

Bacterial Staining

By:

Assistant Lecturer

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What is a Stain?

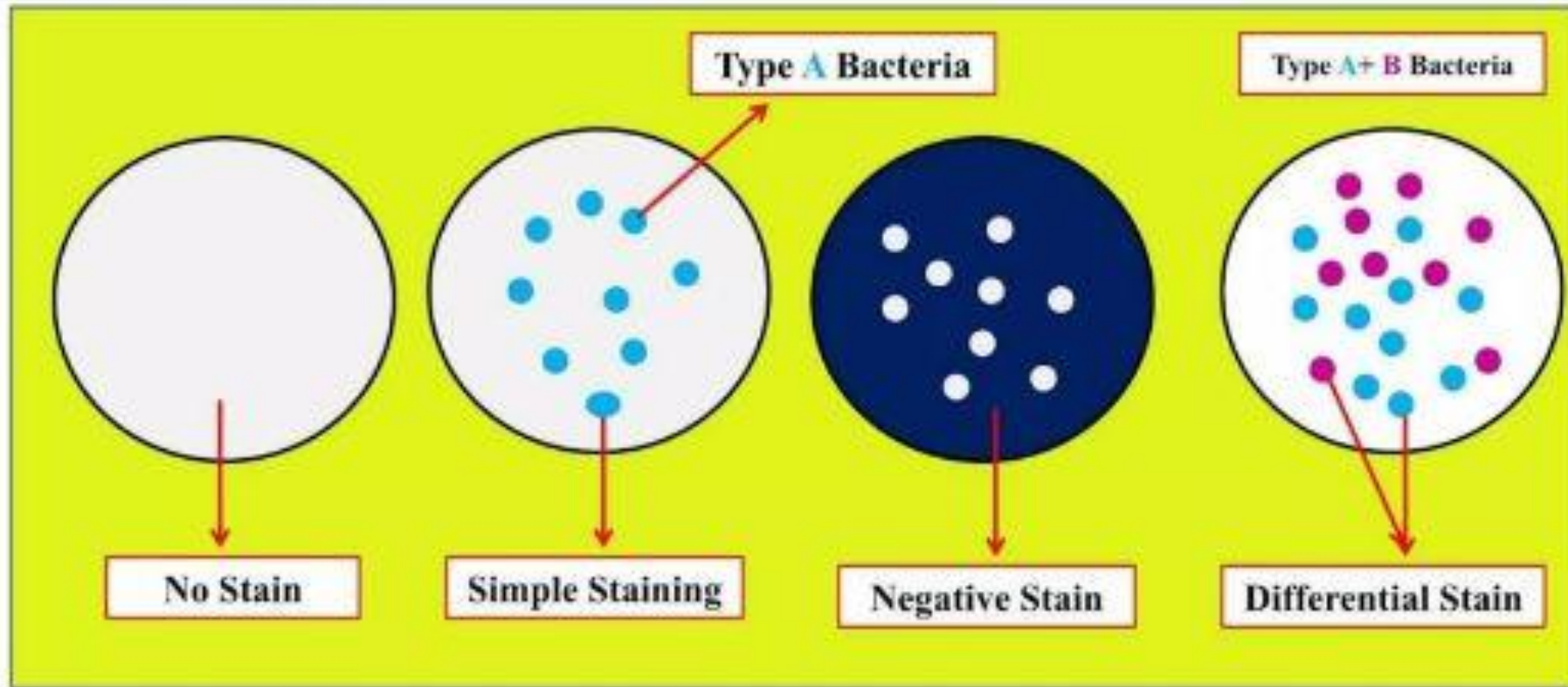
- Bacteria are usually colorless.
- **Stain** is a substance that adheres to a cell giving the cell color.
- **Staining** makes the microscopic observation possible for the internal and external structures of microorganisms.
- Staining bacterial cell for microscopic examination makes it more visible for human eye and to study unique characteristic including cell size, shape, arrangement and other properties.

- The dyes used to stain bacteria are basic dyes.
- These dyes contain ionizable groups (positive charge) that are attracted to the negatively charge components of the bacterial cell.

Types of Stains

- There are three types of stains in microbiology lab:
- Simple stains.
- Differential stains (Gram stain & Acid-Fast stain)
- Structural stain (capsular, endospores, flagellar stains)

Staining Methods



Based on the Nature of Chromogen

1. Acidic stain

(Anionic stain) with -ve charge

- Nigrosin
- Malchite green

1. Basic stain

(Cationic stain) with +ve charge

- Crystal violet
- Methylene blue
- Safrinin
- Basic fuchsin

1. neutral stain:

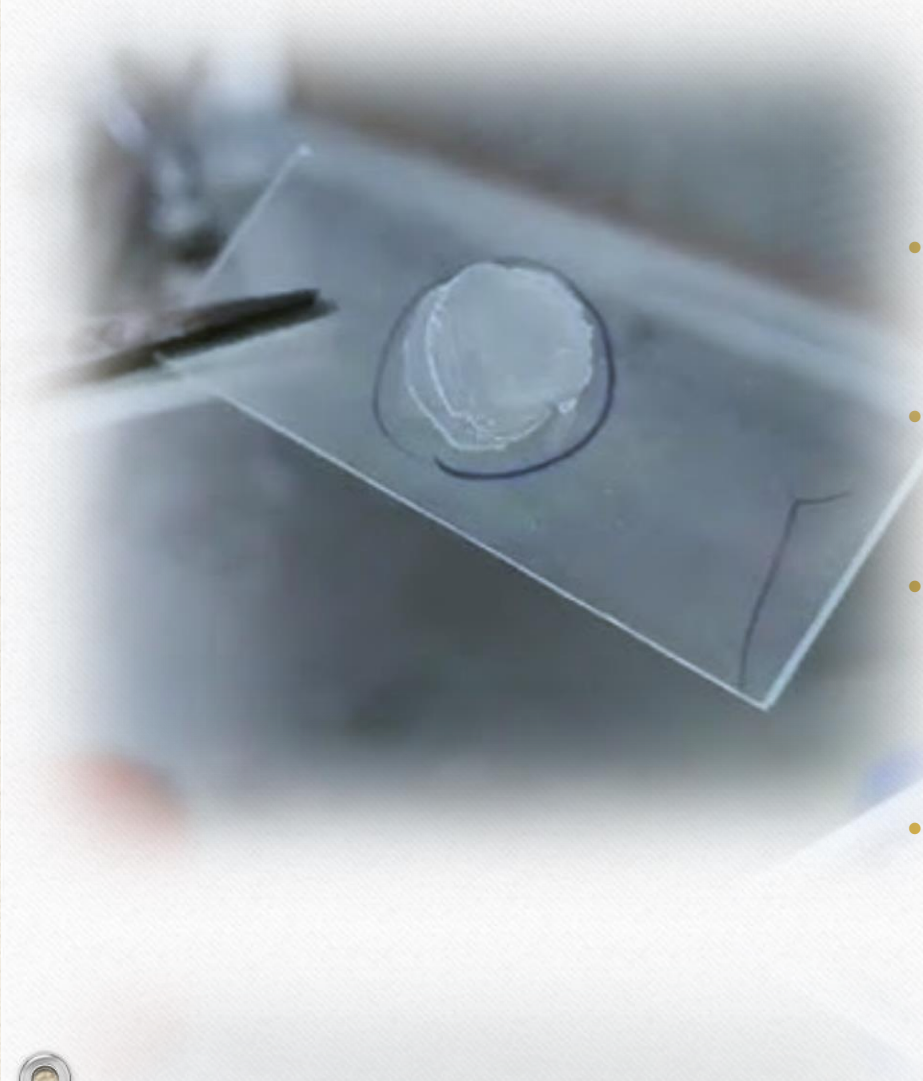
- Eosinate of methylene blue
- Giesma stain

Steps of Bacterial Staining

- Smear Preparation
- Staining

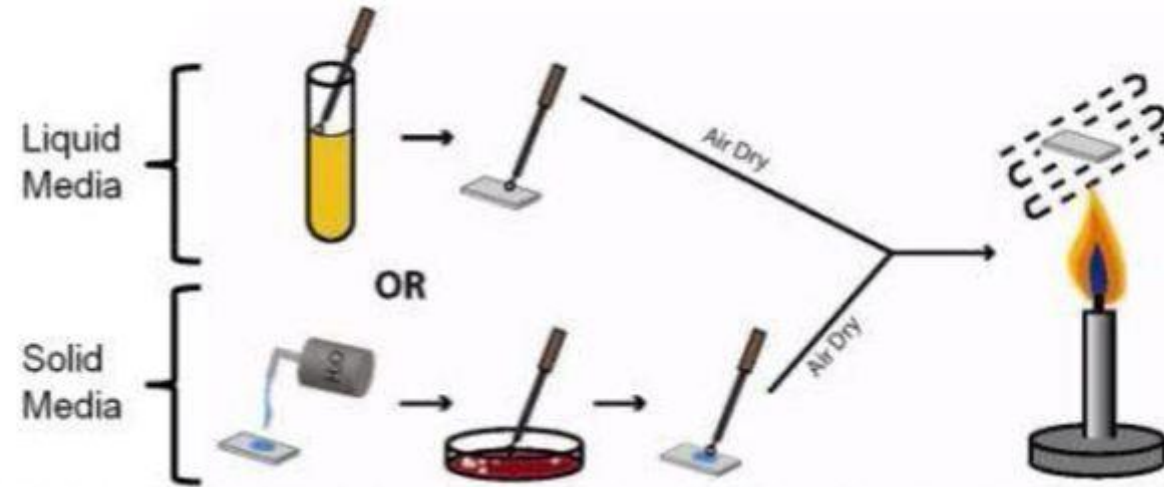
Smear

- **Smear:** is a thin film of material containing microorganism which spread over the slide.
- **Specimens:** urine, stool, blood, CSF, Pus, pleural fluid, broth or agar media and others.
- In a **smear preparation**, cells from a culture or a specimen spread in a thin film over a small area of a microscope slide, dried, and then fixed to the slide by heating or other chemical fixatives.
- The **purpose** of making a smear is to fix the bacteria on the slide and to prevent the sample from being lost during a staining procedure.



Smear preparation

Step 1. Heat Fix Smear



Procedure:

1. **Label** a clean glass slide using wax marker. "**Note** that it is important to recognize the side of the glass slide that you put your bacterial sample on".
2. **Add** a small drop of saline to the slide.
3. With an inoculation loop or stick, **pick up** a small amount of bacteria. **Mix it** well with the saline and spread the mixture over a wider area of the slide.
4. **Air dry** the bacterial specimen on the slide.
5. **Heat fix** the bacterial specimen by passing the slide slowly over the flame twice.

Smear Preparation

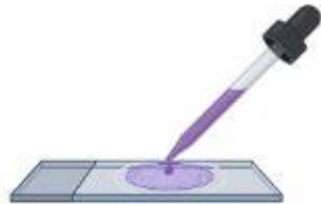
①

Degrease the slides by moving slide on flame



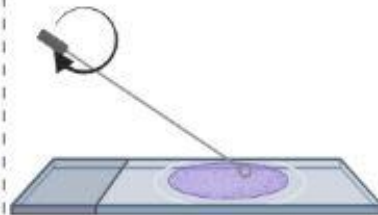
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Add a drop of culture or water



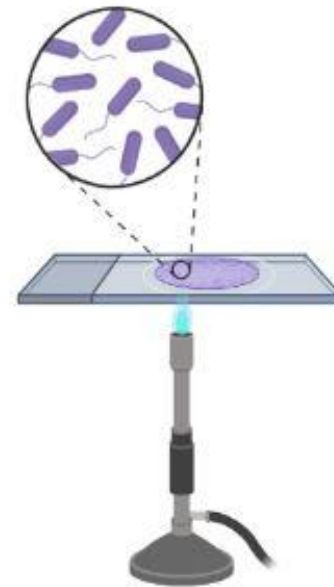
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Spread the drop on slide with the help of loop in circulating fashion

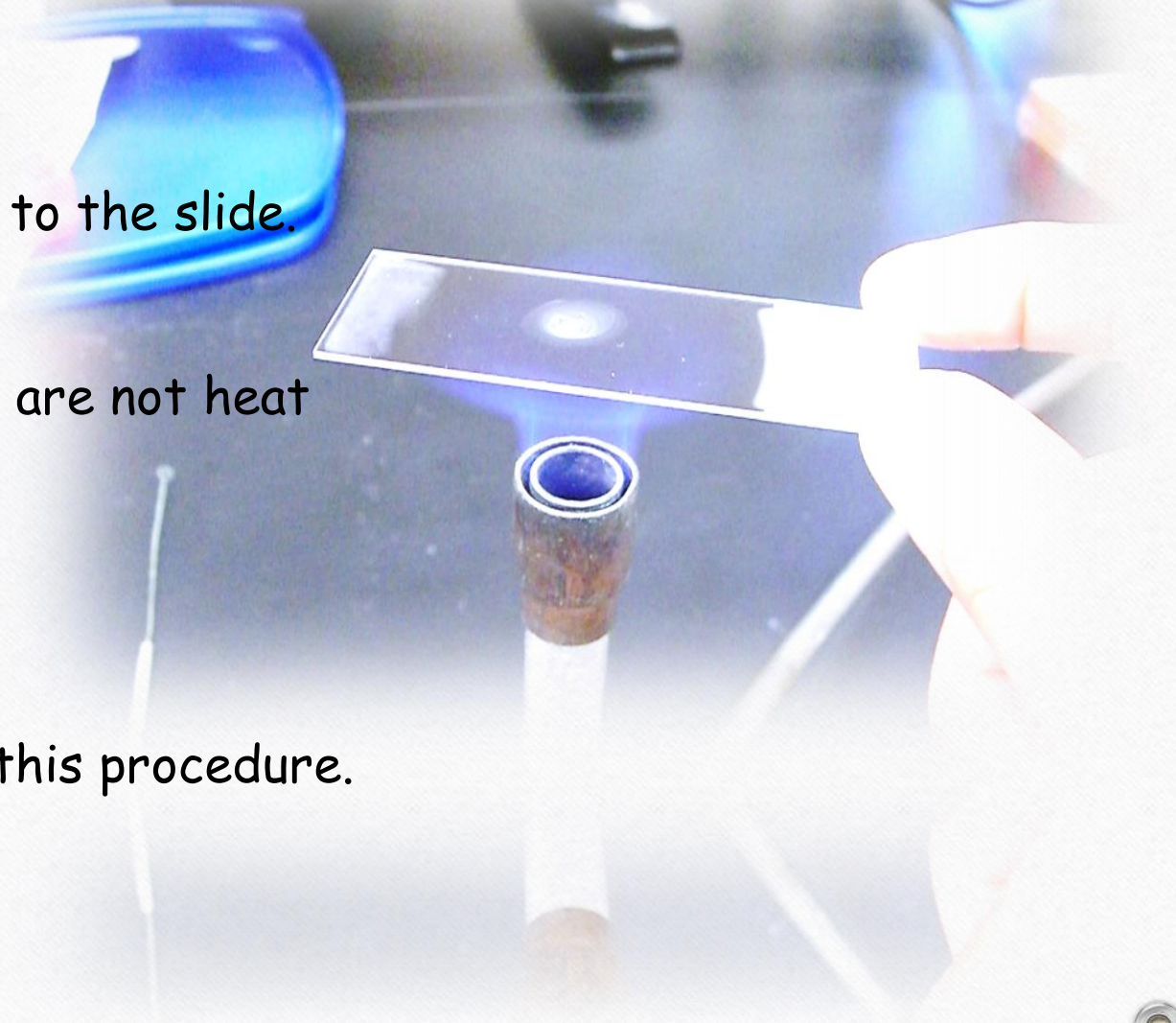


④

let the smear air dry and heat fix it



- * Heat fixing kills cells, and adheres them to the slide.
- * Cells will be rinsed off the slides if they are not heat fixed properly.
- * Be careful not to overheat the slides in this procedure.



**"A good smear preparation
is the key to a good stain"**



Staining

- Simple staining
- Differential staining
- Structural staining

Simple Staining

- **Simple staining** include directly staining the bacterial cell with a positively charged dye in order to see bacterial detail.
- using **one stain only** such as safranin, crystal violate or methylene blue and appears in one color.


Simple Staining is used to determine:

- **Size** of microorganism
- **Shape:** spherical (cocci), rod (bacilli), pleomorphic (coccobacilli) and spiral (spirillum).
- **Grouping or arrangement:** single, pairs, chain or cluster.


common bacterial arrangement

Shape

Spherical


coccus
(pl., cocci)

Rod-shaped


bacillus
(pl., bacilli)

Spiral


spirillum
(pl., spirilla)

Incomplete spiral


vibrio
(pl., vibrios)

Irregular or variable shape


pleomorphic

Arrangement

diplococcus
(pairs)



streptococcus
(chains)



staphylococcus
(random or
grapelike clusters)



micrococcus
(square groups
of four cells)



streptobacillus
(chains)

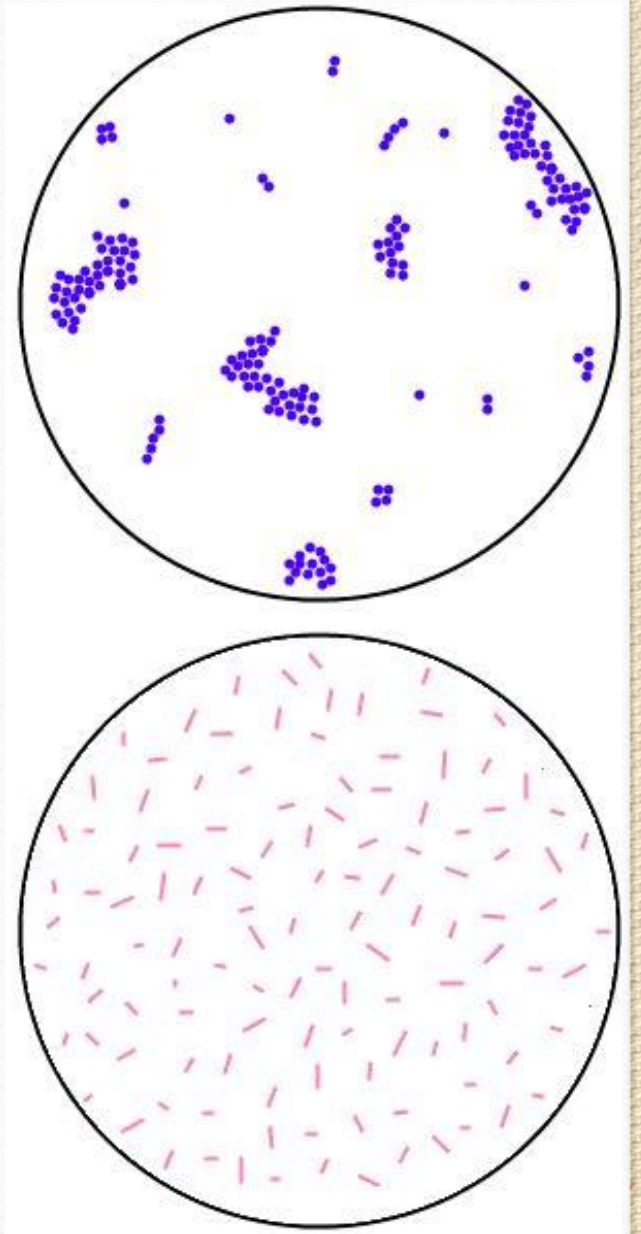


sarcina
(cubical packets
of eight cells)



Simple Stain Procedure:

1. Prepare a fixed smear of bacteria.
2. Flood the slide with simple stain for (1 minute).
3. Wash off the stain with tap water.
4. Dry the slide (place the slide between the layers of filter papers).
5. Examine under oil immersion lens.



Differential Staining

- Using two or more dyes successively to differentiate between organisms according to their response to these dyes, include:
 1. Gram stain
 2. Acid fast stain

"Gram Stain"

- **Gram stain** is the most common differential stain used in microbiology.
- The unique cell wall components of the bacteria will determine how they will react to the different dyes.
- This method of staining is used to classify bacterial species into two large important groups:
 1. **Gram-positive** bacteria such as *Staphylococcus aureus*
 2. **Gram-negative** bacteria Such as *E.coli*.

Gram stain

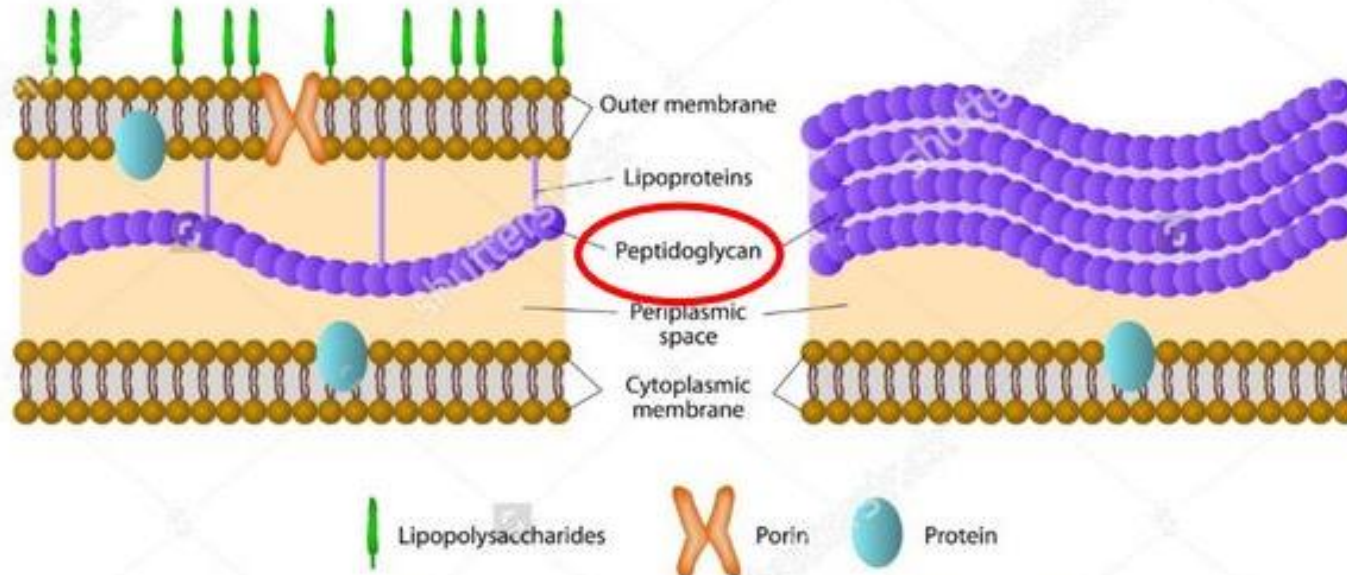
- Hans Christian Gram , Danish scientist
- Classify Bacteria into Gram **negative** & Gram **positive** .

GRAM-NEGATIVE

GRAM-POSITIVE

Thin cell wall

Thick cell wall



We have two stains used in this technique:

- ❑ primary stain (**crystal violet**): appear **dark purple** and are called **gram positive**.
- ❑ counter stain (**safranin**) or (**basic fuchsin stain**): appear red are termed **gram negative**.

Gram staining includes four basic steps:

1. Applying a **primary stain** (crystal violet).
2. Adding a **mordant** (Gram's iodine).
3. Rapid **decolorization** with ethanol, acetone or a mixture of both.
4. **Counterstaining** with safranin.

Gram-positive

Gram-negative



Fixation



Crystal violet



Lugol's iodine



Decolorization



Safranin

Procedure:

1. Prepare a fixed smear.
2. **Flood** the smear with **crystal violet** for (1 minute), wash with water.
3. **Flood** the smear with **grams iodine** for (1 minute), wash with water.
4. **Decolorize** with ethyl alcohol by adding drops of solution until no more color appear (30 seconds), (The time may vary depending on the thickness of the smear), Wash with water.
5. **Counterstain** with dilute carbol fuchsin (1%) or safranin for (1 minute), wash with water.
6. **Observe** under oil immersion

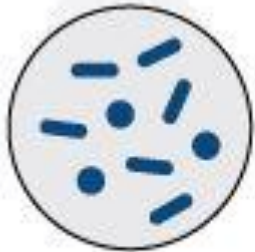
"Gram positive bacteria are dark purple and the Gram negative are red or pink in color"

Gram Staining Procedure

Step 1

Crystal violet

Primary stain added to specimen smear.



- Gram (+) : Blue
- ▬ Gram (-) : Blue

Step 2

Iodine

Mordant makes dye less soluble so it adheres to



- Gram (+) : Blue
- ▬ Gram (-) : Blue

Step 3

Alcohol

Decolorizer washes away stain from gram (-) cell walls.

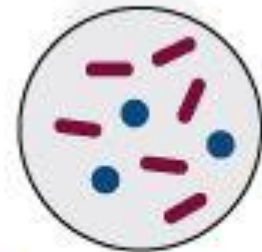


- Gram (+) : Blue
- ▬ Gram (-) : Colorless

Step 4

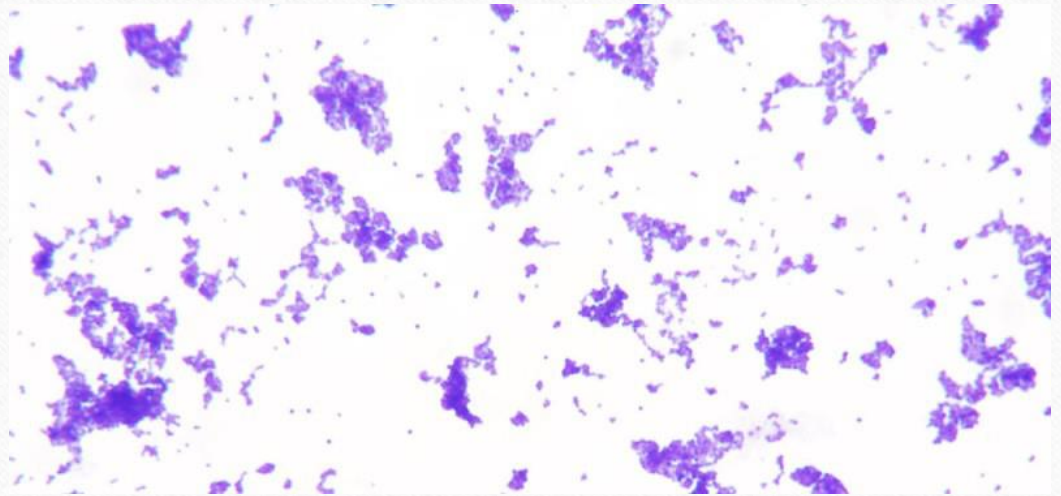
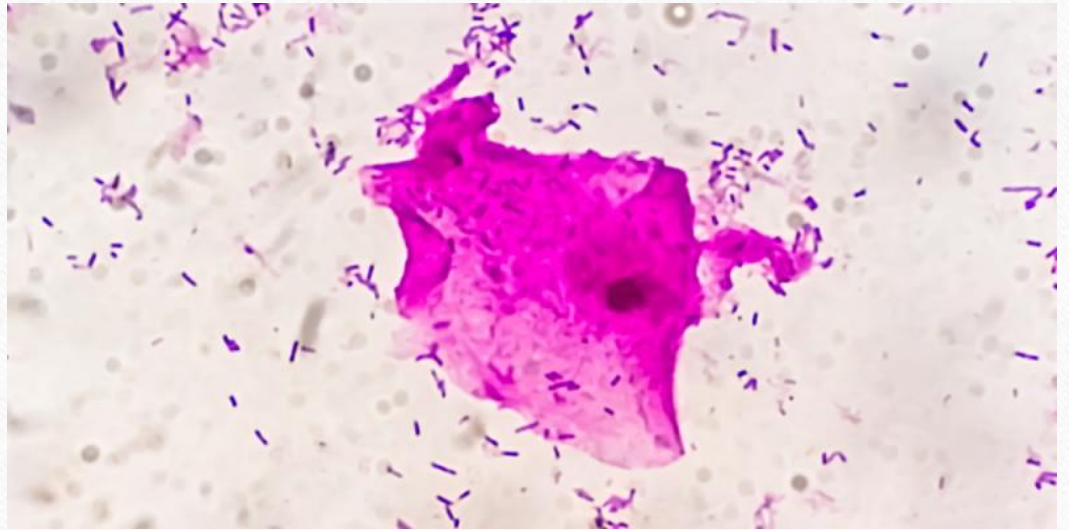
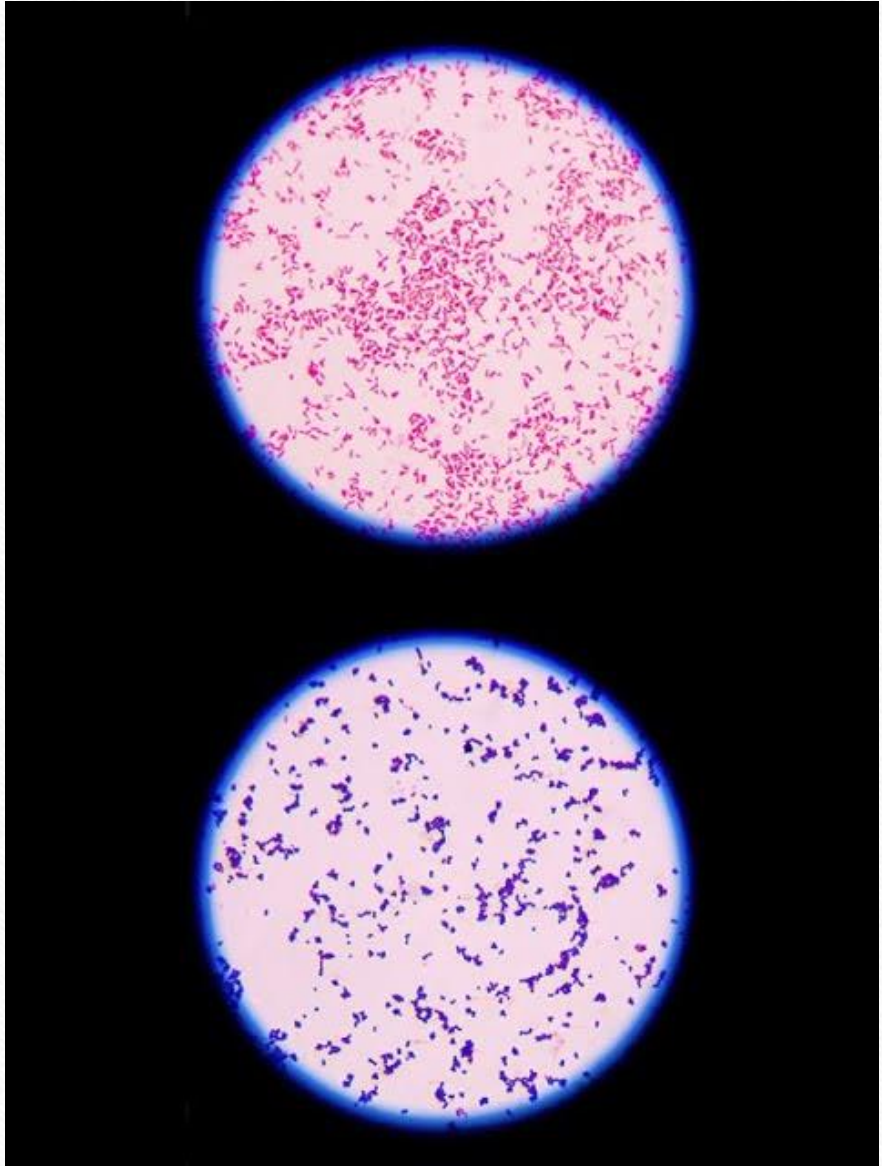
Safranin

Counterstain allows dye adherence to gram (-) cell walls.



- Gram (+) : Purple
- ▬ Gram (-) : red

- The ability to resist decolorization is related to the **chemical composition** and structure of cell wall.
- The decolorization step is the **critical step** that differentiate the bacteria.
- Grams iodine is a **mordant** that increase the affinity of the primary stain to the bacterial cells.



"Acid-fast Stain"

- Acid - fast staining procedure also called the ziehl-neelson technique.
- It's a laboratory test used mainly for diagnosis the causative microbe for tuberculosis (TB) in a sample.
- A differential stain that is used in the identification of **Mycobacterium** and other acid fast bacteria.
- Acid - fast **means** "the ability of a microorganism to resist depolarization by acid alcohol after primary staining".
- **Principle:** "The lipoid capsule of the acid-fast organism takes up carbol fuchsin and resists decolorization with a dilute acid rinse".

ACID-FAST BACTERIA



CARBOL FUSCHIN
(PRIMARY STAIN)

+

HEAT
(MORDANT)

ACID ALCOHOL
(DECOLORIZER)

METHYLENE BLUE
(COUNTERSTAIN)



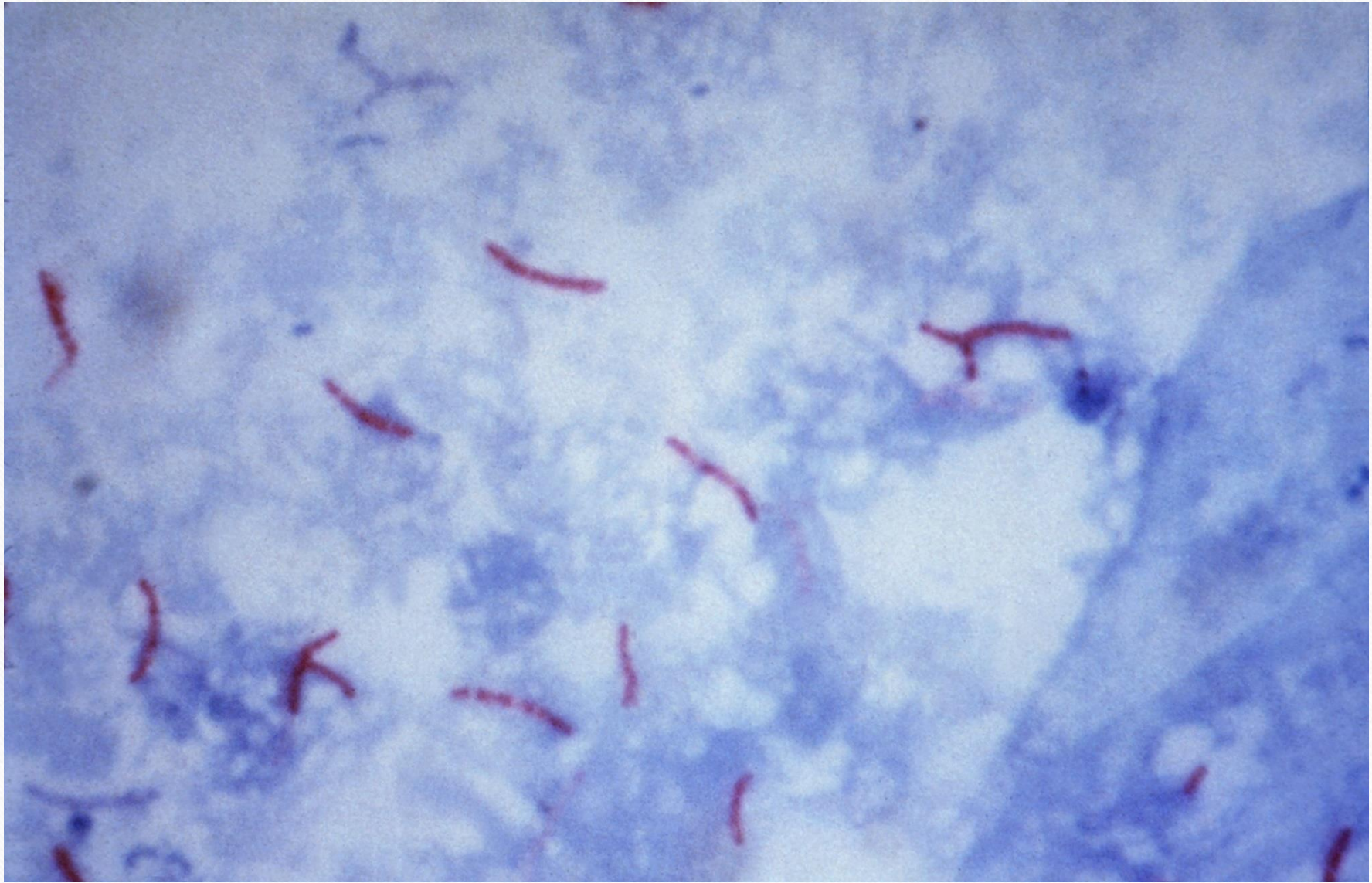
NON ACID-FAST BACTERIA

- Acid alcohol resistant is due to "the presence of a high lipid content (40-60%) in the cell envelope" (gram negative bacteria have no more than (20%) while gram positive bacteria have (1-4%) lipid in their cell envelope).
- The lipoid capsule of an acid-fast organism stains with carbol-fuchsin and resists decolorization with dilute acid rinse. The acid-fast bacilli will stain bright red, and the background will stain blue.

Procedure:

1. Prepare fixed smear of a mixture of two bacteria for example *E.coli* and *Mycobacterium* on the slide.
2. **Flood** the smear with concentrated **carbol fuchsin** with flaming until steaming for (5 minutes), wash with water.
3. Add the **decolorizer** acid alcohol until no more color appears, Wash with water.
4. **Flood** with **methylene blue** for (1 minute), Wash with water.
5. **Dry** the slide and **observe** under oil immersion.

Acid fast bacteria appear **red** and non-acid fast bacteria appear **blue**.





HOPE YOU ENJOYED THE LAB