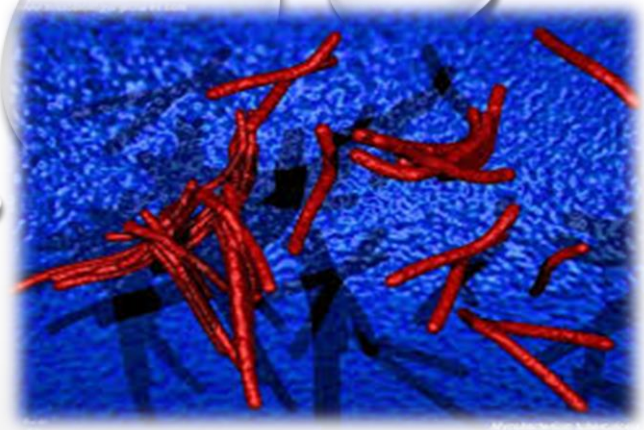
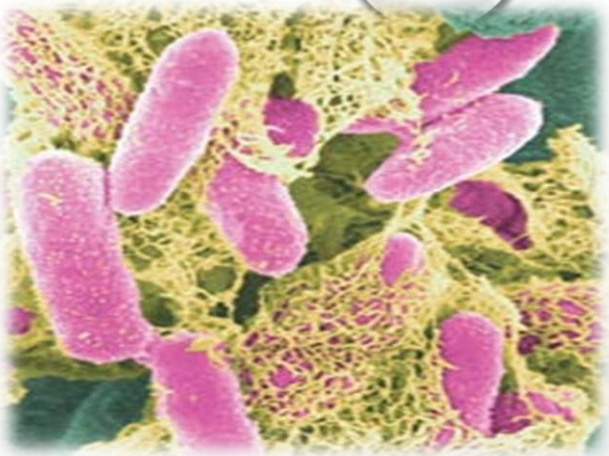




STAINING

Dep. Medical microbial., college of medicine
Ruqayah Alkuhli, B.Sc., M.Sc. Med. Microbiology

Staining of Bacteria

- **Bacteria cells are almost *colorless* and *transparent***

- **A staining technique is often applied to the cells to *color them* →**

  Their shape and size can be easily determined under the microscope.

Principle of staining

- **Stains → combine *chemically* with the bacterial protoplasm.**

- **Commonly used stains are *salts*:**

❖ **Basic dyes: colored cation + colorless anion**

e.g. methylene blue (methylene blue chloride)

$MB^+ + Cl^-$

❖ **Acidic dyes: colored anion + colorless cation**

e.g. eosin (Na^+ + $eosin^-$).

- **Bacterial cells are *slightly negatively charged* (rich in nucleic acids bearing negative charges as phosphate groups)**

→ combine with *positively charged basic dyes*

- ***Acidic dyes* do not stain the bacterial cell → can stain the background material with a contrasting color.**

Types of staining techniques

Simple staining
(use of a single stain)

For visualization of morphological shape & arrangement.

Differential staining
(use of two contrasting stains separated by a decolorizing agent)

- Identification
  - Gram stain
  - Acid fast stain
- Visualization of structure
  - Spore stain
  - Capsule stain

Smear Preparation

Smear Preparation:

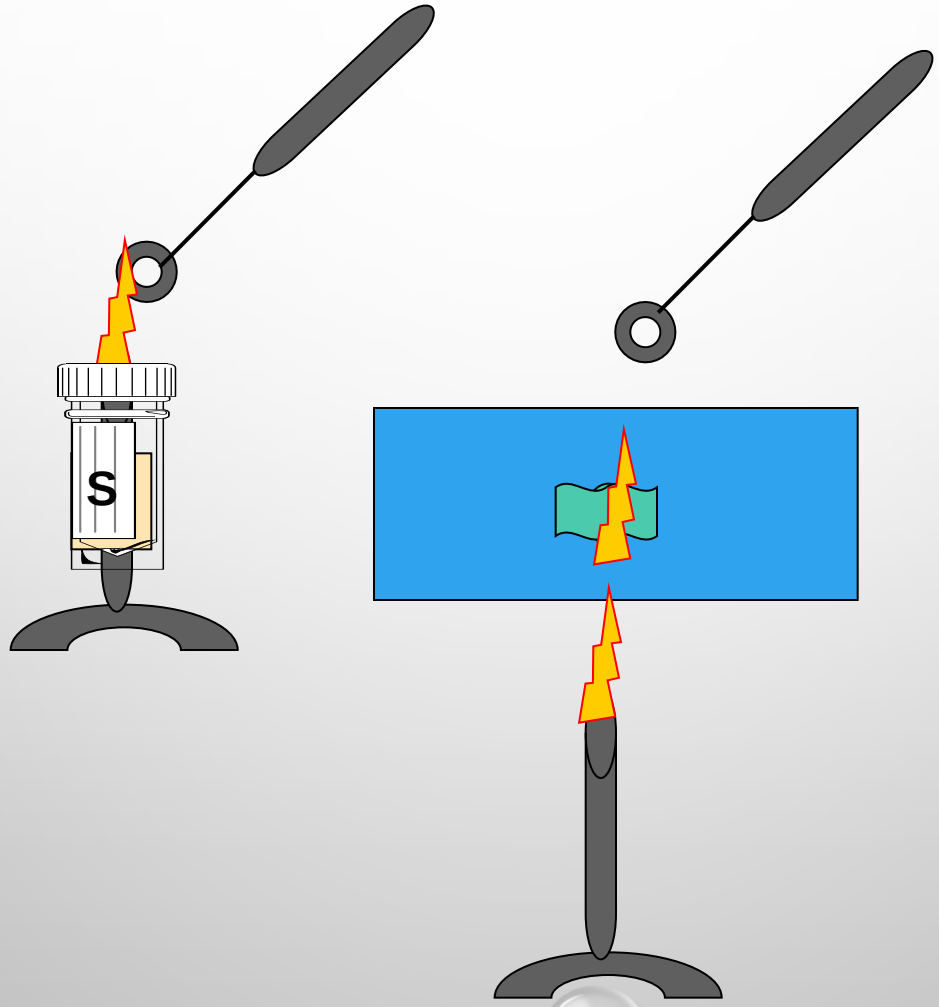
Preparation and Fixation of Bacteria for Staining.

Objective:

To kill the microorganism & fix them to the slide to prevent them from being washed out during the process of staining .

- Heat fix the bacteria to the slide (release of "sticky" proteins from the cell surface of the bacteria adheres the bacterial cell to the slide)

Smear preparation



Fixation

Simple Staining

Definition:

It is the use of *single basic dye* to color the bacterial organism.

e.g. methylene blue,
crystal violet,
safranin.

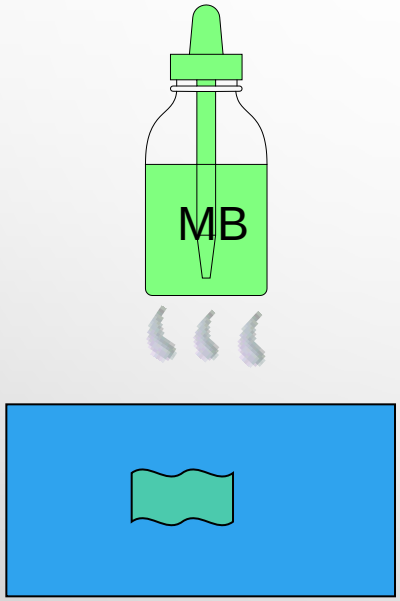
***All bacteria* take *the color of the dye*.**

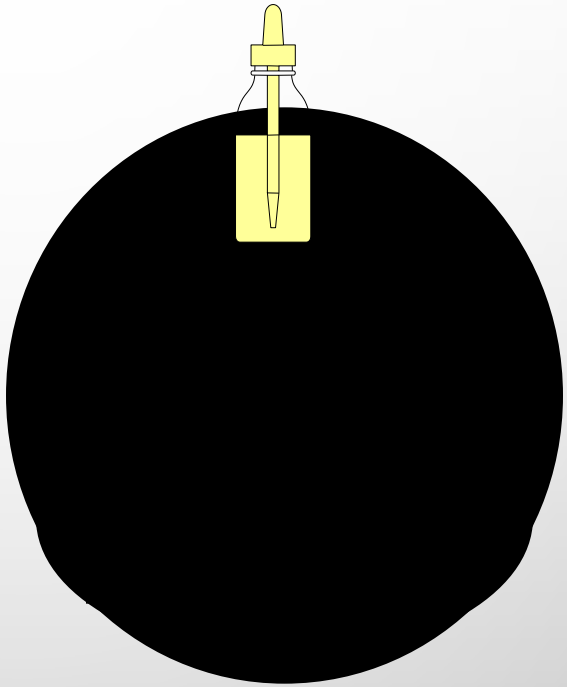
Objective:-

To show the morphological shapes and arrangement of bacterial cells.

Simple Staining

- **Procedure:-**





1-2 min

Bacterial Morphology

Bacterial morphology deals with size, shape, and arrangement of bacterial cells

- **Coccus** or **Cocci** are bacterial cells that are spherical, and resemble tiny balls (*Streptococcus)*
- **Bacillus** or **Bacilli** are bacterial cells that are rod shaped
- **Spiral** bacteria have twisted or helical morphology. They may appear as curved rods, called vibrios, as spirilla or spirochetes having pliant bodies

Arrangement of cells

Arrangement of cocci cells

Singly: Bacteria that appear as single cell, is just called as cocci

Diplococci: These cells are found in pairs and they are found attached to each other

(Streptococcus) These bacteria form long chains and remain attached to each other

(Staphylococcus) These bacteria are arranged irregularly in clusters like grapes

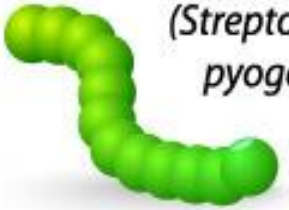
Arrangement of Bacilli

Singly: Bacteria that exists as single cell, called bacilli

BACTERIA SHAPES

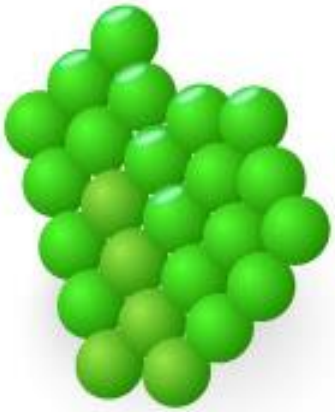
SPHERES (COCCI)

Diplococci
(Streptococcus pneumoniae)



Streptococci
(Streptococcus pyogenes)

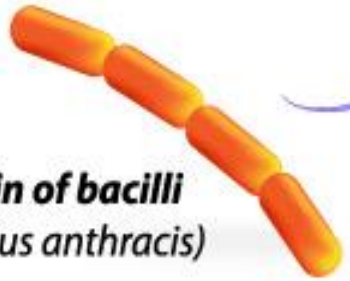
Tetrad



Staphylococci
(Staphylococcus aureus)

Sarcina
(Sarcina ventriculi)

RODS (BACILLI)



Chain of bacilli
(Bacillus anthracis)

Flagellate rods
(Salmonella typhi)

Spore-former
(Clostridium botulinum)

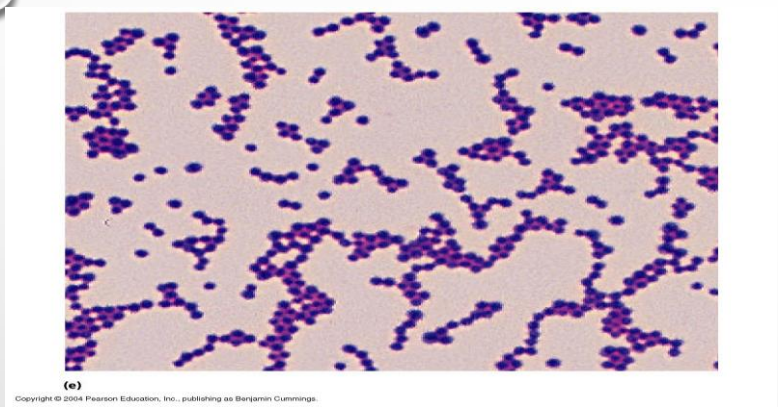
SPIRALS

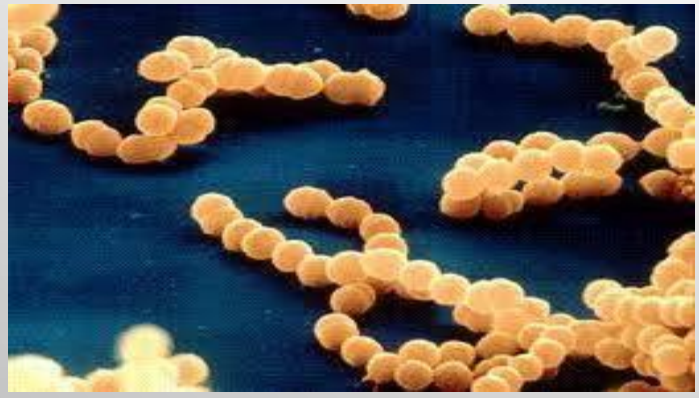
Vibrios
(Vibrio cholerae)

Spirilla
(Helicobacter pylori)

Spirochaetes
(Treponema pallidum)

Basic Shapes of Bacteria





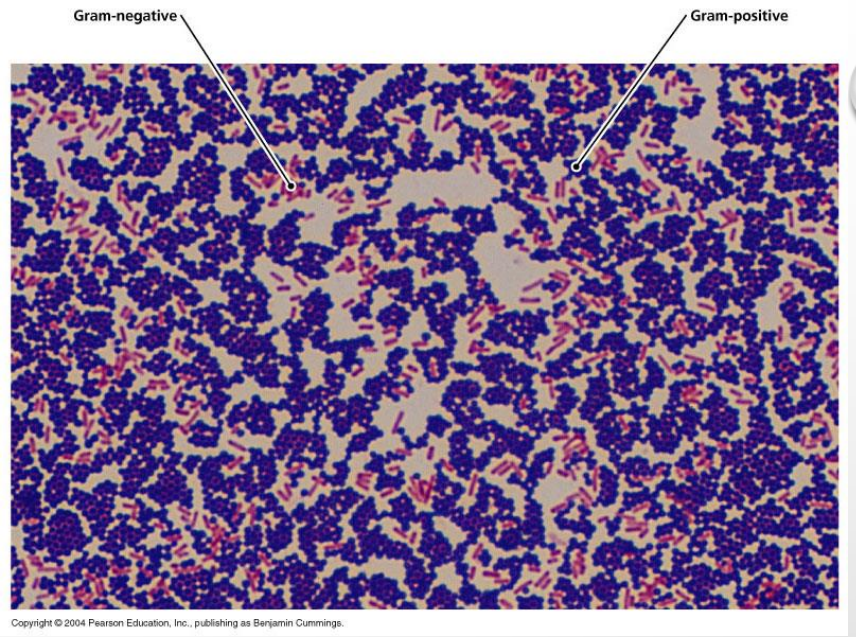
Cocci

Bacilli

Gram Staining

Gram Stain:

- It is the most important differential stain used in bacteriology because
- it classified bacteria into two major groups:



a) Gram positive:

Appears violet after Gram's stain

b) Gram negative:

Appears red after Gram's stain

CRYSTAL VIOLET

↓

IODINE

↓

ALCOHOL

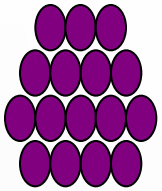
↓

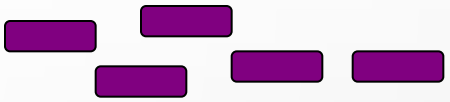
SAFRANIN

Gram +ve
S.aureus

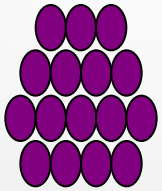
Gram –ve
E.coli

Step 1: Crystal Violet

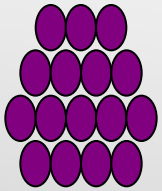


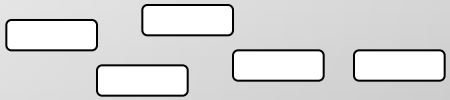


Step 2: Gram's Iodine

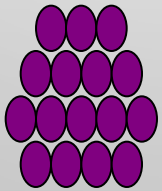


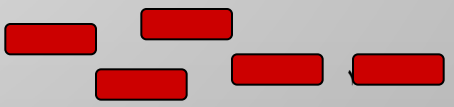
Step 3: Decolorization (Aceton-Alcohol)

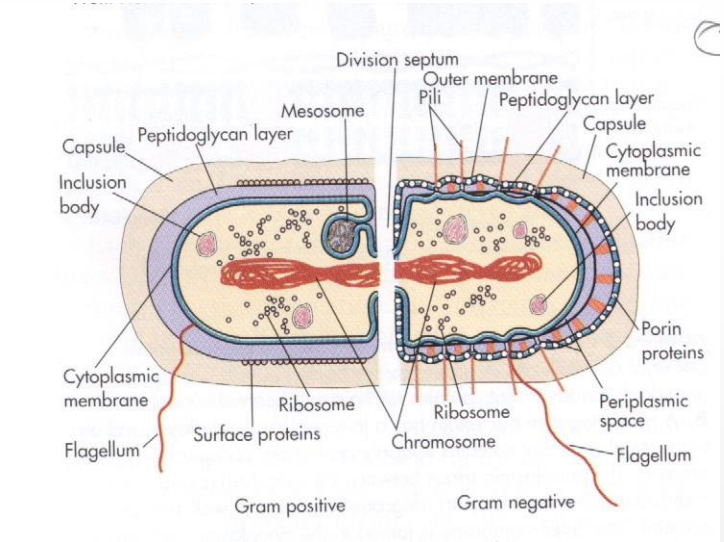


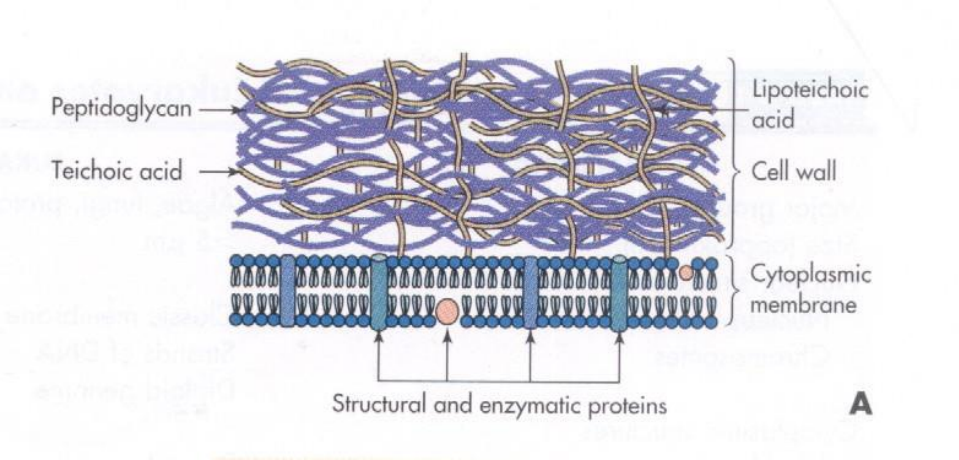


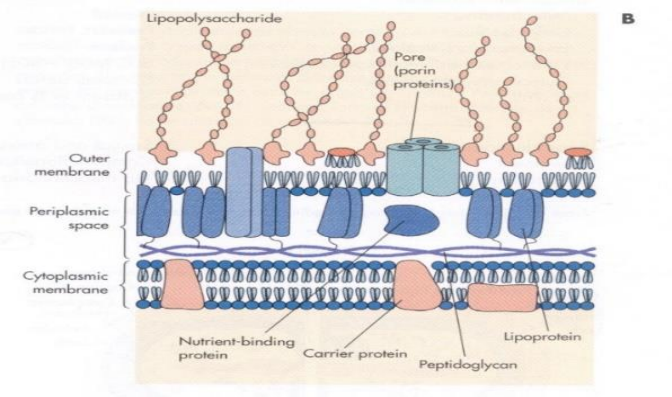
Step 4: Safranin Red





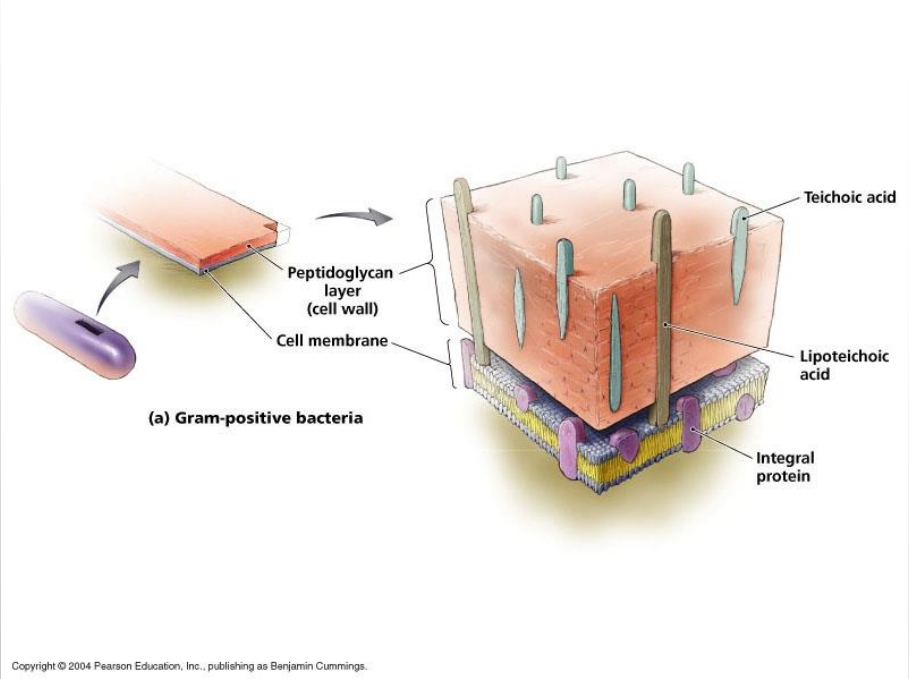


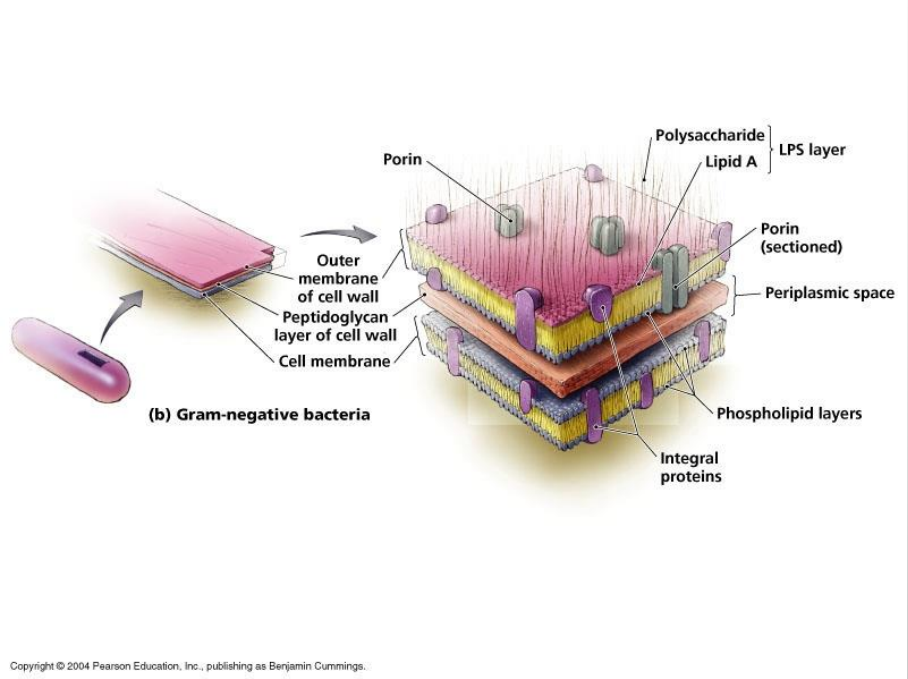




Gram's +ve Bacteria

Gram's -ve Bacteria





Gram's +ve Bacteria

Gram's -ve Bacteria

Gram-positive bacteria

- Have a thick peptidoglycan layer surrounds the cell.
- The stain gets trapped into this layer and the bacteria turned purple.
- Retain the color of the primary stain (crystal violet) after decolorization with alcohol

Gram-negative bacteria

- Have a thin peptidoglycan layer that does not retain crystal violet stain.
- Instead, it has a thick lipid layer which dissolved easily upon decoulorization with aceton-alcohol.
- Therefore, cells will be counterstained with safranin and turned red.

GRAM STAIN

- ***MATERIALS:-***
  - **CULTURES OF *STAPHYLOCOCCUS AUREUS, E.COLI***
  - **GRAM STAIN:**

    CRYSTAL VIOLET (PRIMARY STAIN)

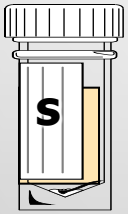
    GRAM'S IODINE (MORDANT)

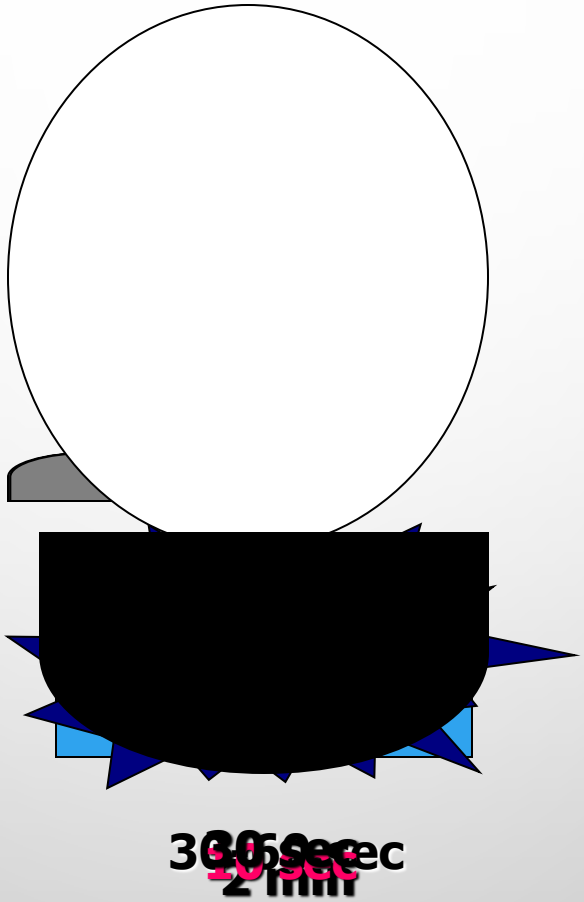
    ACETONE-ALCOHOL (DECOLORIZING AGENT)

    SAFRANIN (COUNTER STAIN)

GRAM STAIN

- ***PROCEDURE:***

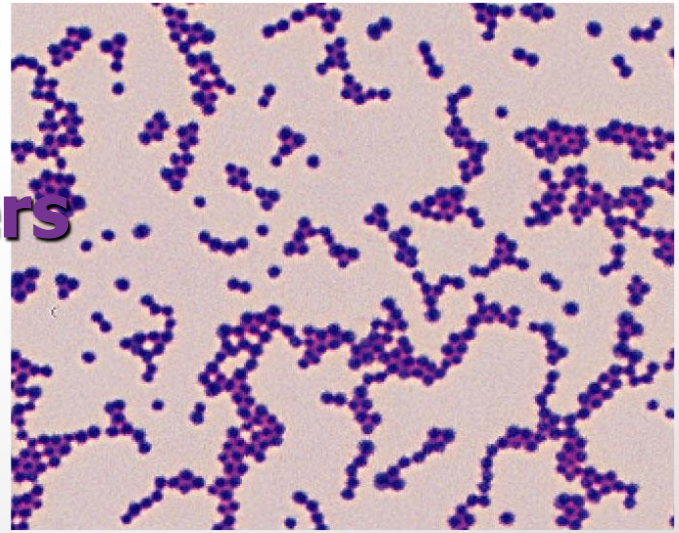




RESULTS:

Shape: Cocci

Arrangment: irregular clusters



(e)

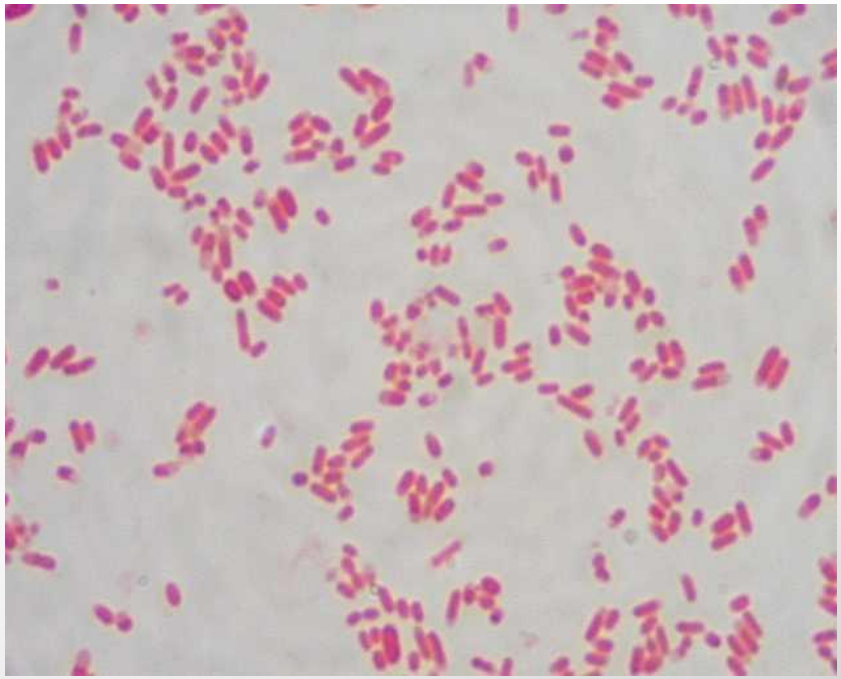
Colour: Violet

Gram's reaction: Gram's +ve

Name of microorganism:

Staphylococci

RESULTS:



Shape: Rods

Arrangment: Single

Colour: red

Gram's reaction: Gram's –ve

Name of microorganism:

Gram negative bacilli

Acid – Fast stain (Ziehl-Neelsen Method)

- It is a special bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria
- 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
  - **Mycobacterium tuberculosis**
  - **Mycobacterium leprae**

Structural Stains

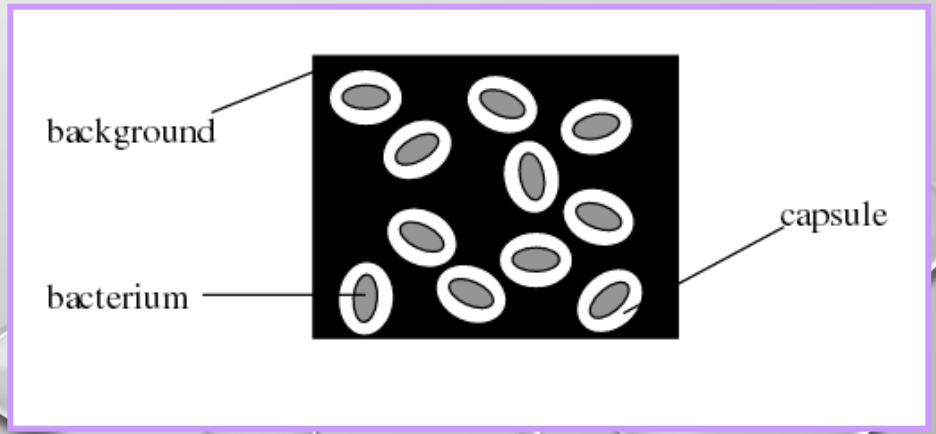
Capsule stain

A capsule: a gelatinous outer layer secreted by the cell & surrounded the cell wall, it is chemically composed of polysaccharide, a *glyco -protein* or polypeptide.

- The capsule is a major virulence factor in the major disease-causing bacteria, such as ***Klebsiella pneumoniae***

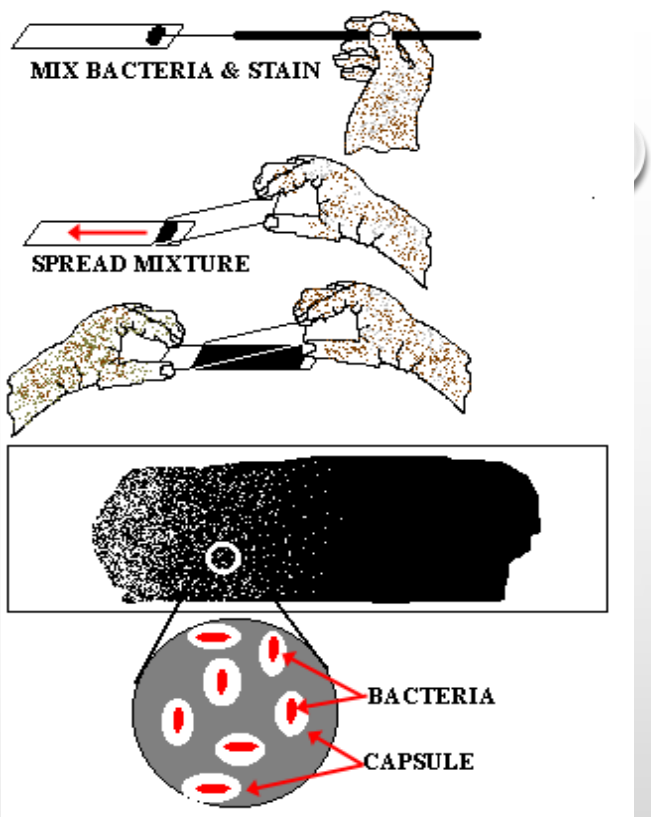
WHY WE USE CAPSULE STAIN ?

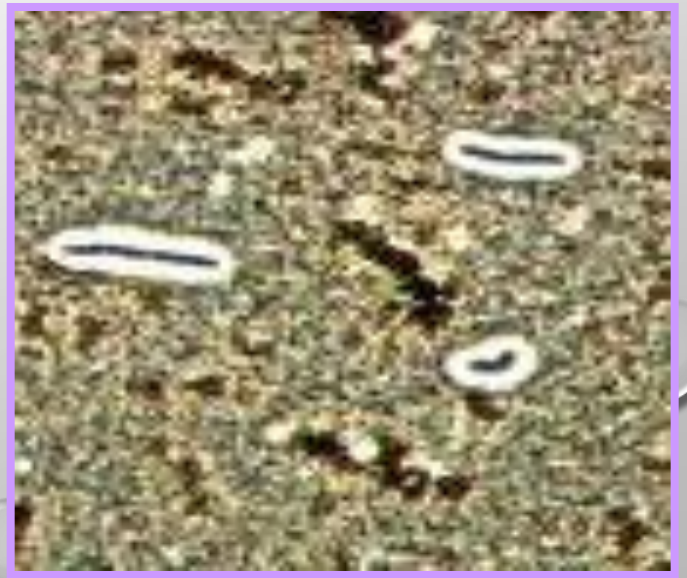
- Heat fixation cause capsule shrinkage
- Bacterial capsules are non-ionic



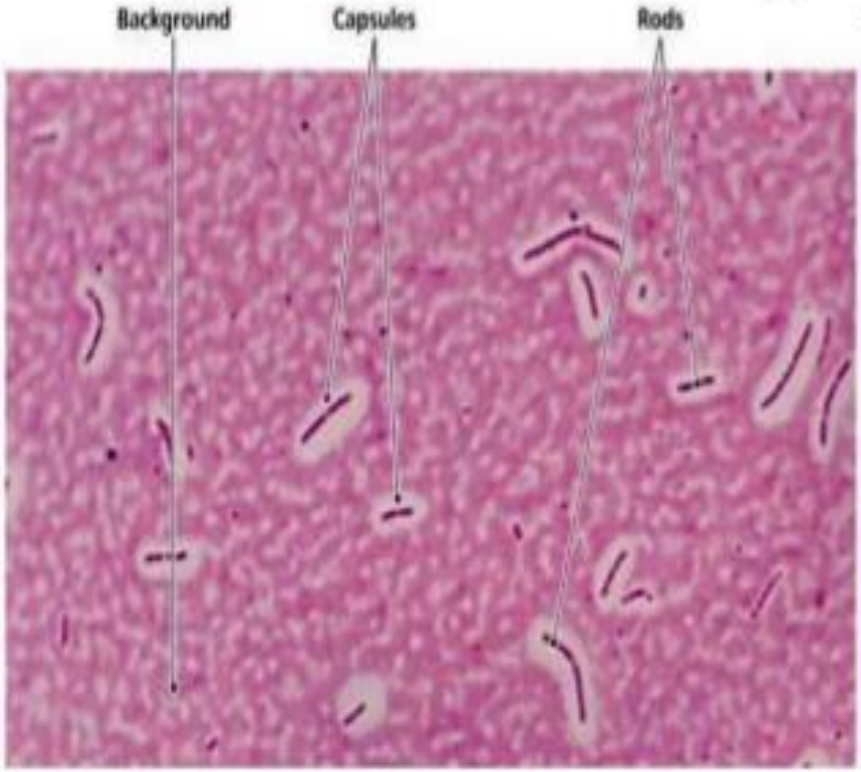
Procedure

- Introduce a drop of crystal violet on to a clean glass slide
- Using a sterile loop, place the sample on to the glass slide
- Obtain another glass slide and at an angle, spread the drop and sample to form a thin film
- Allow the film to dry (air dry) for about 6 minutes
- rinse with 20% copper sulfate solution
- Allow the slide to air dry for about 3 minutes
- Place the slide on to the microscope stage and observe using oil immersion

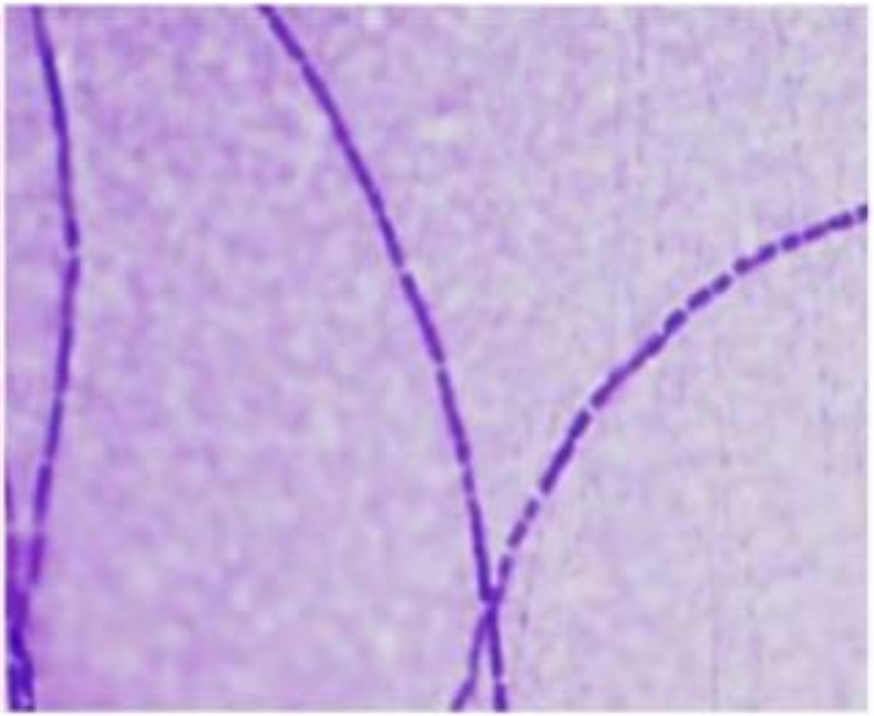




Capsule Staining



Non Capsulated Bacterium



Spore stain

Schaeffer- Fulton method

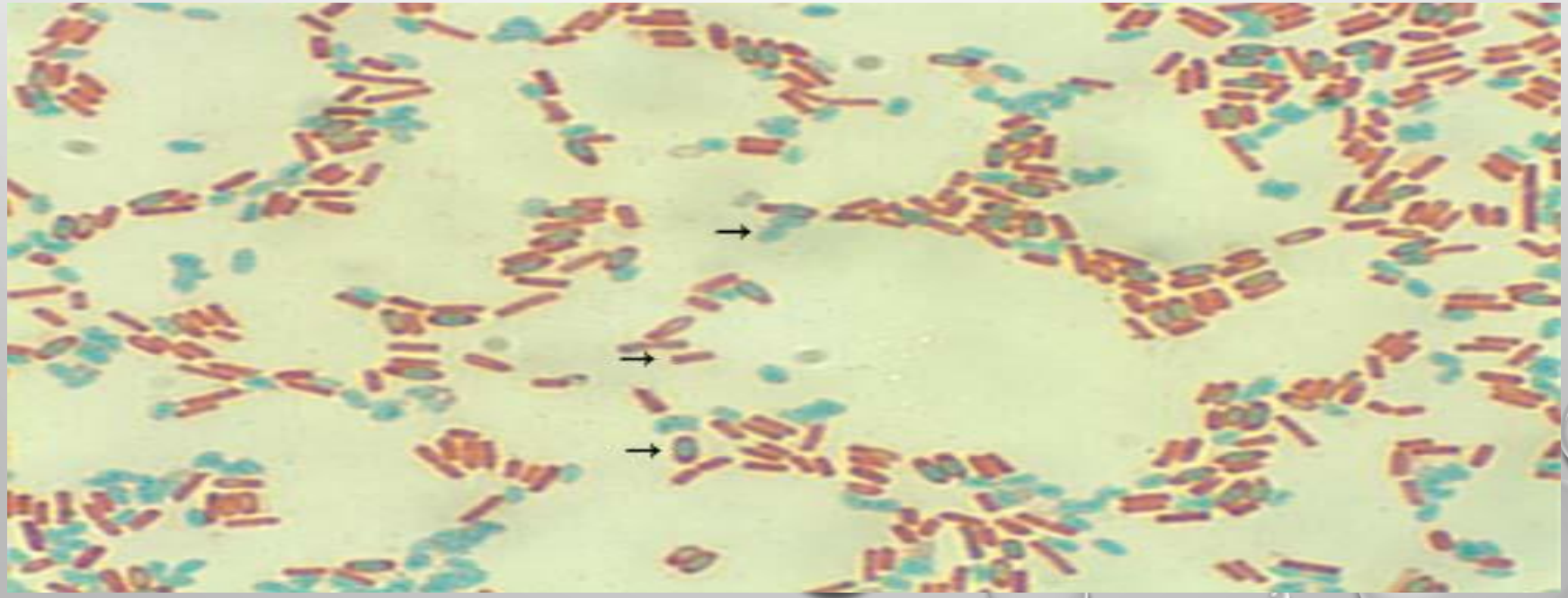
• The endospore consists of the bacterium's DNA and part of its cytoplasm, surrounded by a very tough outer coating.

• The position of the endospore differs among bacterial species and is useful in identification.

• The main types within the cell are terminal, subterminal, and centrally placed endospores.

Procedure

1. Prepare a smear of the bacteria (a spore-producing organism)
2. Flood the smear with malachite green
3. Do not allow the stain to evaporate or completely evaporate.
4. Remove from heat and allow slides to cool
5. Once the slides are cool → rinse with water
6. Flood the sample with safranin (30-60 seconds)
7. Rinse the slide →blot dry →observe under microscopy.



Flagella Staining Technique by Liefson's Method

• **Flagella** is a thin,hair like structure made up protein called as flagellin.

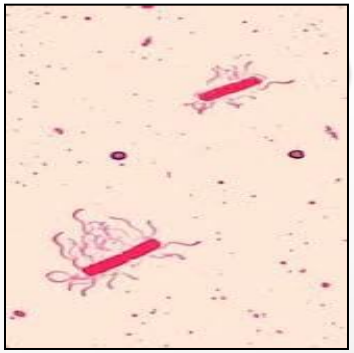
• It size ranges from 20 µ to 200 µ in length.

• Flagella is one of the most important locomotory organ .It is mainly made up of three parts- 1) Basal body 2) Filament 3) Hook.

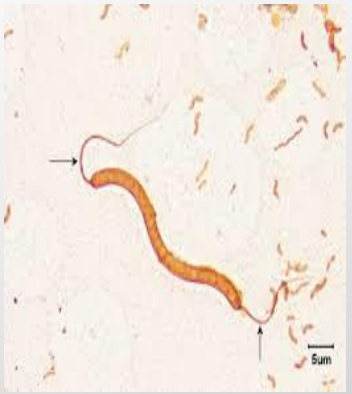
• Flagella is generally present in rod shape bacteria and very few cocci shape bacteria posses flagella.

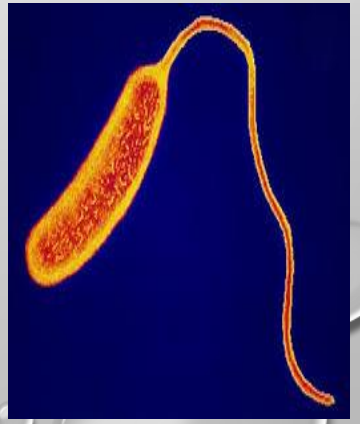
• As flagella are very thin and hair like they cannot be easily observed under microscope. So a special technique is design to increase thickness of flagella as well as stain it..

Procedure



- First of all take two hours old flagellated cell culture slant and add two to three drops of sterile distill water in the slant with the help of sterile pipette.
- Note that the distill water is added slowly without disturbing the growth of cells.
- After addition of distill water incubated the slant for 20 minutes.
- Then take a drop of suspension from the slant and place the drop on a clean slide which is kept in slanting position.
- The drop should flow slowly from one end of slide to other end to avoid folding of flagella on cell.
- Allow smear to air dry here we don’t use heat fixation treatment .
- After air drying the slide is flooded with Leifson’s stain till a thin film of shinny surface appear.
- After this give a gentle stream of water wash treatment to a slide.
- Now treat the slide with 1 % methylene blue treatment for 1 minute.
- Give the slide water wash treatment ,air dry and observe under oil immersion lens.





Thank You