Bacterial taxonomy Lab:1

Staining & Differential stains

Bacterial taxonomy

Bacterial taxonomy includes three main components:

1-Classification: the arrangement of **bacteria** into groups based on similarities in their characteristics such as **morphology, physiology, biochemical properties,** and **genetic relationships**.

2-Nomenclature: the system of naming bacteria according to internationally accepted rules and standards.

2-Identification:the process of determining the identity of bacterial isolates through laboratory methods such as **staining techniques, biochemical tests**, and **molecular analysis.**

Bacterial shapes

Bacteria have three main shapes: cocci (spherical), bacilli (rod-shaped), and spirals (helical or curved), with variations like comma-shaped vibrio, flexible spirochetes, and different arrangements like chains (strepto-) or clusters (staphylo). These shapes help in identification, with subgroups like diplococci (pairs of spheres) or streptobacilli (chains of rods) emerging from how they divide and group.

1-Basic Shapes

- **Coccus (plural: Cocci): Spherical or round, sometimes oval or bean-shaped.**
- **Bacillus (plural: Bacilli): Rod-shaped.**
- **Spiral: Curved or twisted shapes.**
    - **Vibrio: Comma-shaped.**
    - **Spirillum (plural: Spirilla): Rigid, corkscrew-like spirals.**
    - **Spirochete: Long, flexible, and slender spirals.**

2-Common Arrangements (for Cocci & Bacilli)

- **Diplo-: In pairs (e.g., Diplococci, Diplobacilli).**
- **Strepto-: In chains (e.g., Streptococci, Streptobacilli).**
- **Staphylo-: In grape-like clusters (e.g., Staphylococci).**
- **Tetrad: Groups of four cocci.**
- **Sarcina: Cubical packets of eight cocci.**

3-Other Forms

- **Pleomorphic: Bacteria that can vary in shape.**
- **Coccobacillus: An intermediate shape, between cocci and bacilli.**

SHAPES OF BACTERIA

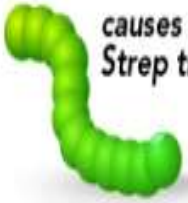
SPHERICAL (COCCI)

Streptococcus pneumoniae

causes Pneumonia

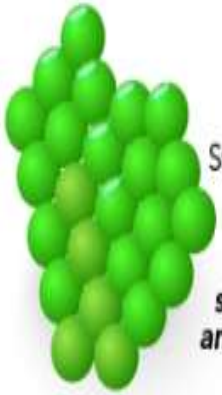
Streptococcus pyogenes

causes Strep throat



Micrococcus luteus

causes armpits to stink



Staphylococcus aureus

can cause sinus infections and food poisoing

ROD-SHAPED (BACILLI)

(Bacillus anthracis)

causes Anthrax

(Salmonella enterica)

causes Typhoid

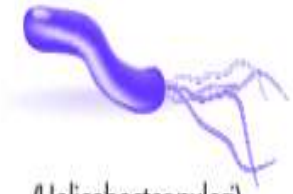
(Clostridium botulinum)

causes Botulism

SPIRAL-SHAPED (AND OTHERS)

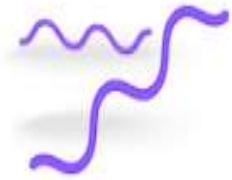
(Vibrio cholerae)

causes Cholera



(Helicobacter pylori)

can cause stomach ulcers



(Treponema pallidum)

causes Syphilis

Staining

Bacterial staining techniques are essential microbiology methods used to enhance contrast, observe morphology (shape/arrangement), and differentiate bacteria. The most critical techniques include Gram staining (dividing bacteria into Gram-positive/purple & Gram-negative/red),acid-fast staining for Mycobacterium and special staining.

The types of dyes

Dyes used in microbiological staining are classified according to their chemical properties and charge into three main types:

1-Basic dyes (cationic dyes):

These dyes carry a positive charge and bind to negatively charged components of bacterial cells, resulting in direct staining of the cells. Examples include crystal violet, methylene blue, and safranin.

2-Acidic dyes (anionic dyes):

These dyes carry a negative charge and are repelled by the negatively charged bacterial surface; therefore, they stain the background rather than the bacterial cells (negative staining). Examples include Eosin, Congo red and Nigrosin.

3-Neutral dyes:

These dyes are formed by combining acidic and basic dyes and are used in certain differential staining techniques.

Common Staining Categories

- **Simple Stain:** Uses one dye (e.g., methylene blue, safranin) to stain all cells the same color.
- **Differential Stain:** Uses multiple dyes to differentiate types (e.g., Gram stain, Acid-fast).
- **Special Stain:** Special stains are staining techniques used to visualize specific structures or components of bacterial cells that cannot be clearly observed using simple or Gram staining methods. These stains include structural stains such as capsule, endospore, and flagella stains.

1-Gram staining

In Biology, specifically in Microbiology Gram Staining is an inevitable technique. It was introduced in the year 1884 by Hans Christian Gram. This staining process can classify a bacteria into Gram Positive and Gram Negative. It can be used as a step in various bacterial diagnosis.

Purpose

Gram staining is used to identify the bacteria which cause certain infections. This can be done using blood, body fluids, etc. This helps to give clear information to start medication. This is not the final or only way of diagnosis. This is only used to classify the bacteria. All the bacteria cannot be classified by this method.

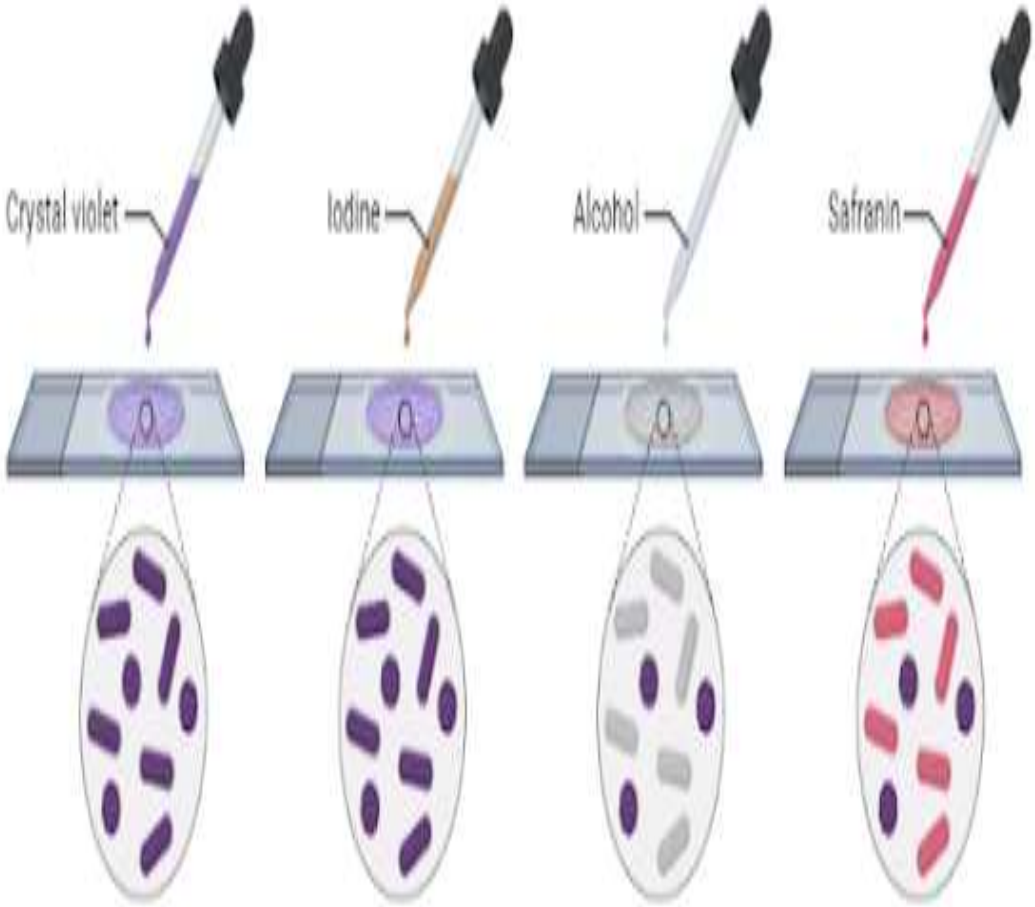
Reagents Used

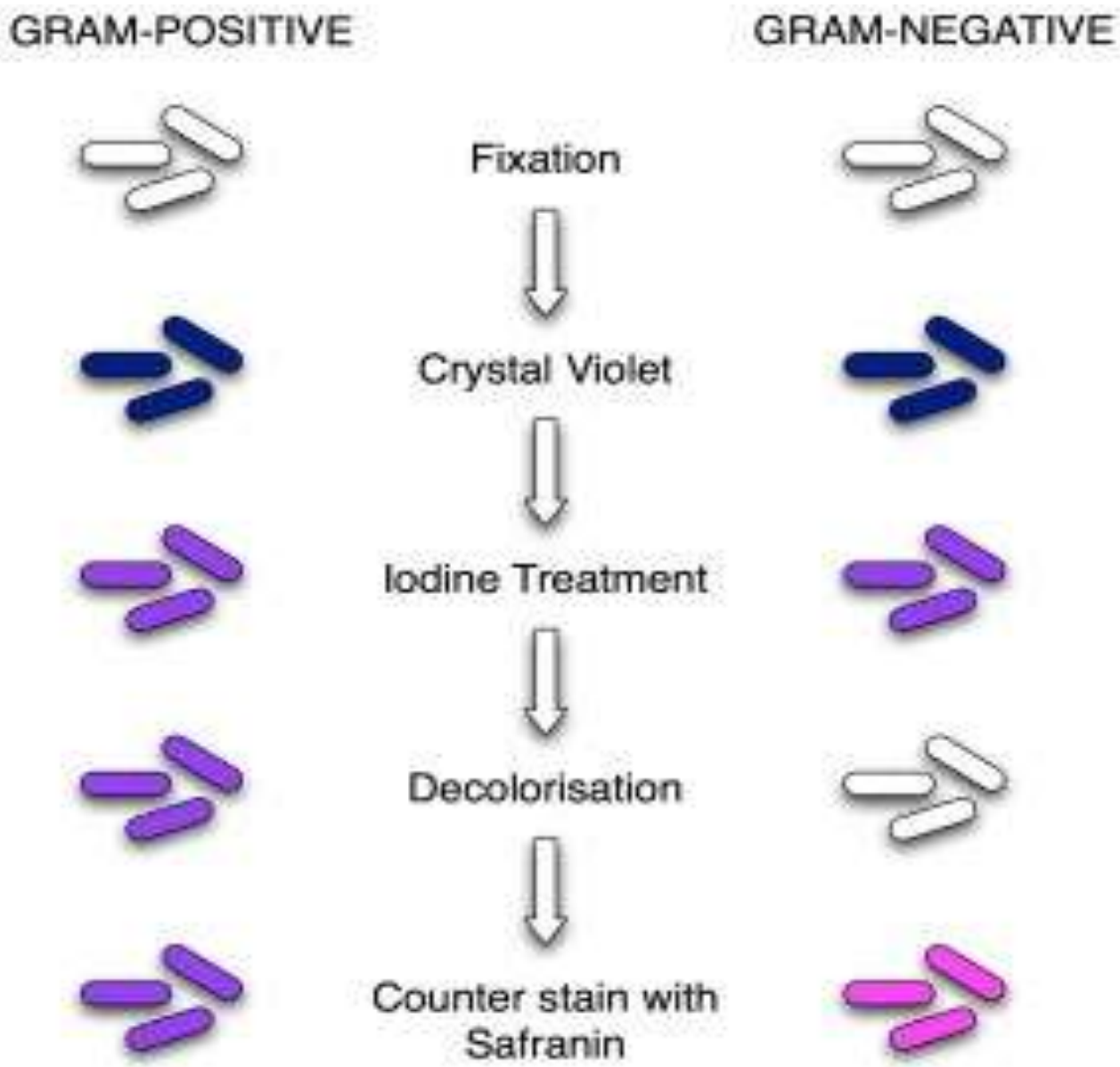
1. **Primary stain – Crystal Violet**
2. **Secondary stain or counter stain – Safranin**
3. **Gram's Iodine (Mordant – Used along with Crystal Violet)**
4. **Acetone and Alcohol based decolorizing agent.**

Principle

It is the process in which the bacteria is stained with a series of reagents used for gram staining. It is initially stained with the primary stain which is Crystal Violet. The primary stain is fixed by iodine. Then it is treated with alcohol so that decolorization will take place. Some bacteria can retain the stain but some cannot. So the bacteria which retains the Crystal Violet – Iodine mixture is called Gram-Positive Bacteria. They will appear in blue or purple color. The retaining of the stain complex is due to the cell wall of the bacteria. During decolorization, the pores in the cell wall containing the stain complex dehydrate and shrink. So the stain cannot move out and decolorization will not take place.

The cell wall of the Gram-Negative bacteria is very thin. It cannot retain the Crystal Violet – Iodine mixture. So during destaining, the stain mixture will be washed off. For identification, it is again stained with safranin. This bacteria take up the safranin and look red/pink in color.


Crystal violet
Iodine
Alcohol
Safranin


GRAM-POSITIVE
GRAM-NEGATIVE
Fixation
Crystal Violet
Iodine Treatment
Decolorisation
Counter stain with Safranin

Preparation of the smear

- **Take a clean grease free slide.**
- **Transfer a loop of the sample (for example, sputum, CSF, or pus) to the microscope slide. If performing a Gram stain from a bacterial colony, first put a drop or a few loopful of water and emulsify the bacterial colony in the water drop.**
- **Spread the sample to an even-thin film over a circle of 15 mm diameter.**
- **Air dry the sample, and once the sample gets air dried, heat fix the smear by passing it through a bunsen burner three times. Heat application helps the cell adhesion (fixation) to the glass slide and prevents its loss during rinsing.**

  Allow the slide to cool to the touch before applying the stain. Alternatively, the smear can be fixed using methanol.

Methanol fixation

Place or hold the slide over a paper towel and flood the slide with absolute methanol for two minutes. Alternatively, dip the slide into a Coplin jar filled with methanol.

Once two minutes have passed, tilt the slide and drain off the excess methanol and let the slide air dry. Do not wipe or blot the slide, as this can remove cells.

Procedure

1. **Take a freshly cleaned glass slide.**
2. **Smear the sample into the slide with the appropriate smearing procedure.**
3. **Dry the sample at room temperature and then gently heat-fix it.**
4. **The primary dye Crystal Violet must be poured over the slide containing the sample and kept for about 60 seconds and rinsed with water.**
5. **Add the mordant (Gram's Iodine), keep it for 60 seconds, and wash it with water.**
6. **Add 95% alcohol or acetone or a mixture of both used for rapid decolorization (20-30 sec).**
7. **Add safranin for 60 seconds (Counter staining).**
8. **Dry the slide and observe under the microscope.**

- **Gram Negative: Red/Pink Color**
- **Gram Positive: Blue Color**

Explain the Steps of Gram Staining

1. **Fixation of clinical materials to the surface of the microscope slide either by heating or by using methanol. (Methanol fixation is recommended rather than heat fixation. Methanol fixation preserves the morphology of host cells and bacteria. Heating the slide causes cell distortion, could increase cell debris, and may cause erroneous Gram stain results.**
2. **Application of the primary stain (crystal violet). Crystal violet is a dark blue to purple dye. It stains all cells blue/purple.**
3. **Application of mordant: The iodine solution (mordant) is added to form a crystal violet-iodine (CV-I) complex; all cells continue to appear blue.**
4. **Decolorization step: The decolorization step distinguishes gram-positive from gram-negative cells. The organic solvent such as acetone or ethanol extracts the blue dye complex from the lipid-rich, thin-walled gram-negative bacteria to a greater degree than from the lipid-poor, thick-walled, gram-positive bacteria. The gram-negative bacteria appear colorless, and gram-positive bacteria remain blue.**
5. **Application of counterstain (safranin): The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain purple.**

Gram-Positive Bacteria

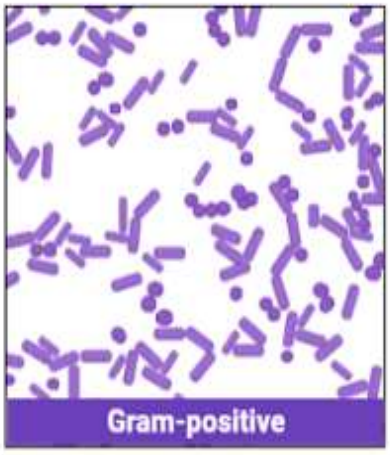
It is a type of bacteria which have a thick peptidoglycan outer cell wall. This peptidoglycan layer causes Gram-Positive bacteria to look Blue by Gram Staining.

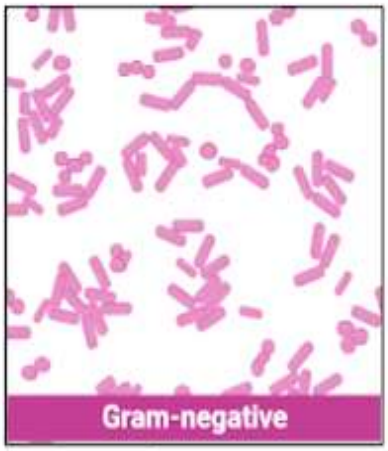
Examples: *Streptococcus* spp, *Clostridium* spp

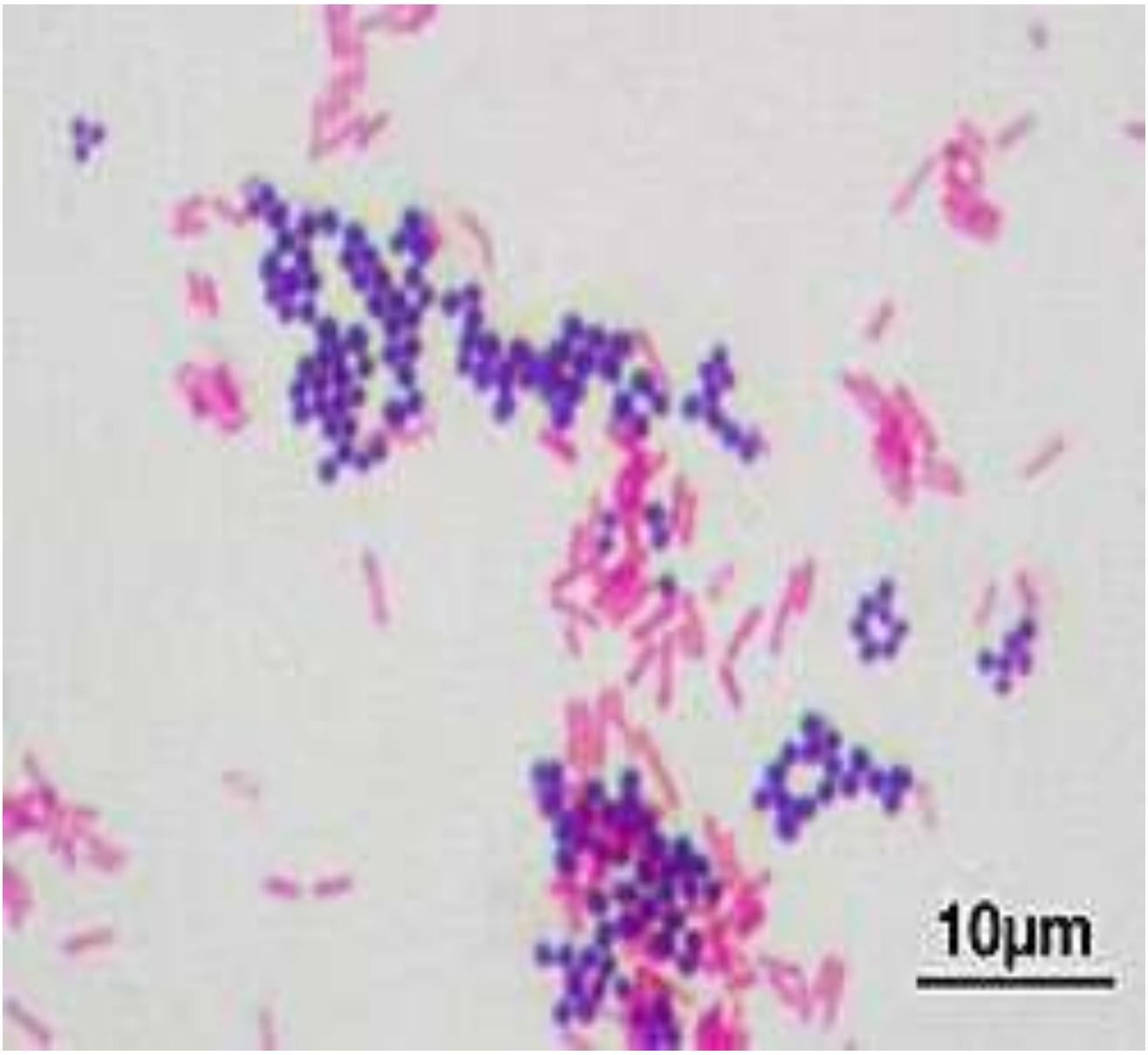
Gram-Negative Bacteria

It is a type of bacteria having thin layers of a peptidoglycan layer. This causes the Gram stain to get washed away and retains the counter stain safranin to retain and appear Red.

Examples:
***Escherichia Coli*, *Pseudomonas* species**






10µm