FACULTY OF AGRICULTURE SCIENCES AND ALLIED INDUSTRIES

Course Material

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Course Instructor

Dr. Kartikay Bisen
Faculty of Agricultural Sciences and Allied Industries
Rama University, Kanpur

LECTURE 7

Bacteria: cell structure

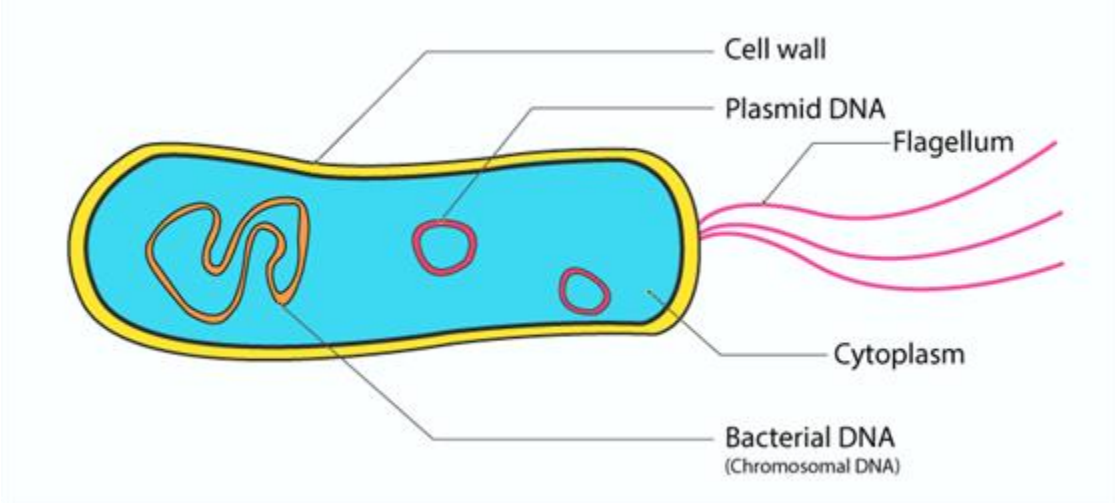
Today, bacteria are considered as one of the oldest forms of life on earth. Even though most bacteria make us ill, they have a long-term, mutual relationship with humans and are very much important for our survival. But before we elaborate on its uses, let us know the structure of bacteria, its classification, and the bacteria diagram in detail.

Bacteria Definition

"Bacteria are unicellular organisms belonging to the prokaryotic group where the organisms lack a few organelles and a true nucleus".

Bacteria Diagram

The bacteria diagram given below represents the structure of bacteria with its different parts. The cell wall, plasmid, cytoplasm and flagella are clearly marked in the diagram.



Bacterial Size

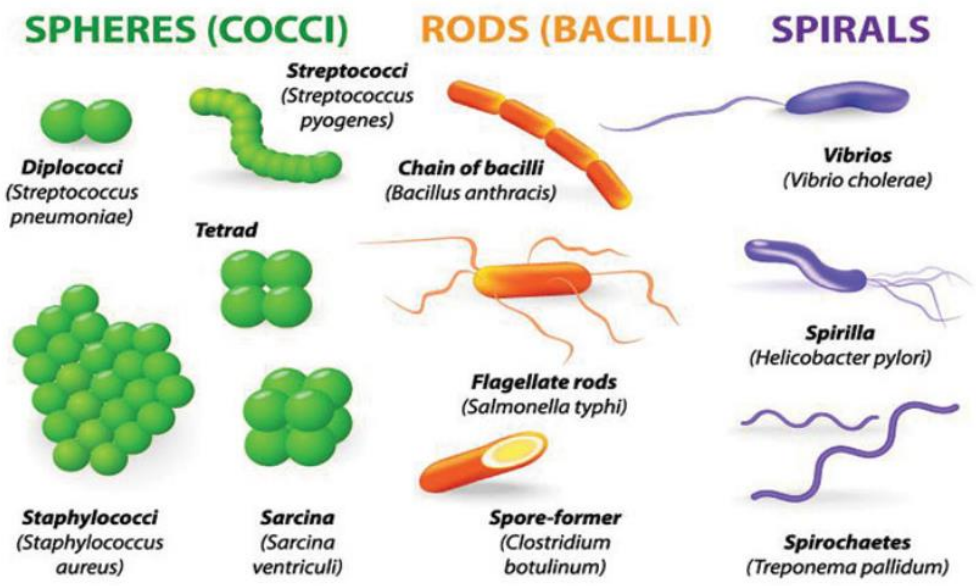
Bacterial cells are about one-tenth the size of eukaryotic cells and are typically 0.5– 5.0 micron in length. "Micron" or micrometre (denoted as µm) is the unit of measurement that is used to measure the bacterial size. One micron equals to one thousandth of a millimetre or 10-6 of a meter. It is worth mentioning that the limit of resolution with the unaided eye is merely about 200 microns while most commonly the bacteria are 0.2 – 1.5 µm in diameter and 3 – 5 µm in length. Thus, bacteria are smaller and can only be visualized with the aid of light microscope.

The power of a light microscope is limited by the wavelength of visible light, which is about 0.5 µm.

Bacterial Morphology

Bacterial morphology is very diverse. The bacterial shapes directly affect biological functions, including mode of nutrition, motility, dispersion, stress resistance and interactions with other organisms. Although, bacterial shape is genetically determined, but physical or environmental forces (may be internal and/or external) exerted on cells are increasingly recognized as responsible players in deciding bacterial shapes.

Classification of Bacteria on the Basis of Shape



In the year 1872 scientist Cohn classified bacteria to 4 major types depending on their shapes are as follows:

A) Cocci: These types of bacteria are unicellular, spherical or elliptical shape. Either they may remain as a single cell or may aggregate together for various configurations. They are as follows:

- **Monococcus:** they are also called micrococcus and represented by single, discrete round

  Example: *Micrococcus flavus*.

- **Diplococcus:** the cell of the Diplococcus divides ones in a particular plane and after division; the cells remain attached to each other. Example: *Diplococcus pneumonia*.
- **Streptococcus:** here the cells divide repeatedly in one plane to form chain of cells. Example: *Streptococcus pyogenes*.
- **Tetracoccus:** this consists of four round cells, which defied in two planes at right angles to one another. Example: *Gaffkya tetragena*. *Staphylococcus* here the cells divided into three planes forming a structured like bunches of grapes giving and irregular configuration. Example: *Staphylococcus aureus*.
- **Sarcina:** -in this case the cells divide in three planes but they form a cube like configuration consisting of eight or sixteen cells but they have a regular shape. Example: *Sarcina lutea*.

B) Bacilli: These are rod shaped or cylindrical bacteria which either remain singly or in pairs. Example: –*Bacillus cereus*.

C) Vibro: The vibro are the curved, comma shaped bacteria and represented by a single genus. Example: – *Vibro cholerae*.

D) Spirilla: These types of bacteria are spiral or spring like with multiple curvature and terminal flagella. Example: –*Spirillum volutans*.

In addition to above defined shapes, some other structural diversity also exists. The **Actinomycetes or Streptomycetes** are the bacteria which form branched filamentous hyphae having resemblance with fungal hyphae. Actinomycetes are so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus).

Mycoplasma is another example of structural variant. Mycoplasmas are the bacteria which are cell wall deficient and hence do not possess a stable morphology. They grow as round or oval bodies as interlacing filaments. The different shapes and arrangement of bacterial cells are described in Table 2.1. In addition to these mentioned shapes, there are some other morphological characters in bacteria such as endospore formation, presence of capsule, presence and arrangement of flagella etc. which are useful in their identification

Bacterial structure

Bacteria, being unicellular prokaryote, have simpler internal structure. Unlike eukaryotes, it lacks all membrane bound cell organelles such as nucleus and nucleolus, mitochondria, lysosome, golgi, endoplasmic reticulum, chloroplast, peroxisome, glyoxysome, and true vacuole. The outer layer of the bacterial cell consists of two components, the outer rigid cell wall and inner plasma membrane. The cell envelope surrounds the cytoplasm and other inclusions such as ribosomes and mesosomes, granules, vacuoles and the nuclear body. Thus, the bacterial structure can best be studied by bifurcating them as the structures outside the cell wall and those inside the cell wall. These structures are listed in table as follow:

A. Structure/components outside cell wall	B. Structure/components inside cell wall
a. Capsule	a. Cytoplasmic membrane
b. Flagella	b. Cytoplasm
c. Pili	c. Ribosome
d. Slime	d. Mesosome
	e. Cytoplasmic Inclusions
	f. Nucleoid
	g. Spore

Flagella

In bacteria, flagella are the organs of locomotion. They are very delicate and fragile and cultures are to be handled carefully for their staining.

The flagella vary from 10-12 nm in width which is smaller than wavelength of light, therefore, cannot be seen by ordinary staining.

Mordants like potassium sulphate and mercuric chloride are generally precipitated on flagella making the width more for making them visible under light microscope.

Parts of a Flagellum

Filament: It is the outermost region of flagellum, and is helical, composed of flagellin with a molecular weight of 30000-40000 and is synthesized in the cell, which moves to the hollow core of the flagellum to the tip. Flagellin is a protein with 14 amino acids and is characterised by higher content of aromatic amino acids and absence of cysteine in many cases.

Hook: Filament is attached to hook which is wider than the flagellum. This is 45 nm wide and made up of different types of protein. The hook of gram positive bacterium is longer than that of gram negative bacteria.

Basal body: The third part called basal body consists of small central rod which is inserted into a system of rings. The gram positive and gram negative bacteria are different in the number of rings. The inner pair of rings (S and M) are embedded in cell membrane and are formed in both gram positive and gram negative bacteria. L and P rings are formed only in gram negative bacteria. S and M rings are important for movement of flagella.

Pili

In some bacteria, small hair like structures is also present which are called pili. These are shorter than the flagella and are thicker (3-15 nm in diameter). The term fimbriae is sometimes also used for pili, but the term pili is reserved for those which are involved in conjugation. They are made up of protein sub-units pilin of molecular weight of 70000. It consists of a helically coiled fibre with a central hole of 2 nm in diameter. Fimbriae may be involved in attachment, whenever there is infection. Both flagella and pili originate from cell membrane and extend outward through the cell wall.

Cell wall

The cytoplasm of all the bacteria is enclosed within cell membrane, external to which a very rigid cell wall is present that gives shape to the bacterial cell. Cell wall constitutes a significant portion of the dry weight of the bacterial cell and is very essential for bacterial growth and division.

The major functions of the bacterial cell wall are as follow:

(i) Protection from osmotic lysis: the cell wall prevents the cell from expanding and eventually bursting due to water uptake (the pressure inside the cell = 300 lbs/in2)

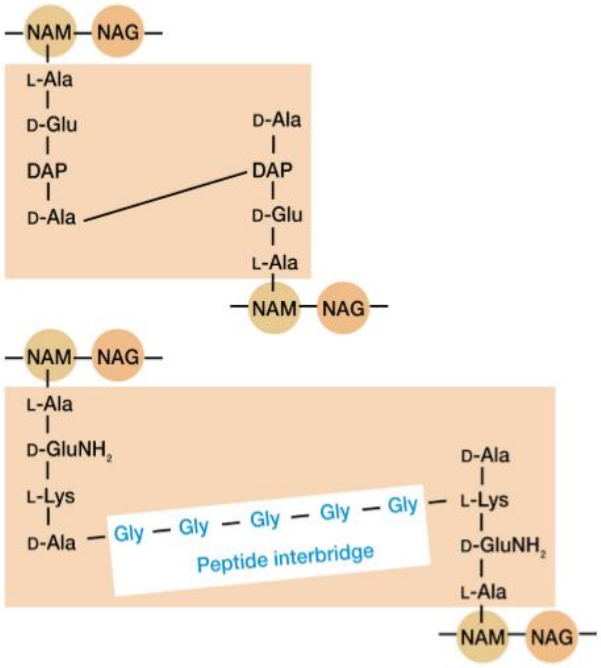
(ii) Virulence factor: cell wall can be responsible for causing virulence in host organisms.

(iii) Defence against host immune response

(iv) Protection from some toxic substances

Chemical composition of bacterial cell walls

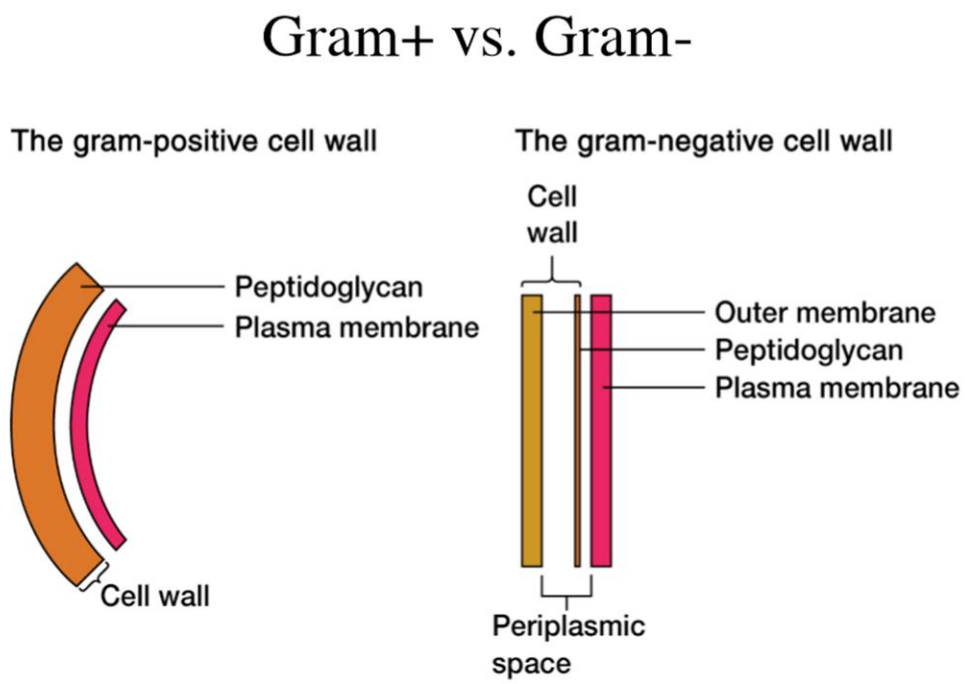
Chemically the cell wall is composed of peptidoglycan or murein which is made up of sugar and amino acids. The structure of peptidoglycan consists of long polymers of two sugar derivatives N acetyl glucosamine (NAG) and N acetyl muramic acid (NAM) with side chains of four alternating D-and L-amino acids attached to the NAM. The peptidoglycan and peptide chains are cross linked to provide rigidity to the cell wall.



A polyalcohol called **Teichoic acid** is embedded in it and responsible for linking peptidoglycan to cytoplasmic membrane and thus provides rigidity to the peptidoglycan.

On the basis of cell wall composition, bacteria are classified into **two** major groups i.e. **Gram Positive** and **Gram negative.**

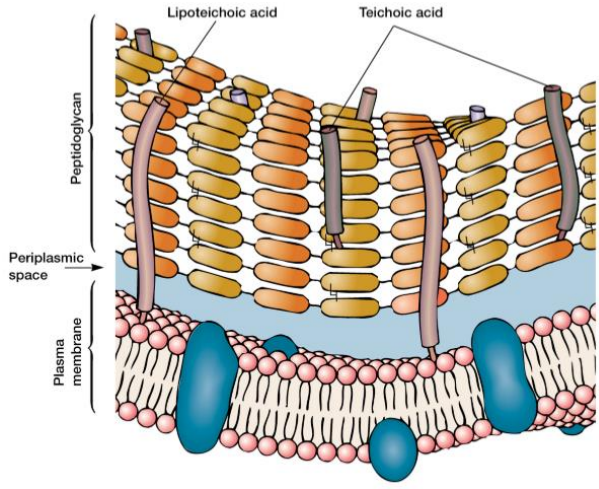
Actually, Bacteria are termed gram negative or gram positive based on a staining called Gram staining and that primarily depends on the type of cell wall that bacteria have.



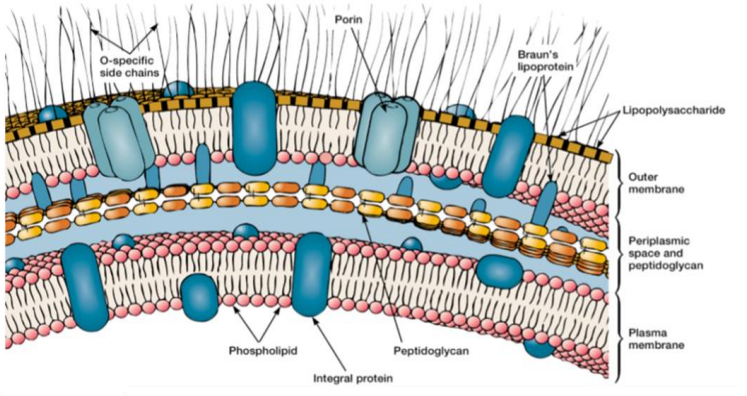
Gram positive bacteria have cell walls made up of peptidoglycan. The cell wall of the gram-positive bacteria also contains teichoic acid, which is made up of alcohol (glycerol or ribitol) and phosphate. These bacteria would retain the gram stain and observed as violet colored after the application of iodine (as mordant) and alcohol (Ethanol as decolorizer). The bacteria whose cell walls are made up of an outer membrane in addition to the inner peptidoglycan layer are called as gram-negative. The outer membrane is made up of Lipopolysaccharides, lipoproteins, and phospholipids. These bacteria would be decolorised by alcohol owing to the lipidous outer

membrane and lose the stain crystal violet, so they have to be counterstained by secondary stain safranin to appear pink in color.

Gram positive



Gram-



Major differences between Gram-positive and Gram-negative bacteria

Bacterial characters	Gram Positive	Gram Negative
Cell Wall	Their cell wall is smooth and single layered	They have a wavy and double layered cell-wall
Cell Wall thickness	The thickness of the cell wall is 20 to 80 nanometres	The thickness of the cell wall is 8 to 10 nanometres
Peptidoglycan Layer	It is a thick layer	It is a thin layer
Teichoic acids	Presence of teichoic acids	Absence of teichoic acids
Outer membrane	The outer membrane is absent	The outer membrane is present
Porins	Absent	Occurs in Outer Membrane
Morphology	Cocci or spore-forming rods	Non-spore forming rods.
Flagella Structure	2 rings in basal body	4 rings in basal body
Lipid content	Very low	20 to 30%
Lipopolysaccharide	Absent	Present
Toxin Produced	Exotoxins	Endotoxins or Exotoxins
Resistance to Antibiotics	More susceptible	More resistant
Examples	*Staphylococcus, Streptococcus* etc.	*Escherichia, Salmonella*, etc
Gram staining characteristics	These bacteria retain the crystal violet color even after they are washed with acetone or alcohol and appear as purple colored when examined under the microscope after gram staining.	These bacteria do not retain the stain color even after they are washed with acetone or alcohol and appear as pink colored when examined under the microscope after gram staining.

Gram staining

- Gram staining is a differential staining technique that is used for microscopic examination of bacteria.
- In differential staining, specimen is subjected to a series of stains (dyes) in which different organisms or different parts of the cell are stained differently so that they can be distinguished from each other.
- Gram staining differentiates bacteria into two groups; Gram positive and Gram negative

- It was developed by **Hans Christian Gram in 1884 and modified by Hucker in 1921.**

Principle:

- The difference in the chemical composition of bacterial cell walls accounts for the Gram staining differences between the two major groups of bacteria (Gram positive and Gram negative).
- Gram-positive cell walls contain thick peptidoglycan layer with numerous teichoic acid cross linkages whereas, the peptidoglycan layer in Gram-negative cells is much thinner and surrounded by outer lipid containing layers.
- Peptidoglycan, a polysaccharide composed of two chemical subunits, N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) are cross-linked by short chains of peptides by means of a trans-peptidase enzyme, resulting in the shape and rigidity of the cell wall.
- In case of Gram-negative bacteria, the cross-linking of the peptidoglycan layer is direct because the bacteria do not have short peptide tails.
- Gram-positive bacteria retain the primary dye, crystal violet (CV), following the application of the mordant, iodine (I). **Mordant** is a substance that increases the cells' affinity for a stain.
- The iodine and crystal violet form a complex (CV-I) within the peptidoglycan. When a decolorizer is applied to the cells, the CV-I complex remains within the cell, making it appear dark purple to blue.
- In Gram-negative cells, following the application of the crystal violet and iodine, the CV-I complexes are not trapped within the peptidoglycan.
- Application of the acid-alcohol decolorizer dehydrates the outer cellular membrane, and also dissolves the lipids leaving holes in the membrane and effectively washing or removing the CV-I complex from the cells.
- The cells appear colorless. Therefore, a counter stain, safranin, is applied, to make the cells distinctly visible (either red or pink).

- Gram-positive organisms that have lost cell wall integrity because of anti-biotic treatment, old age, or action of autolytic enzymes may allow the crystal violet to wash out with the decolorizer and appear Gram-variable, with some cells staining pink and others staining purple.

Requirements:

1. **Primary stain:**2 gm Crystal violet, 20 ml 95% ethyl alcohol, 0.8 gm ammonium oxalate, and 100 mL distilled water
2. **Gram's iodine (Mordant):**2 gm potassium iodide, 1 gm iodine crystals, and 100 ml distilled water
3. **Decolorizer:**Acetone and ethanol (50 ml each)
4. **Counterstain:**0 gm Safranin, 200 ml 95% ethanol, and 800 ml distilled water
5. **Fresh culture sample**: 24-hour agar culture of *Staphylococcus epidermidis*, 24-hour agar culture of *Bacillus subtili*s and 24-hour agar culture of *Escherichia coli*
6. Bunsen burner
7. Inoculating loop
8. Microscope
9. Distilled water or tap water
10. Soft cotton or blotting paper
11. Grease free glass slides

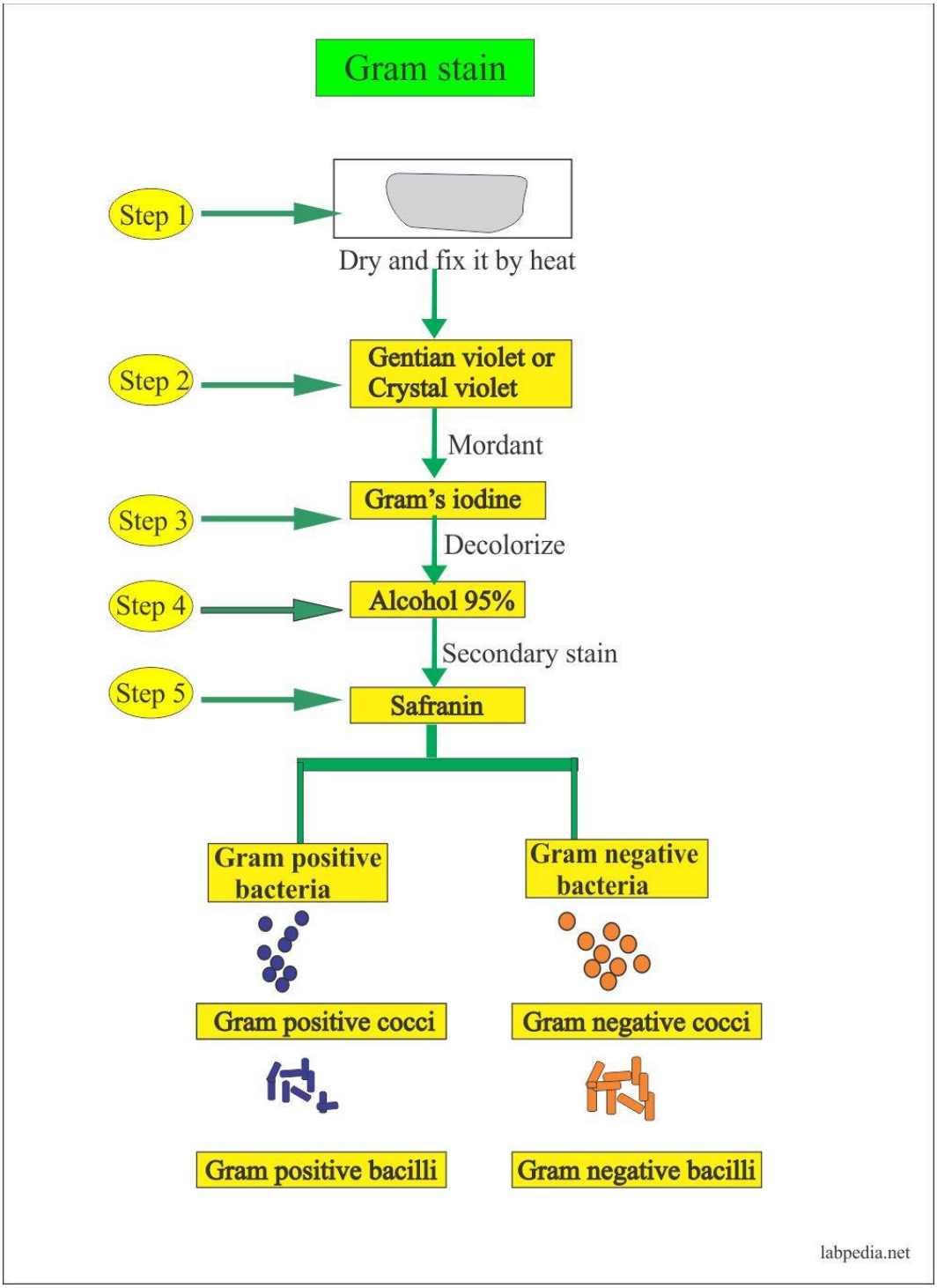
Procedure:

1. Fix the material (any of the specimen or culture sample) on the clean grease free slide with methanol or heat. If slide is heat fixed, allow it to cool to the touch before applying stain.
2. Flood the slide with crystal violet (purple) and allow it to remain on the slide without drying for 10-30 seconds.
3. Rinse the slide with distilled water or tap water, shaking off all excess.

4. Flood the slide with iodine (mordant) to increase the affinity of crystal violet and allow it to remain on the surface without drying for twice as long as crystal violet was in contact with the slide.
5. Rinse the slide with tap water, shaking off all excess.
6. Flood the slide with acid-alcohol decolorizer for 10 seconds and rinse immediately with tap water. Repeat this step until the blue dye no longer runs off the slide with the decolorizer. Thicker smears require more prolonged decolorizing . rinse with tap water and shake off excess.
7. Flood the slide with counter stain, safranin and allow it to remain on the slide without drying for 30 seconds. Rinse with tap water and gently blot the slide dry with soft cotton or blotting paper or air dry. For delicate smears, such as certain body fluids, air drying is the best method.
8. Examine microscopically under an oil immersion lens at 1000X.

Microscopic Examination:

- **Gram-positive cells :**Blue/Purple Color e.g. *Staphylococcus epidermidis*, *Bacillus subtili*s
- **Gram-Negative cells :**Red/Pink Color e.g. *Escherichia coli*


Gram stain
Step 1
Dry and fix it by heat
Step 2
Gentian violet or Crystal violet
Mordant
Step 3
Gram's iodine
Decolorize
Step 4
Alcohol 95%
Secondary stain
Step 5
Safranin
Gram positive bacteria
Gram negative bacteria
Gram positive cocci
Gram negative cocci
Gram positive bacilli
Gram negative bacilli
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