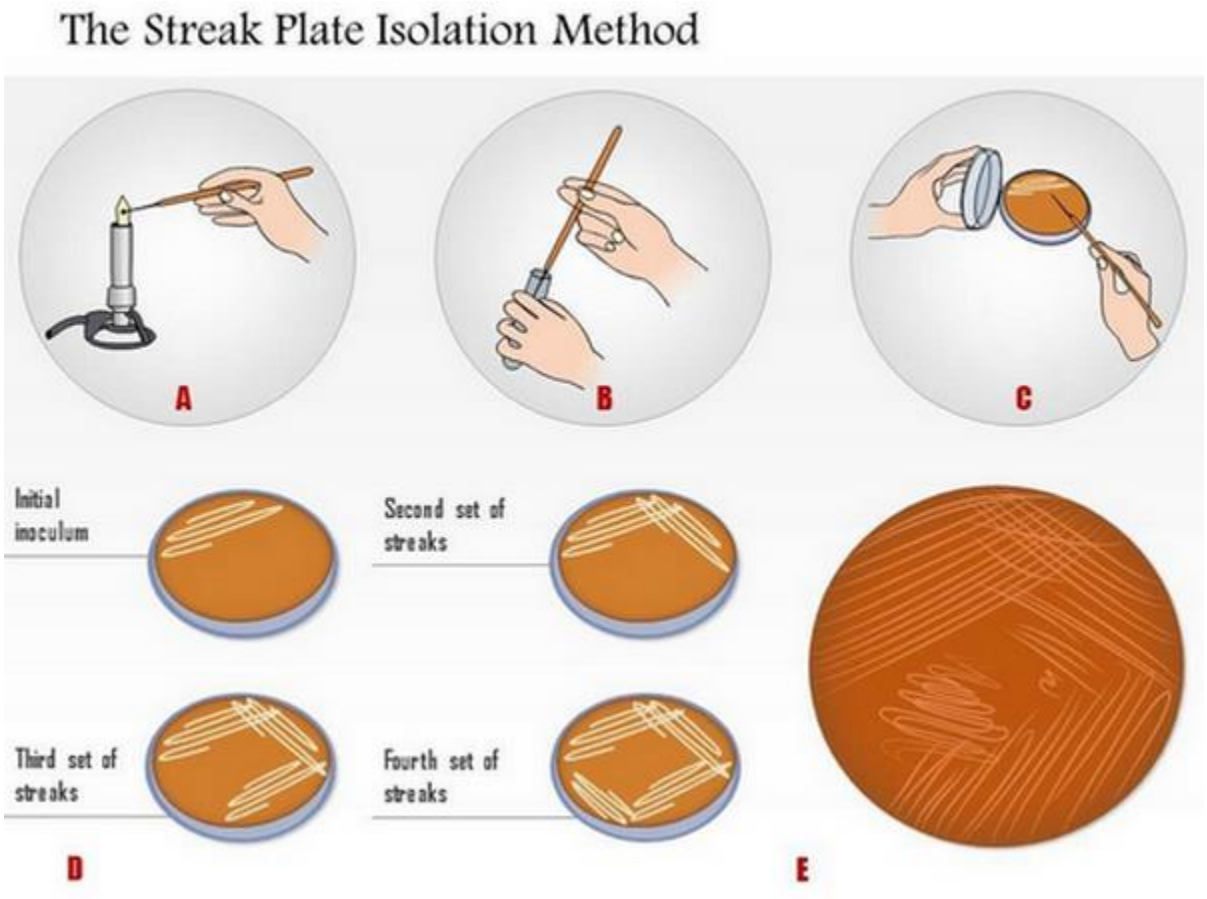
The procedure involves diluting bacteria by streaking the bacteria over the surface of the agar in the Petri dish. That way, an isolated colony can be obtained and grow into a number of cells. The culture is called a microbiological culture if the organism grows in the agar surface.

## What is the principle of the streak plate method?

The streak plate method requires the number of organisms in the inoculums be reduced. The procedure includes a dilution technique which requires spreading a loopful of culture over the agar plate surface.

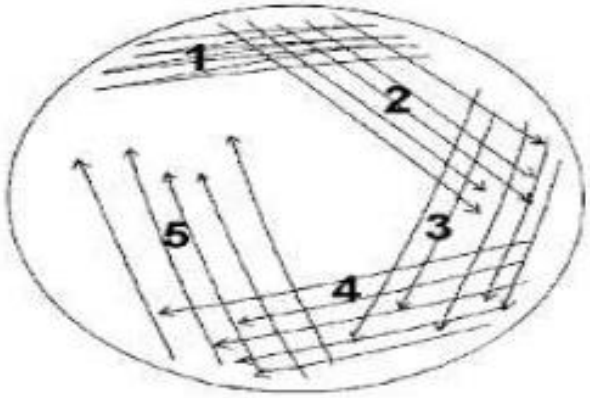
This is to make sure that the individual cells fall apart on the agar medium surface so as separation of the different species takes place. This procedure is also called rapid qualitative isolation method.

# STREAK PLATE METHOD

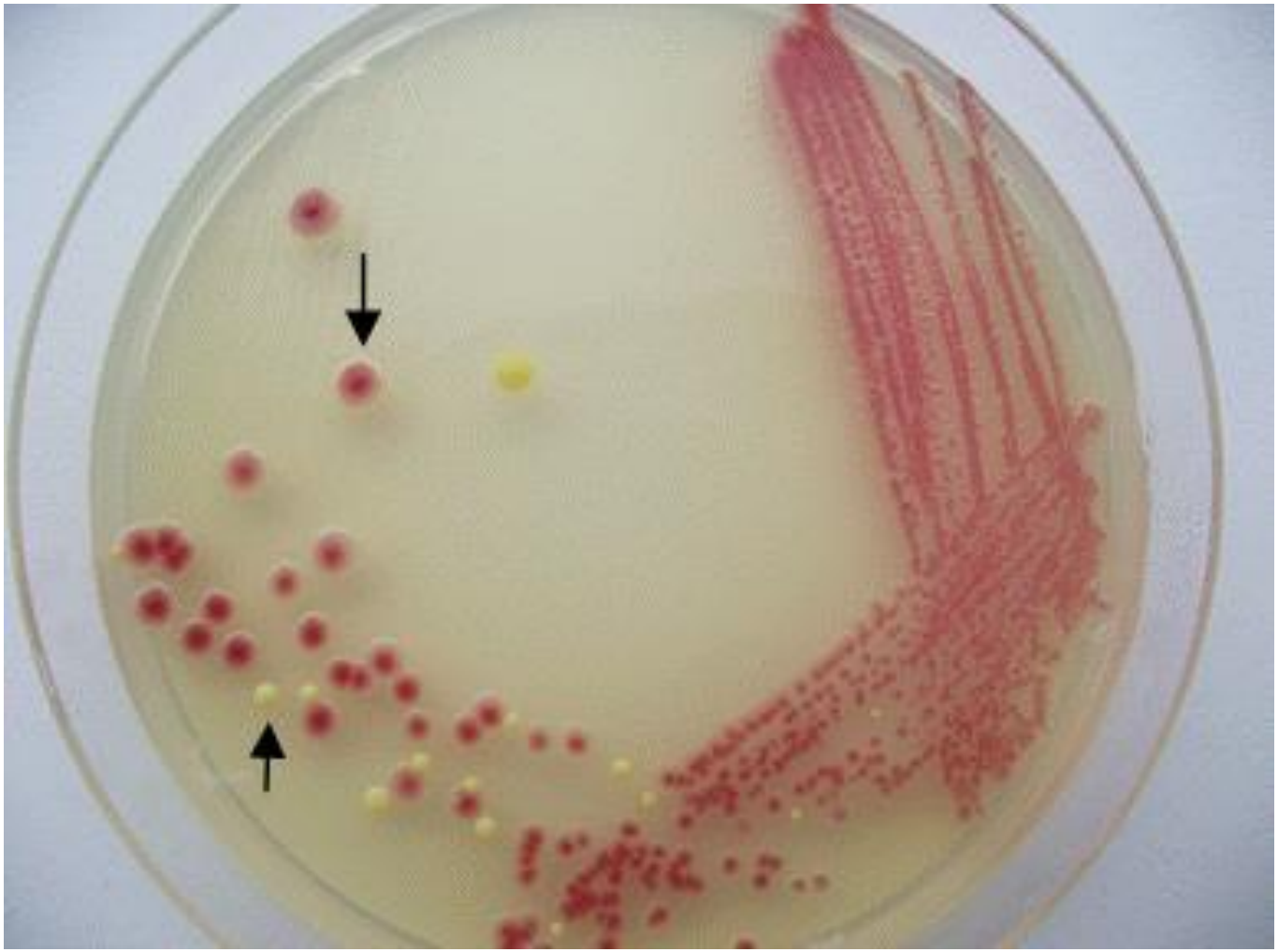


# 1.Streak plate technique

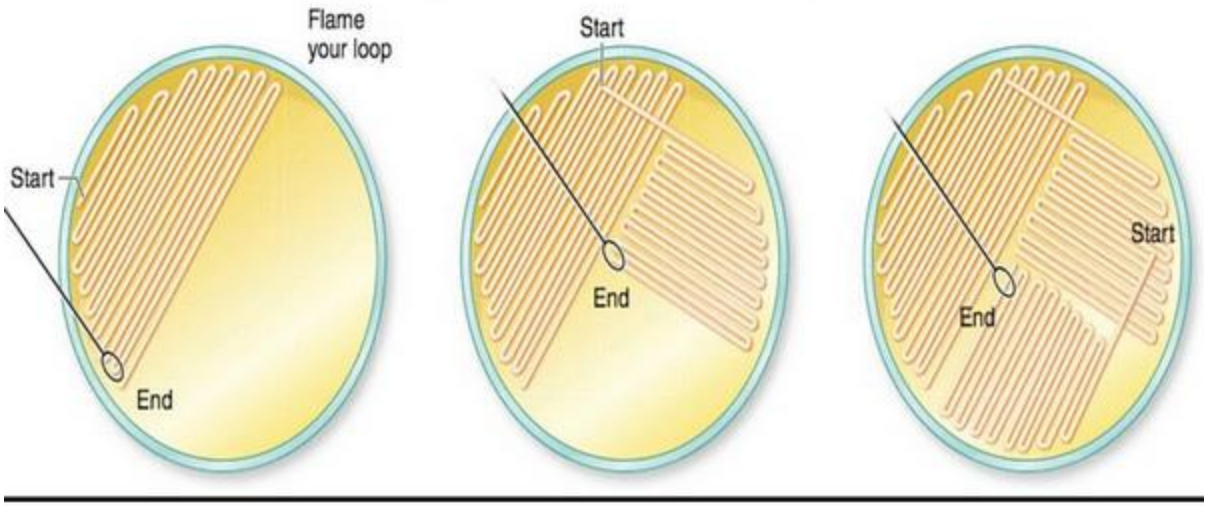
- Streaking is the process of spreading the microbial culture with an inoculating needle on the surface of the media.
- Sterilize the inoculating needle by flame to make red hot and allow it to cool for 30 seconds.



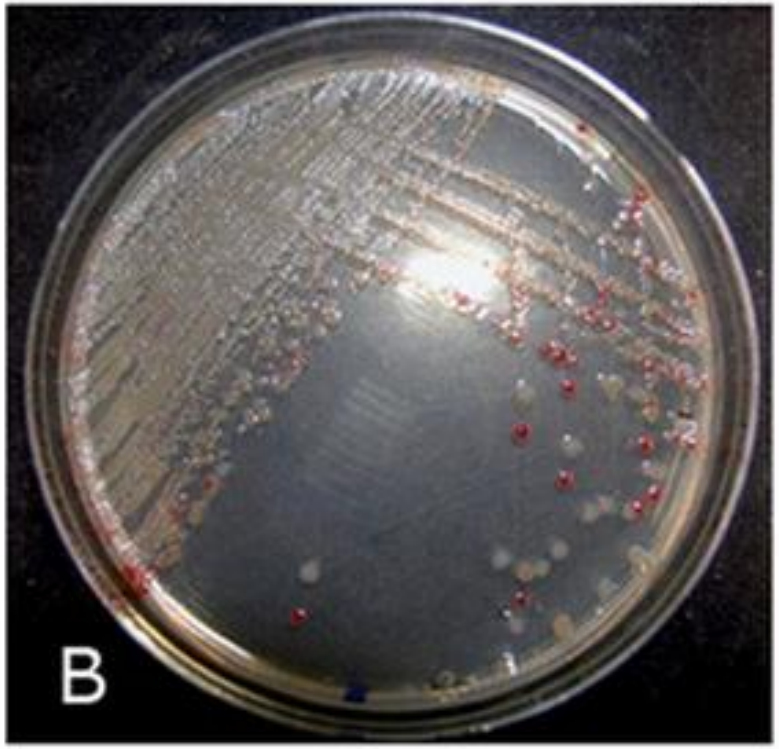

**ISOLATED COLONIES OF DIFFERENT BACTERIA SPECIES**

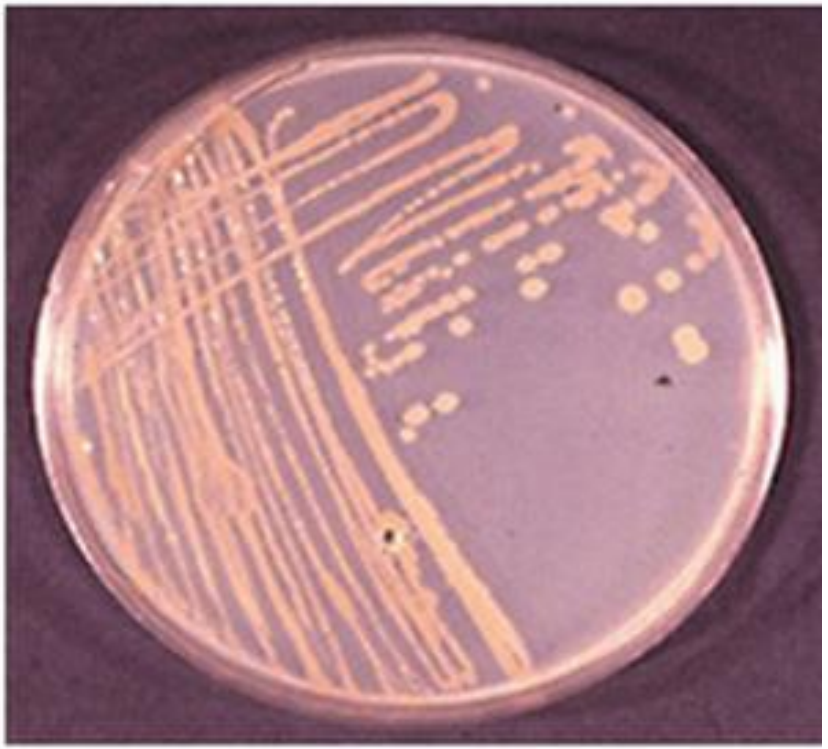


# INOCULATING A PLATE: THE STREAK PLATE TECHNIQUE



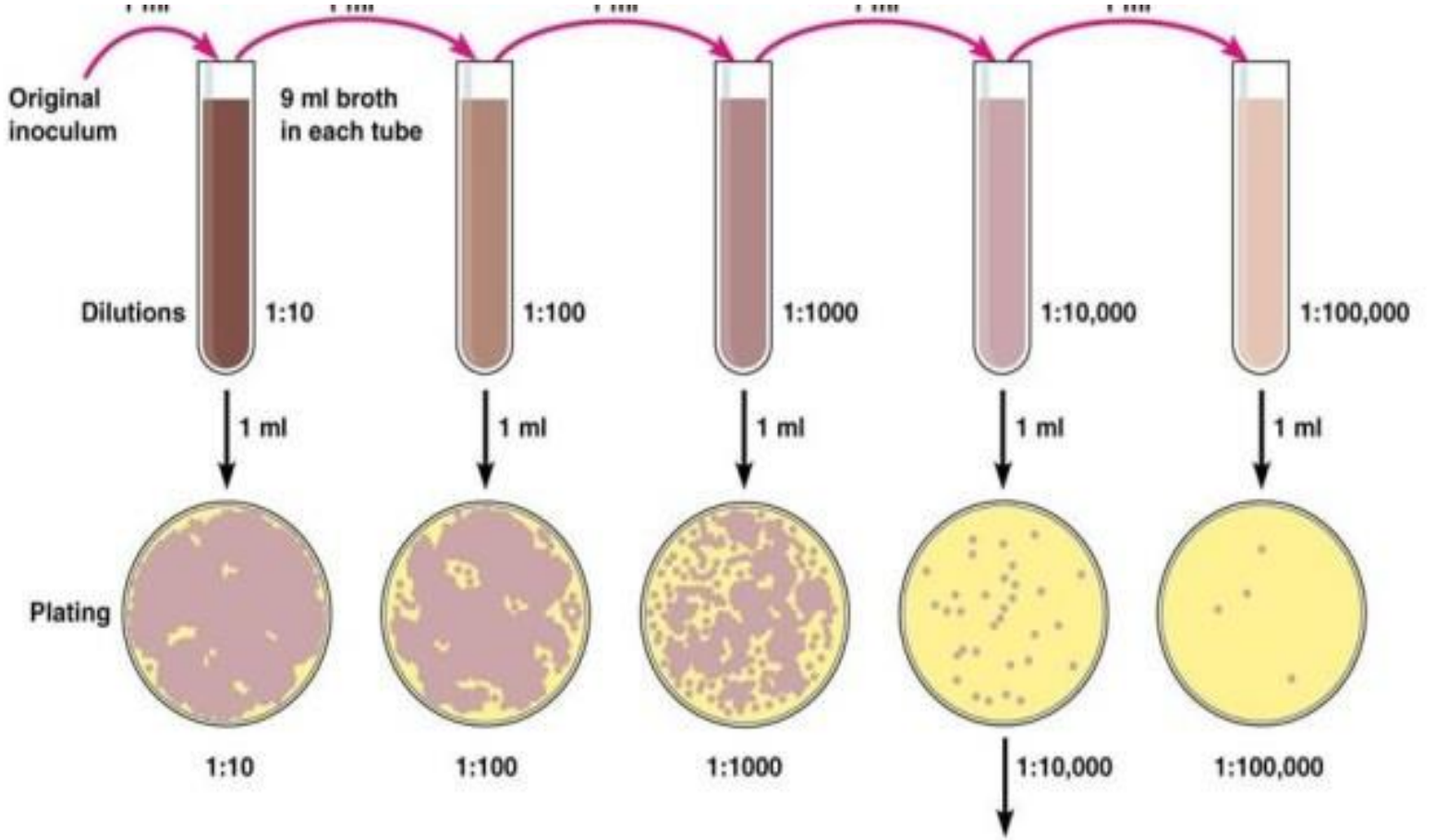
# Results



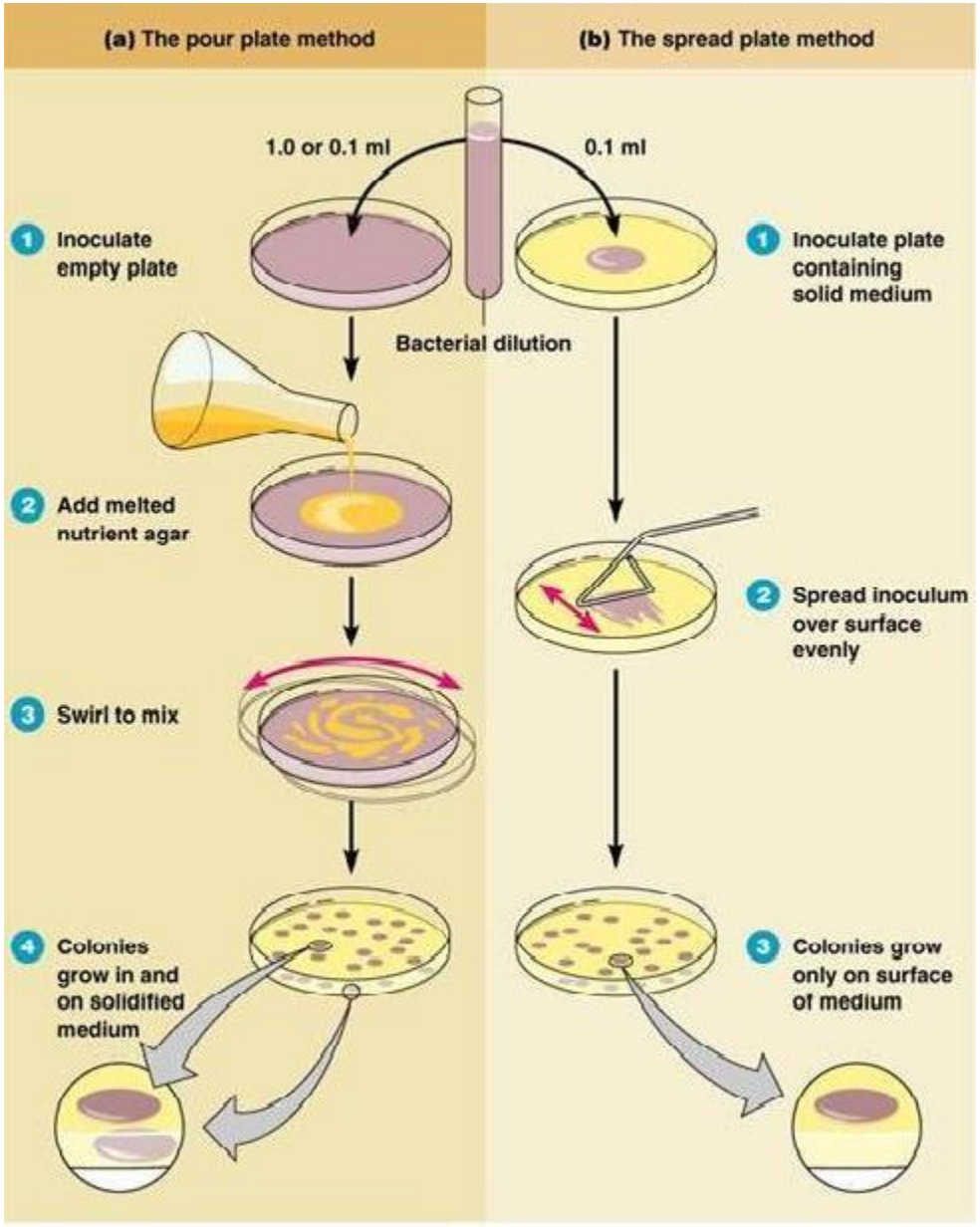



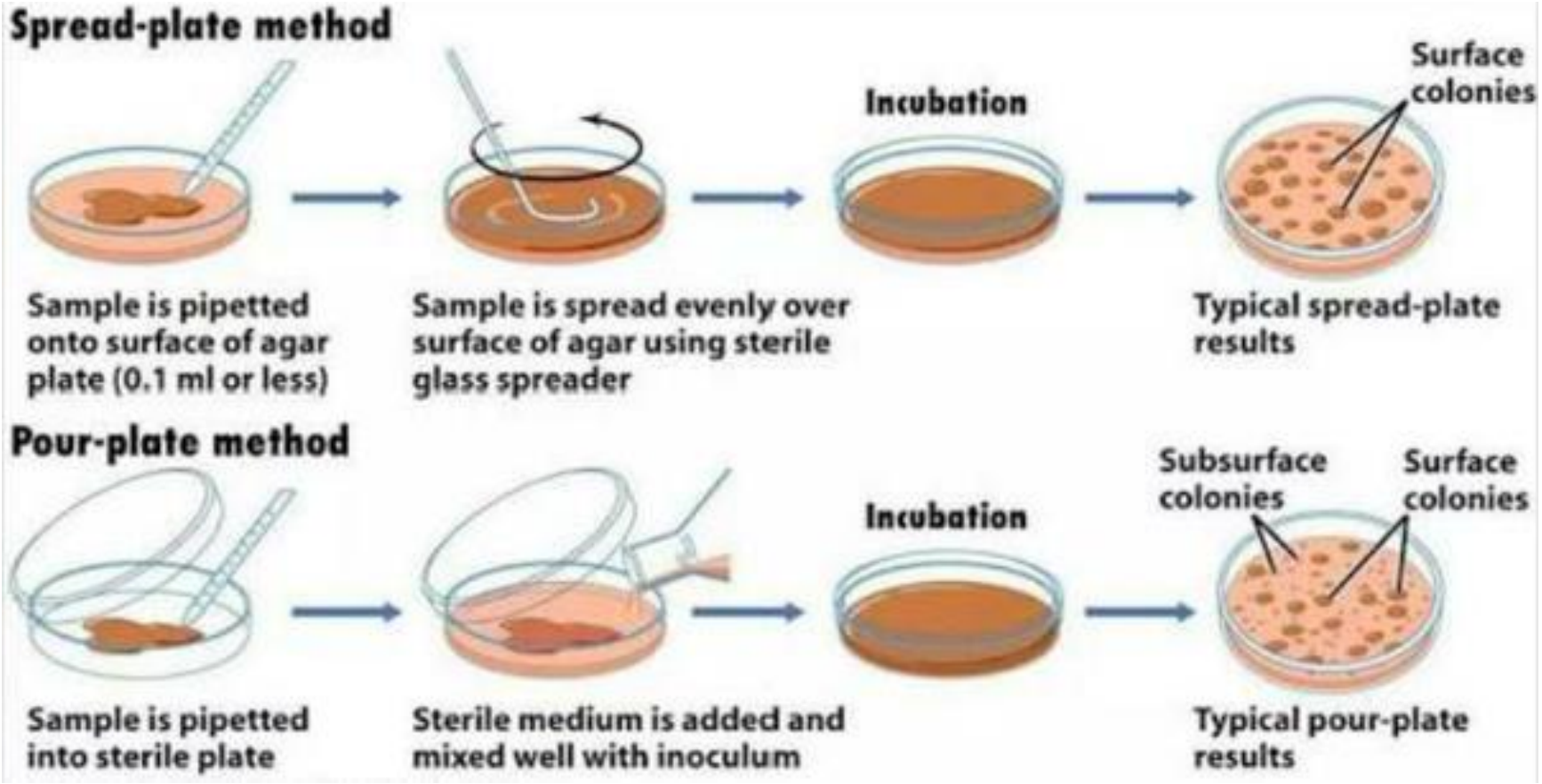
These plates are good examples of streak plate technique. Isolated colonies are visible. Plate B shows 3 types of colonies, the other plate shows a pure culture.

# Serial dilutions for pour plate method




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.)

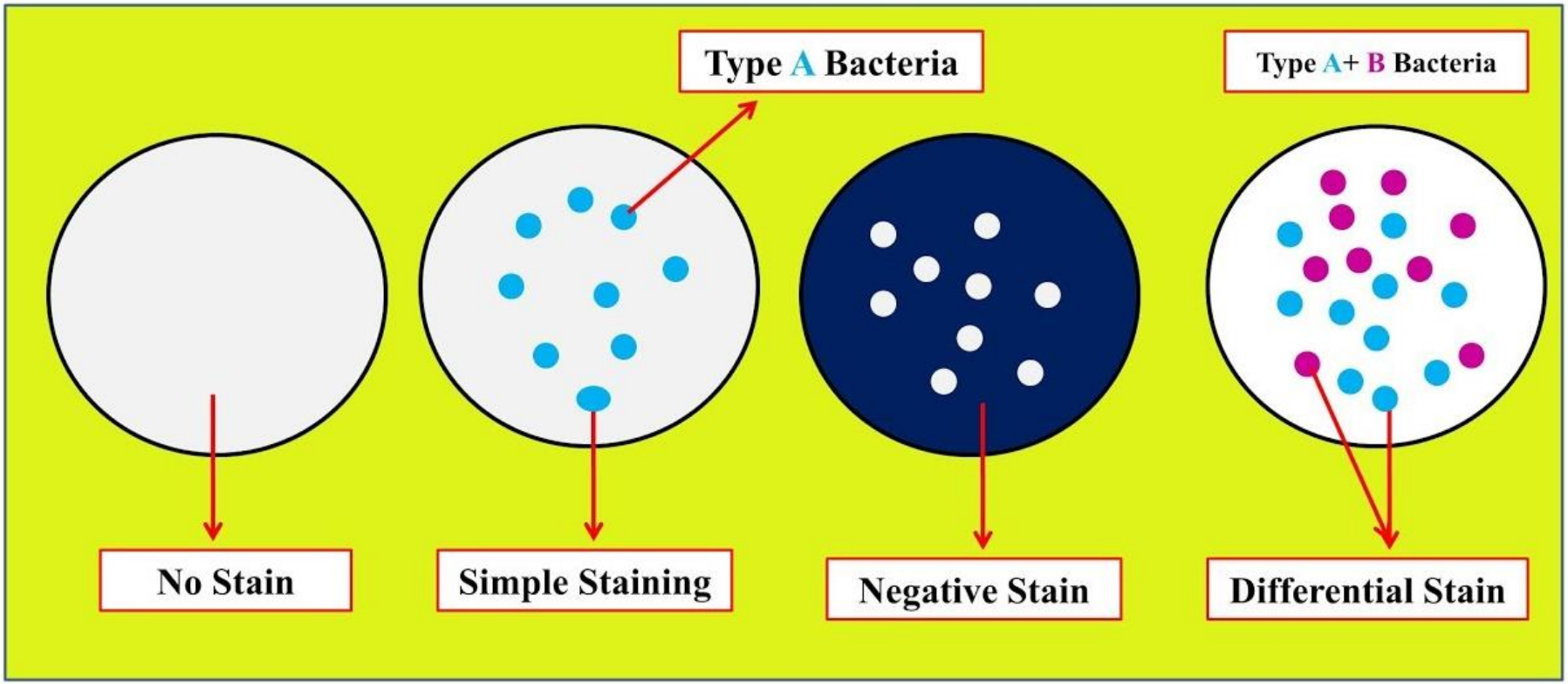

(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
Bacterial dilution
1 Inoculate empty plate
2 Add melted nutrient agar
3 Swirl to mix
4 Colonies grow in and on solidified medium
1 Inoculate plate containing solid medium
2 Spread inoculum over surface evenly
3 Colonies grow only on surface of medium


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

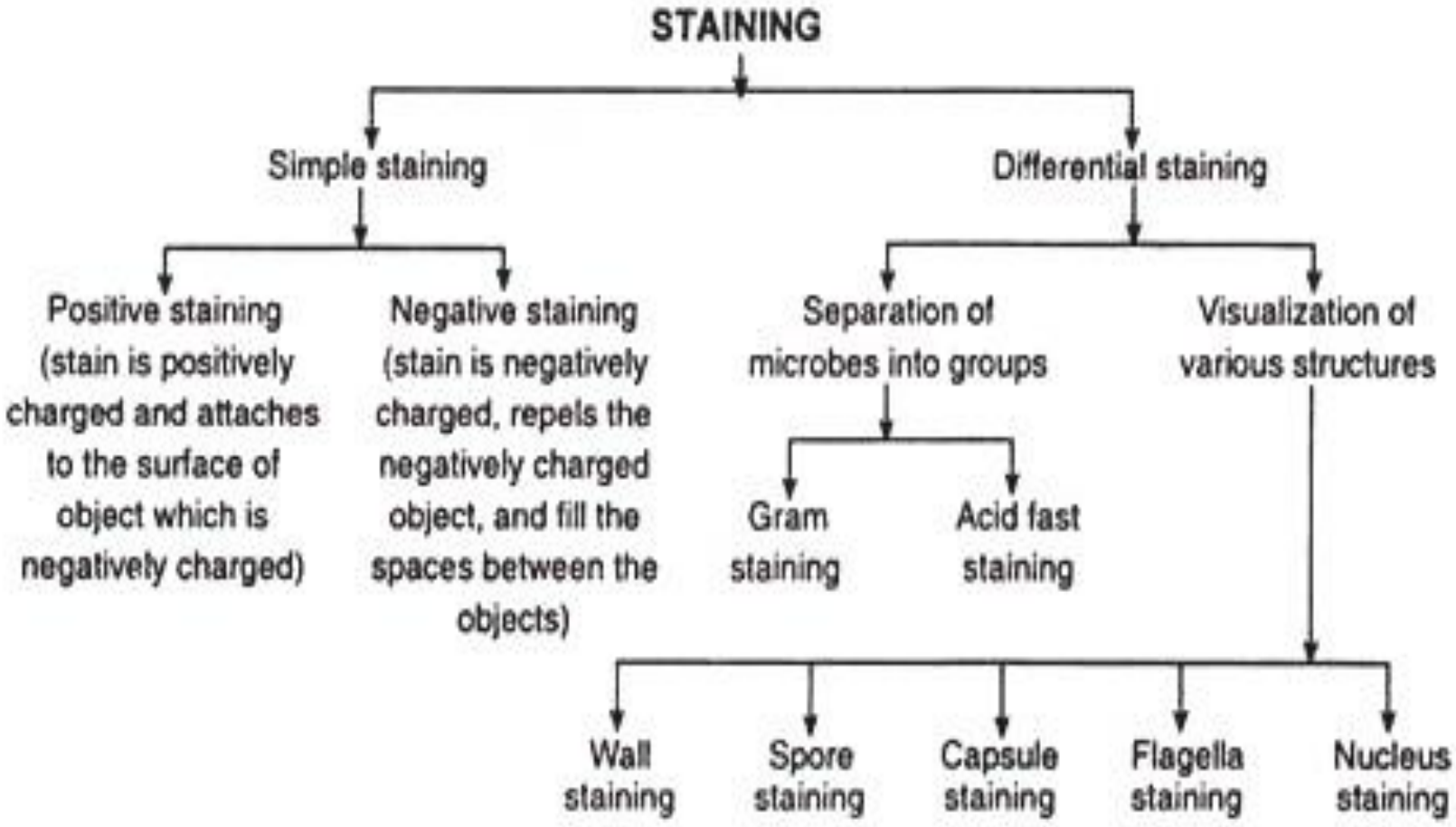
# Staining Bacteria-Why needed?

- Bacteria have same refractive index as water, therefore,when they are observed under a microscope they are opaque on nearly invisible to the naked eye
- Different types of staining methods are used to make the cells and their internal structures more visible under the light microscope

# Importance of staining



# Different Staining of Bacteria



# SIMPLE STAINING:

- Simple to perform- only one basic stain used.

E.g. Crystal violet, Methylene blue, Basic fuschin etc.,

- **Principle:**

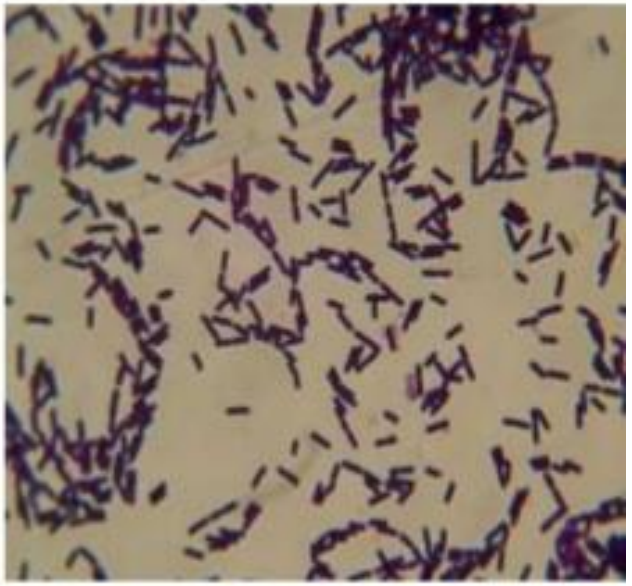
  - All bacteria in smear takes stain and appears in colour of stain.
  - Basic stain more affinity towards bacterial surface & stains the bacteria.

- **Uses:**

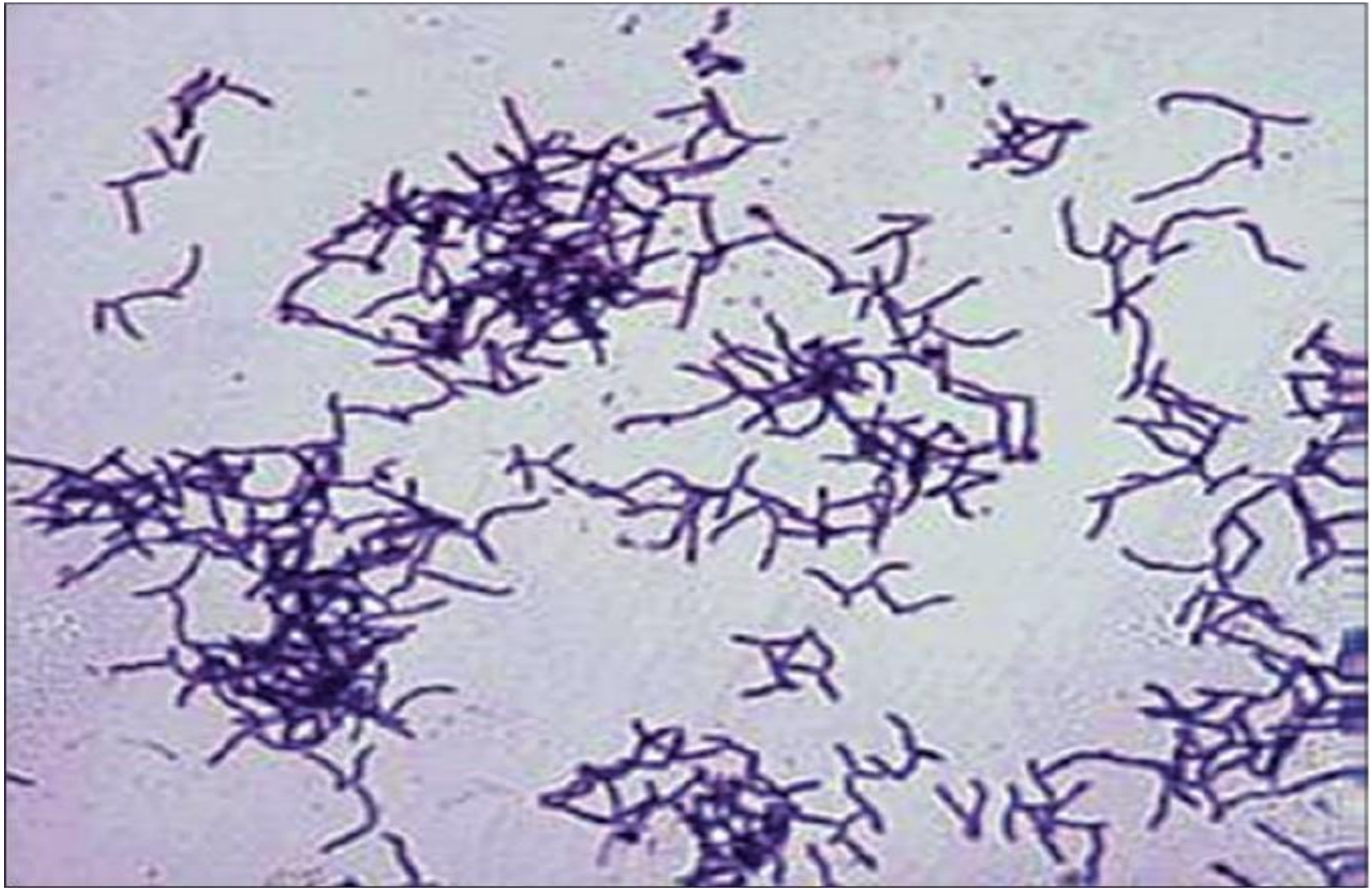
  To study morphology and arrangement of bacteria.

PROCEDURE:

- A bacterial smear is prepared, air-dried and heat-fixed.
- A Heat-fixed smear is flooded with either one of the basic stain and allowed to react for 1-2 minutes and then washed under running tap water.
- Air dried and focused with 10x,45x & 100x.

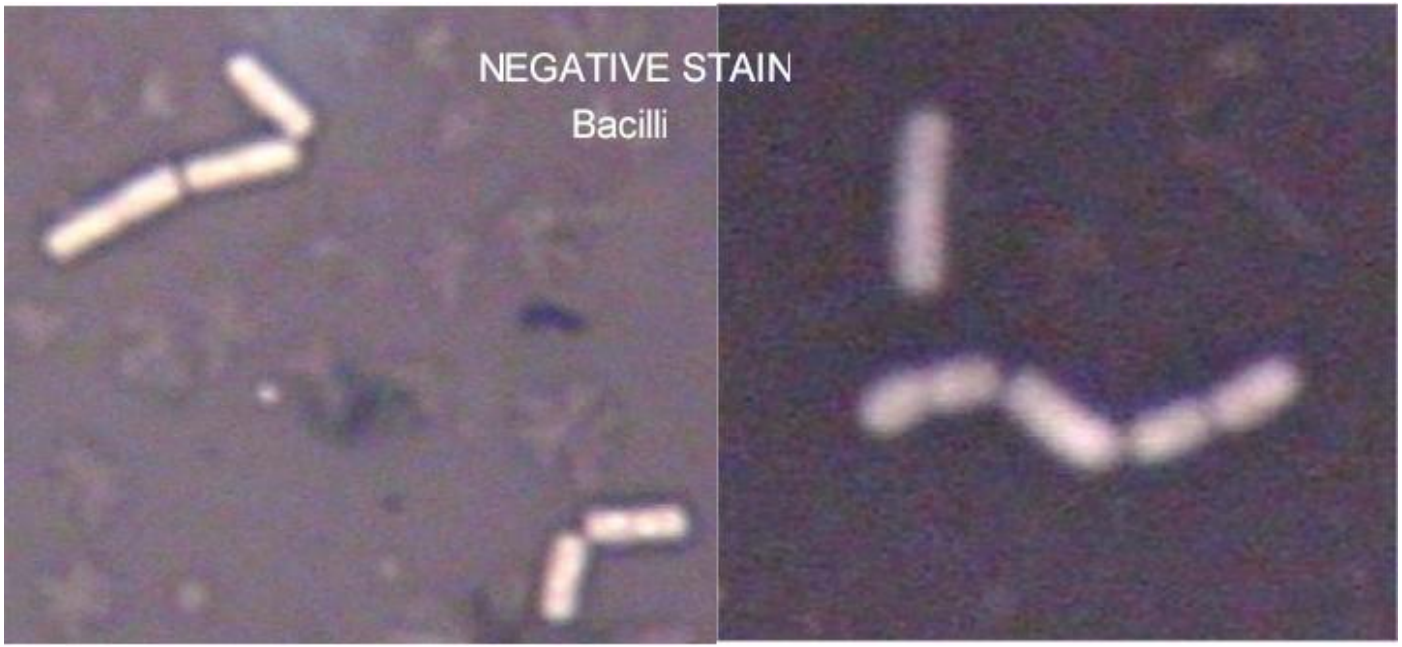


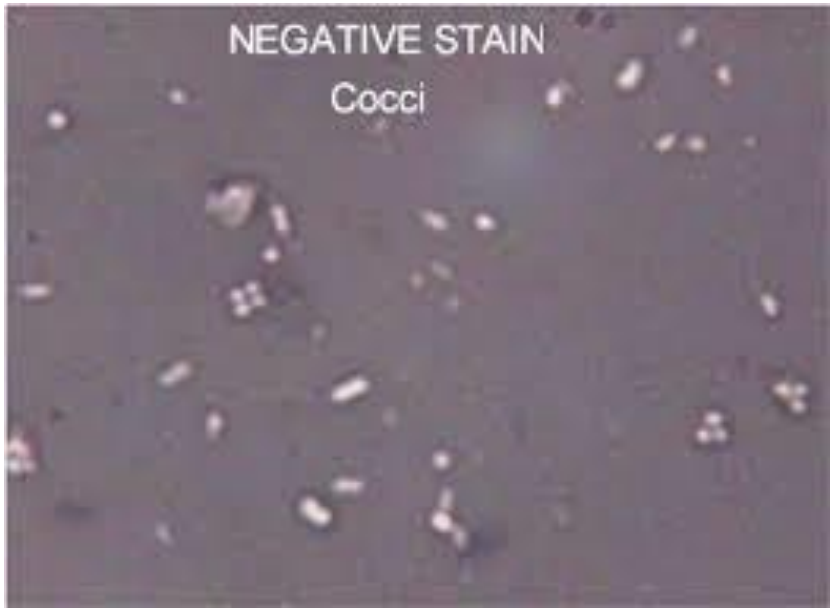
# Simple stain images

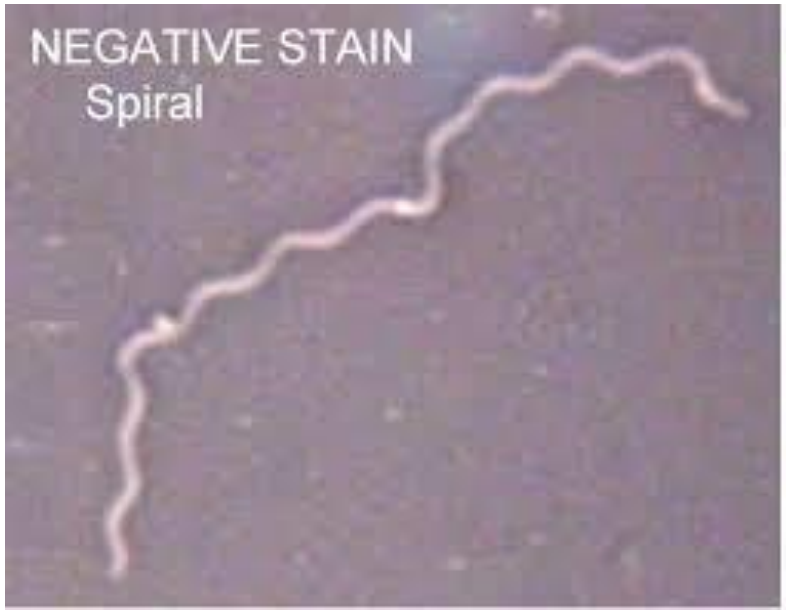


# Indirect staining with acidic dye (Negative staining)

- **The negative stain technique does not stain the bacteria but stain the background.**
- **The bacteria will appear clear against a dark background.**
- **No heat fixation or strong chemicals are used, so the bacteria less distorted than in other staining procedure.**
- **Example: Nigrosine are acidic stain (negatively charged), so the –ve stain doesn't stain the bacteria due ionic repulsion of bacterial cell wall**


NEGATIVE STAIN
Bacilli

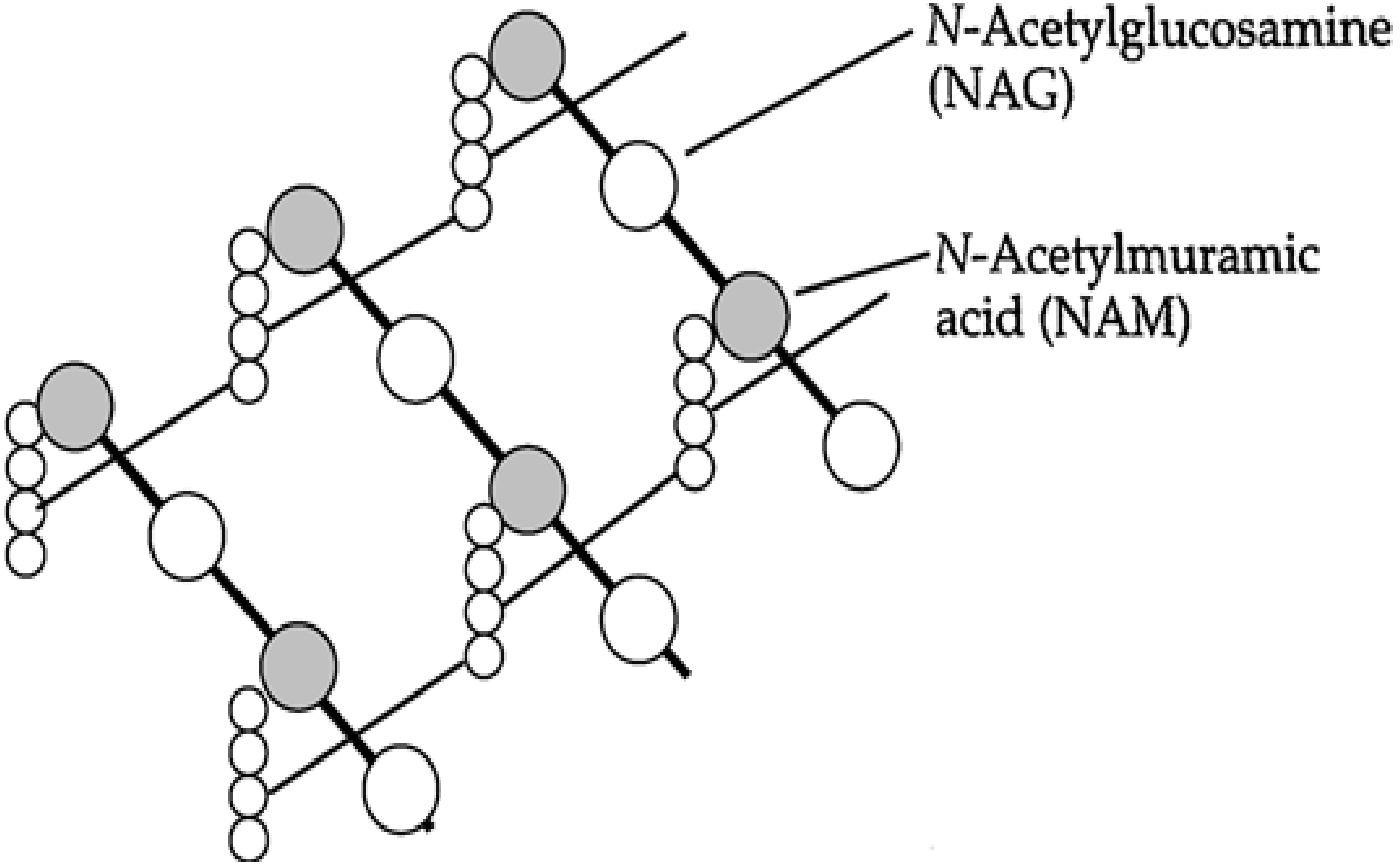

NEGATIVE STAIN
Cocci


NEGATIVE STAIN
Spiral

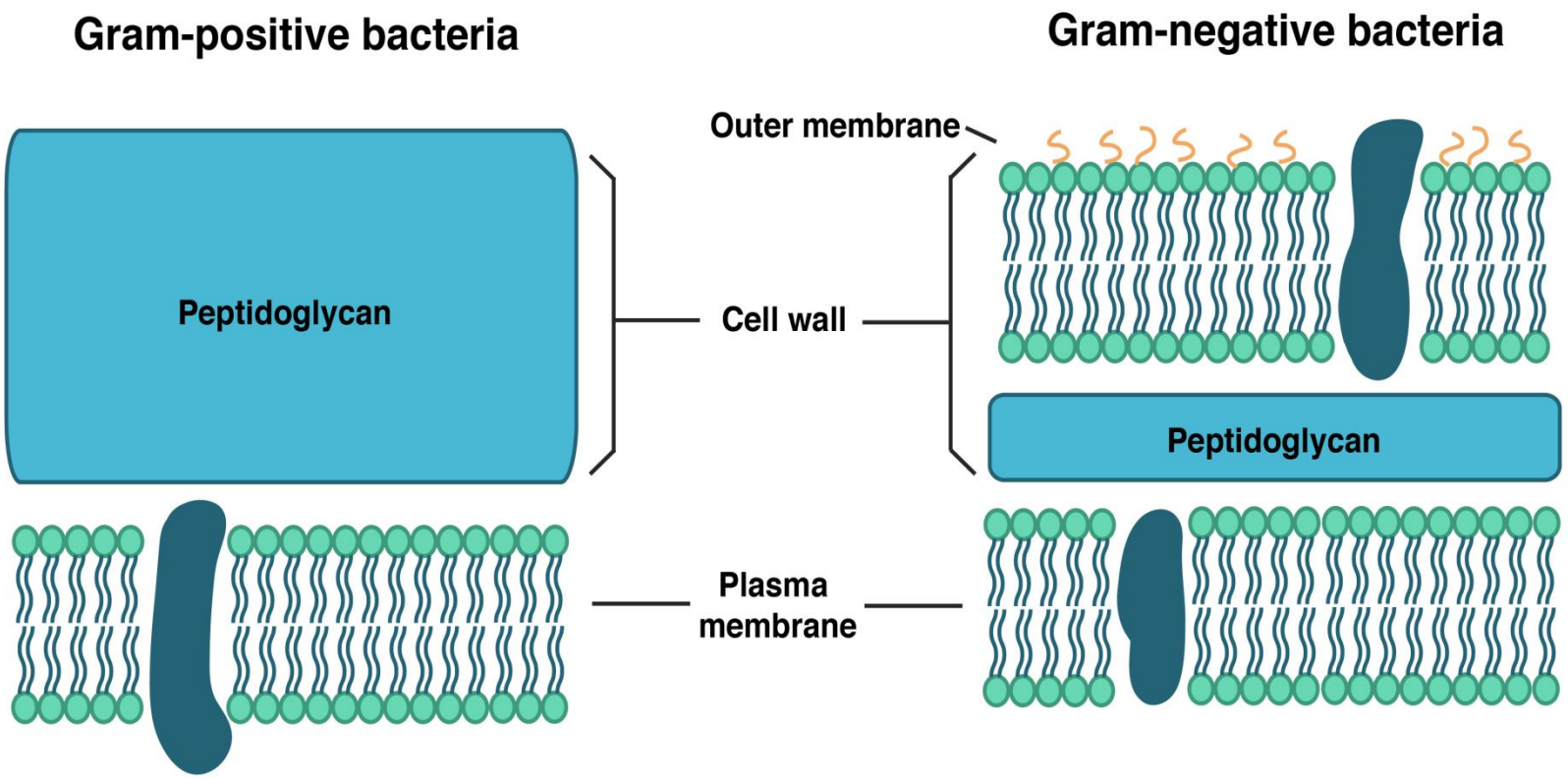
# GRAM STAINING

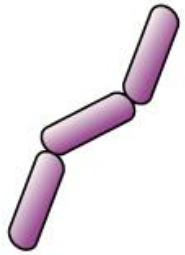
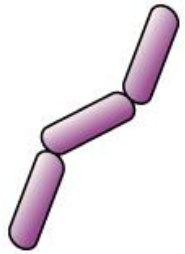
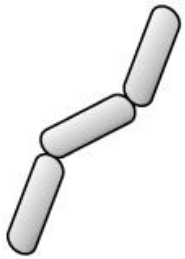
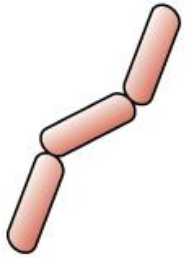
Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the de-coloring process.

# Petidoglycan structure

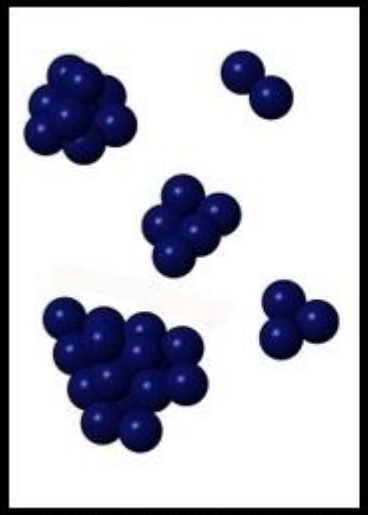


# Bacterial Cell wall(Gram +Ve/Gram-Ve)



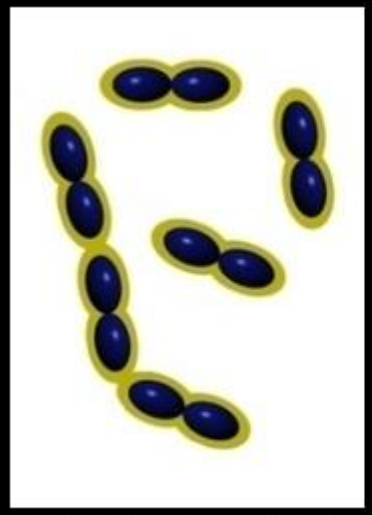
| Gram stain process | | | |
|---|---|---|---|
| **Gram staining steps** | **Cell effects** | **Gram-positive** | **Gram-negative** |
| **Step 1**<br>**Crystal violet**<br>*primary stain added to specimen smear.* | Stains cells purple or blue. |  |  |
| **Step 2**<br>**Iodine**<br>*mordant makes dye less soluble so it adheres to cell walls.* | Cells remain purple or blue. |  |  |
| **Step 3**<br>**Alcohol**<br>*decolorizer washes away stain from gram-negative cell walls.* | Gram-positive cells remain purple or blue. Gram-negative cells are colorless. |  |  |
| **Step 4**<br>**Safranin**<br>*counterstain allows dye adherance to gram-negative cells.* | Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red. |  |  |

## GRAM - POSITIVE



*Staphylococcus aureus*

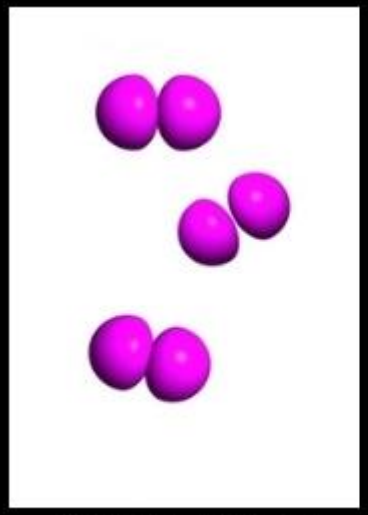
*Streptococcus agalactiae*



*Streptococcus pneumoniae*

*Listeria monocytogenes*

## GRAM - NEGATIVE



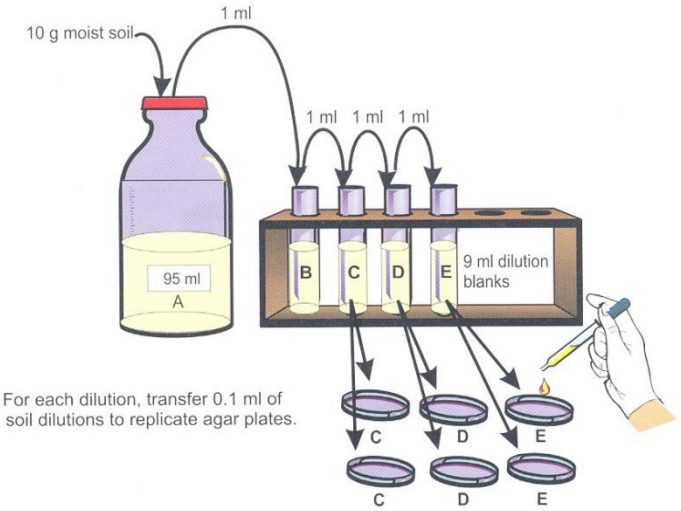
*Neisseria meningitidis*

*Haemophilus influenzae*

*Klebsiella pneumoniae*

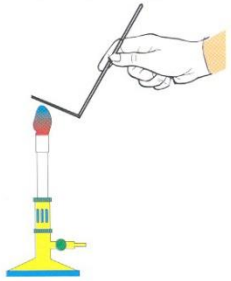
*Escherichia coli*

Step 1. Make a 10-fold dilution series.




Step 2. For each dilution, transfer 0.1 ml of soil dilutions to replicate agar plates.

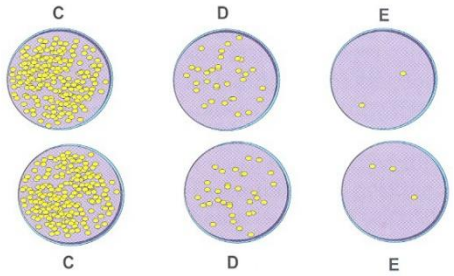
Step 3a. A glass spreading rod is flame sterilized.



Step 3b. Sample is spread on the surface of the agar. This is done by moving the spreader in an arc on the surface of the agar while rotating the plate.

Step 4. Incubate plates under specified conditions.

Step 5. Count dilutions yielding 30-300 colonies per plate. Express counts as CFUs per g dry soil.



# Acid-fast stain (Ziehl-Neelsen stain )

- It is a special bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria. Mycobacterium tuberculosis is the most important of this group because it is responsible for tuberculosis (TB) and other important Mycobacterium species.

- Acid fast organisms like Mycobacterium contain large amounts of waxy lipid substances within their cell walls called mycolic acids. These acids resist staining by ordinary methods such as a Gram stain. It can also be used to stain a few other bacteria, such as Nocardia.
- The reagents used are Ziehl–Neelsen carbolfuchsin, acid alcohol, and methylene blue. Acid-fast bacilli will be bright red after staining.