Preparation of Bacterial Smear

Simple Staining

And

Gram staining

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Bacterial Smear:

Smear is a distribution of bacterial cells on a slide for the purpose of viewing them under the microscope.

Method:

-Aseptically a small sample of the culture is spread over a slide surface.

-This is then allowed to air dry.

-The next step is heat fixation to help the cells adhere to the slide surface.

-The smear is now ready for staining.

Preparation of bacterial smear

1-Put one drop of distilled water in the center of clean , dry slide

Sterile wire loop with burner flame , then take a small amount from bacterial growth .

2-Emulsify the growth with distilled water drop and spread it on slide .

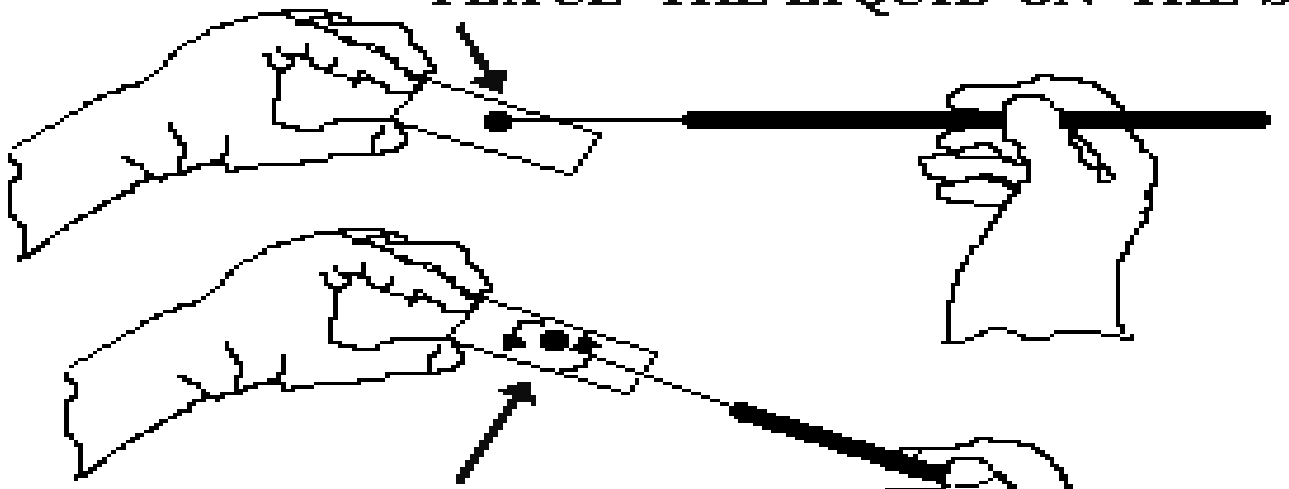
3-Leave the smear to dry .

4-Fixed the smear by flame ,by passing the slide quickly 2-3 times on flame .

5-The smear ready now for staining .

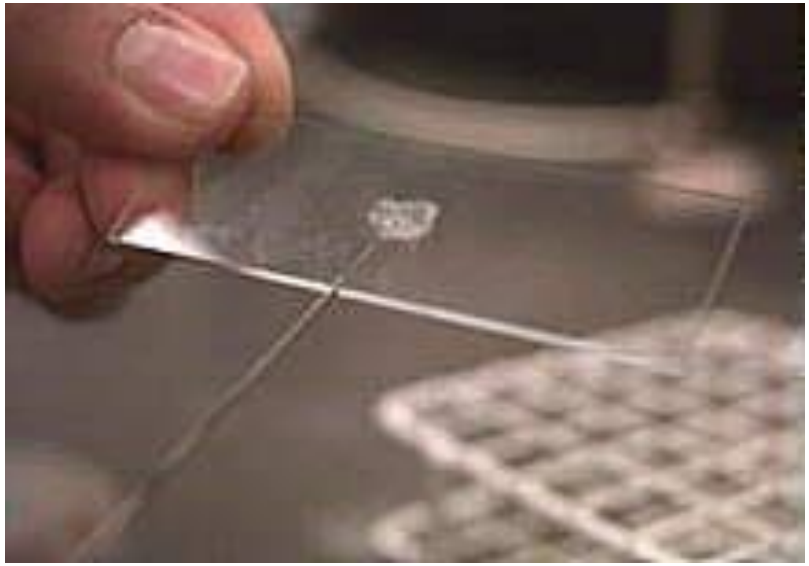
LIQUID CULTURE OR STERILE WATER

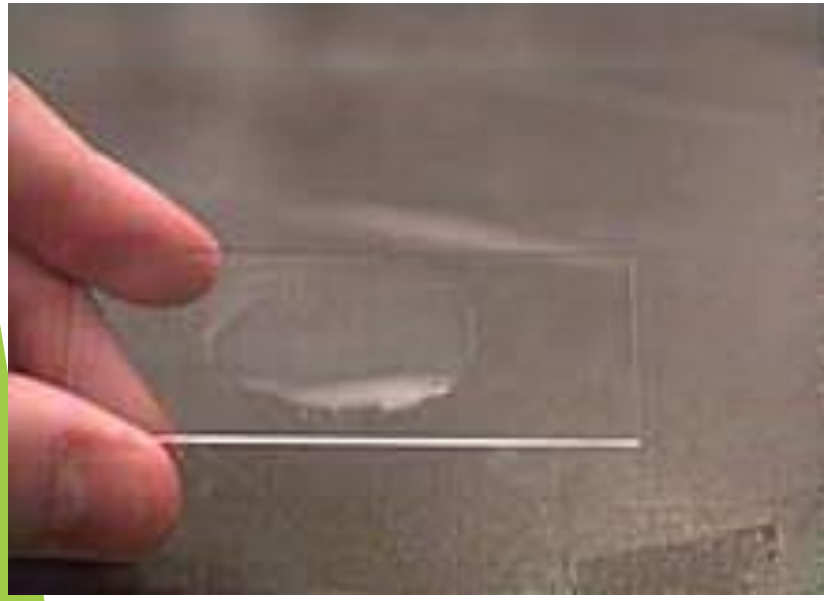
PLACE THE LIQUID ON THE SLIDE



ADD THE MICROBES TO THE LIQUID AND SPREAD OVER A 1 CM AREA

AIR DRY OR HEAT GENTLY. WHEN DRY BRIEFLY HEAT FIX THE CELLS TO THE SLIDE





Bacterial Staining :

- **Staining:** a process in which the bacterial cells are usually stained with different types of dyes and different types of techniquse,in order to study the morphology of bacteria like (cell shape ,cell size, the arrangement and the special parts of bacterial cells .)

Dyes : They are synthetic chemical products ,usually aniline derivatives which may be divided in to :

1- acidic dyes : the dyes which bearing a negative charge like acid fuchsin (red colour dye).

2-basic dyes : the dyes which bearing a postive charge like crystal violet (purpule colour dye) or methylne –blue(the dark-blue coloure dye).

Staining procedure can also be classified in different ways:

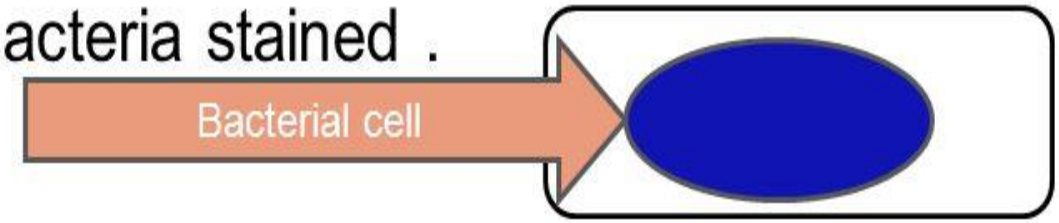
Simple staining involves the use of only 1 dye and is used primarily as a means to study the morphology and structure of organisms.

Differential staining uses more than 2 dyes and is also used to differentiate the organisms into one of two groups.

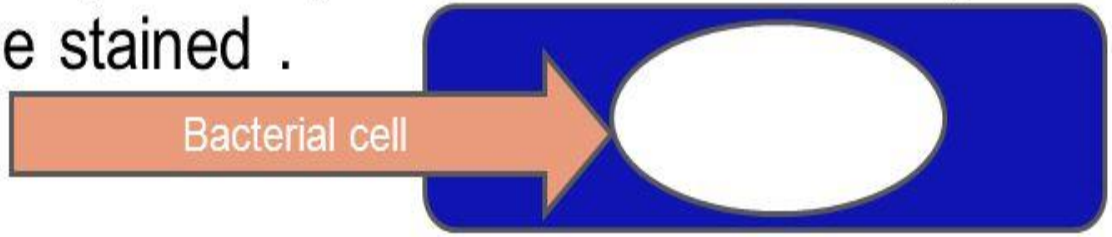
Simple staining – there are two methods:
positive staining - where the actual cells are themselves colored and appear in a clear background;

negative staining – where the cells remain clear (uncolored) and the background is colored to create a contrast to aid in the better visualization of the image.

Basic Dyes : chromophore is the positive ion dye attracted by the bacteria so the cells of bacteria stained .



Acid Dyes : chromophore is the negative ion dye rejected by the cell and the background of slide stained .



- Bacteria are slightly negative, so are attracted to the positive chromophore of the **BASIC DYE**

Procedure :

1 – put the slide on the staining rack.

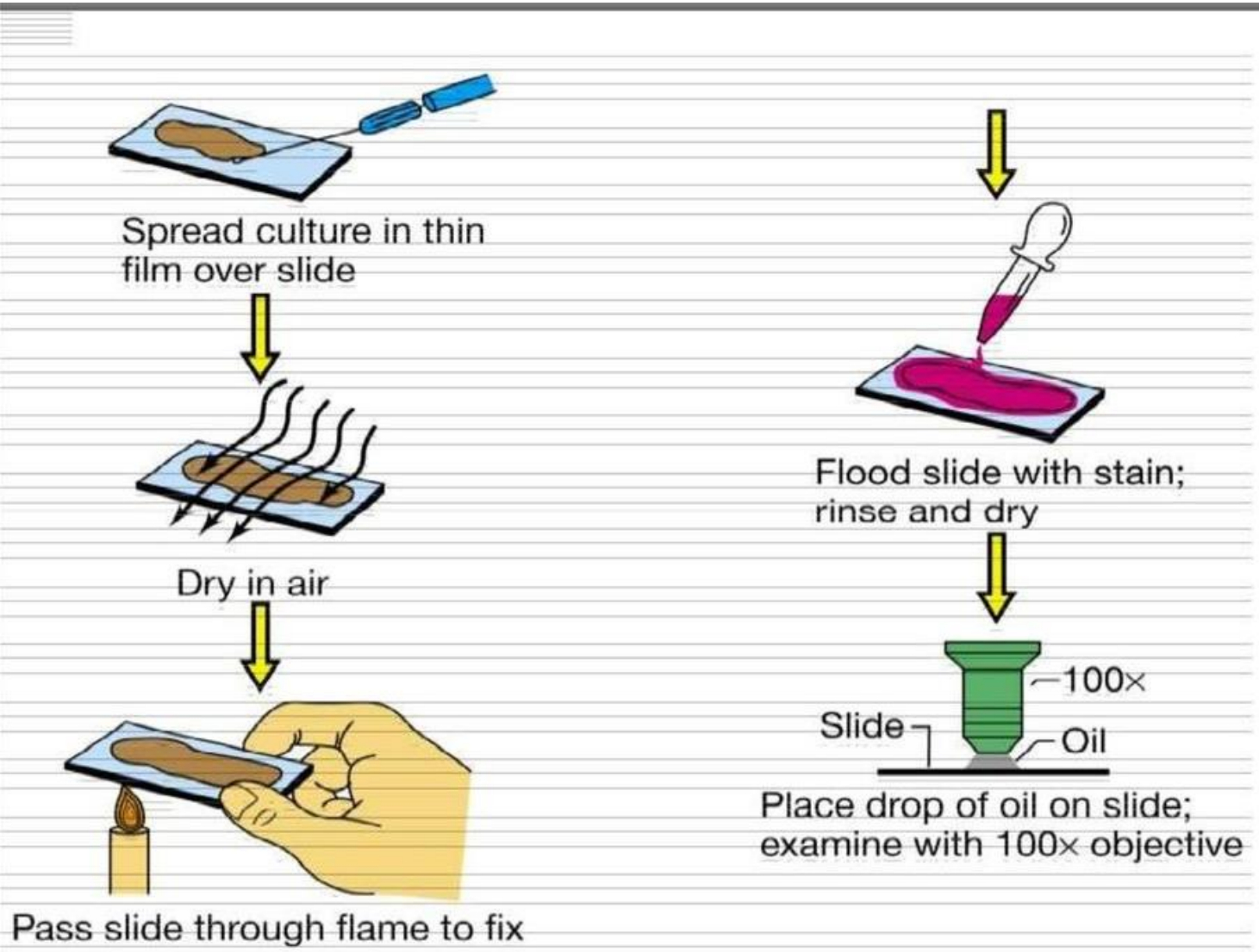
2 – flood it with crystal violet stain ,leave it for 30 seconds .

3 – Wash the slide carefully by washing bottle

4– dry the slide with filter paper .

5 – examine your slide under microscope , then by oil immersion lens .

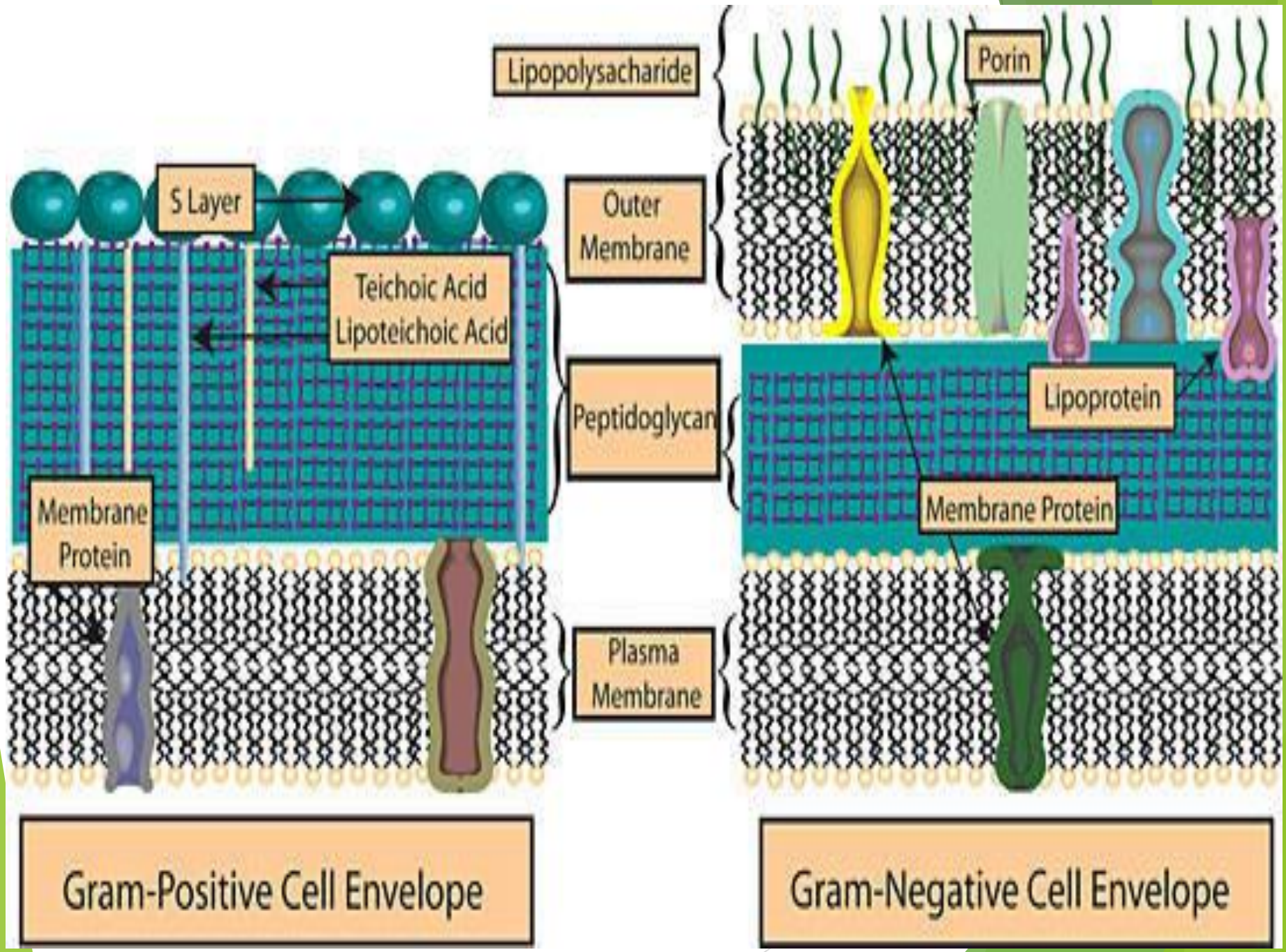
simple staining :



Differential staining (Gram staining)

Bacteria classified into Gram positive and Gram negative bacteria.

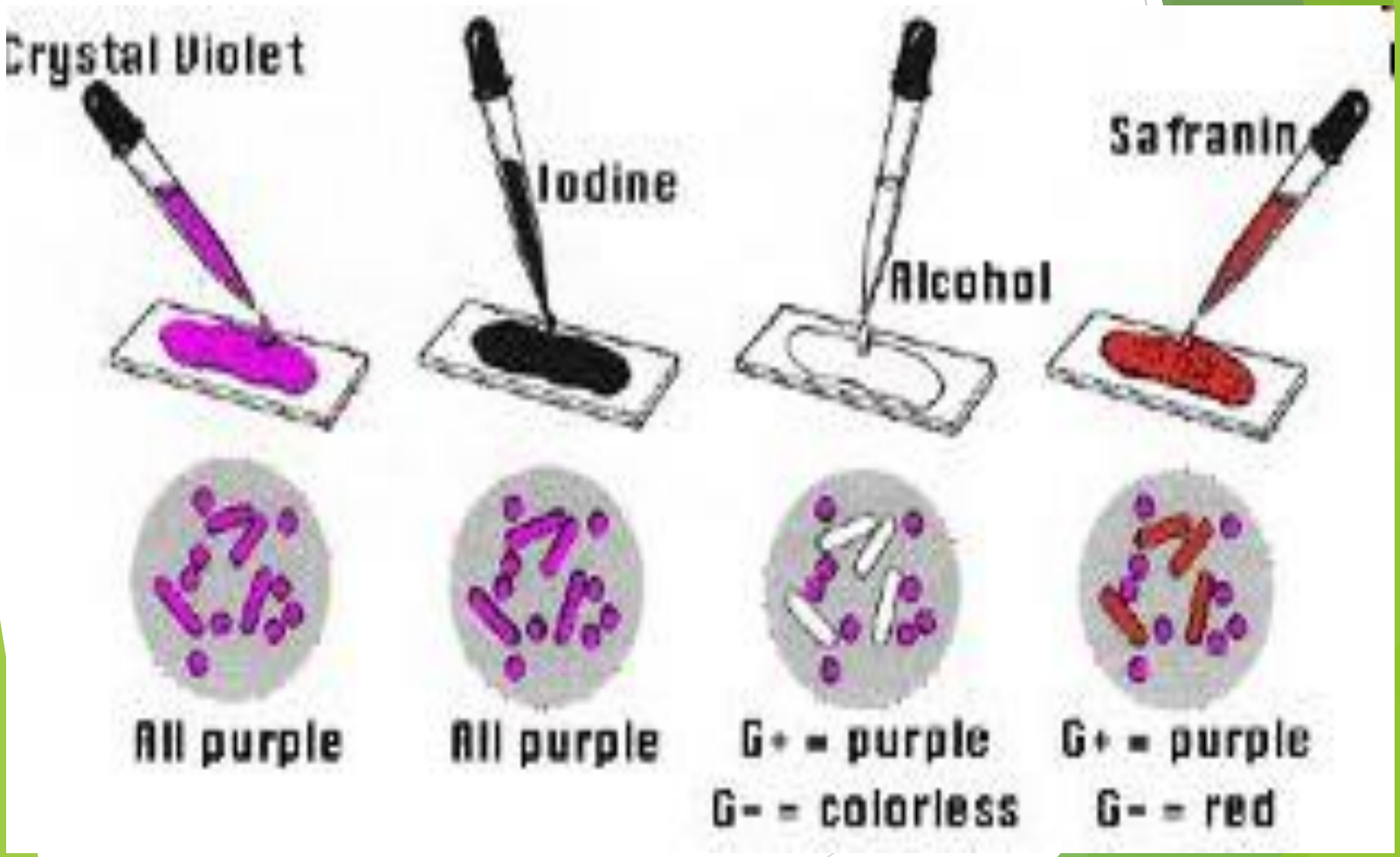
The cell wall composition differences makes difference

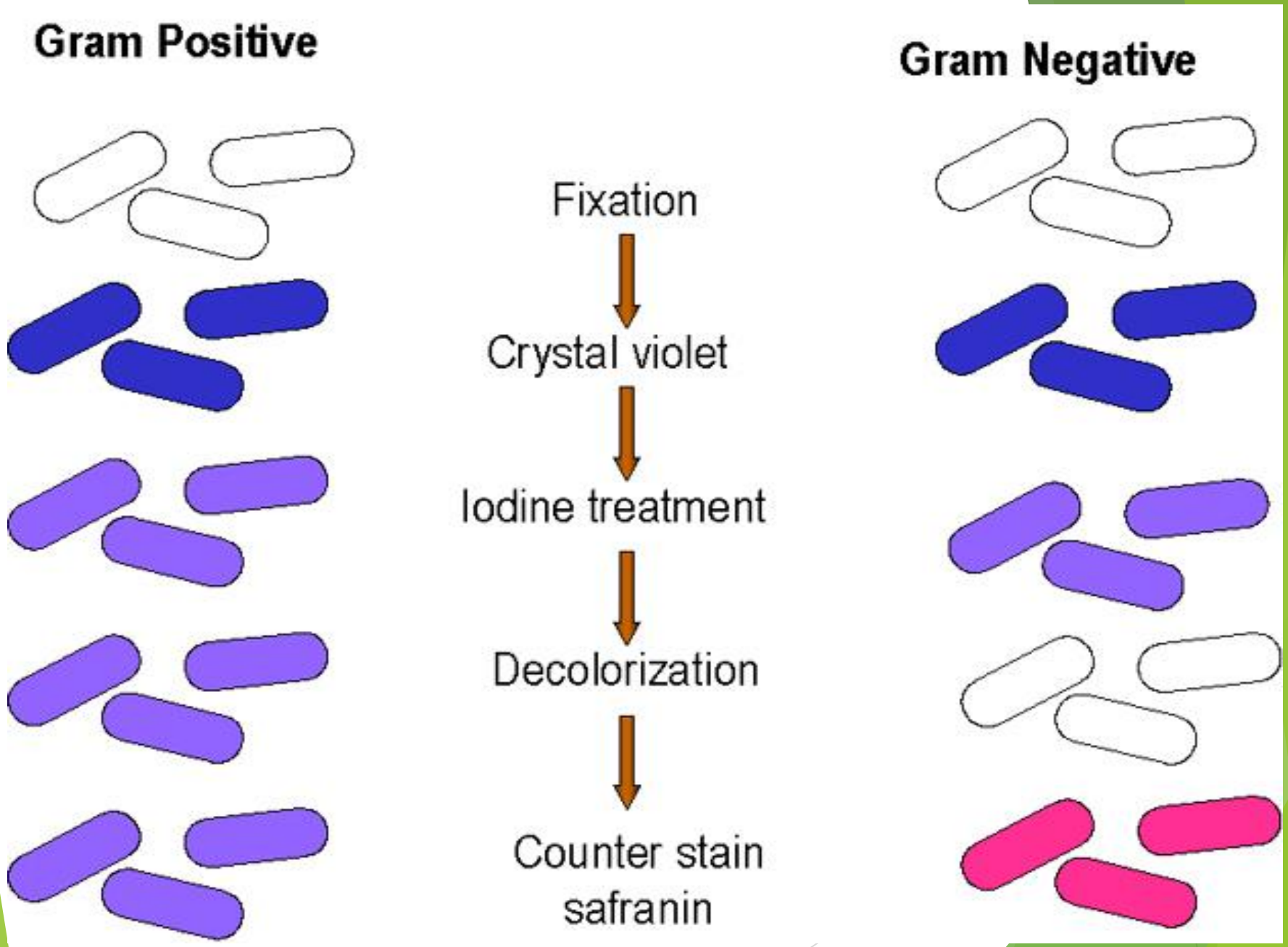

Lipopolysacharide
Porin
S Layer
Outer Membrane
Teichoic Acid
Lipoteichoic Acid
Peptidoglycan
Lipoprotein
Membrane Protein
Membrane Protein
Plasma Membrane
Gram-Positive Cell Envelope
Gram-Negative Cell Envelope

Procedure :

- Flood the smear with crystal violet (30 sec. to 2 min)
- Quickly and gently wash off excess stain (2 seconds)
- Fixation the smear with Grams iodine (1 minute)
- Decolorize with alcohol (10-20 seconds)
- Quickly and gently wash off excess stain (2 seconds)
- Flood the smear with safranin (30 sec to 2 min.)
- Quickly and gently wash off excess stain (2 seconds)
- Dry the slide with filter paper.
- Examine your slide under the microscope.

Procedure :


Crystal Violet
Iodine
Alcohol
Safranin
All purple
All purple
G+ = purple
G- = colorless
G+ = purple
G- = red


Gram Positive
Gram Negative
Fixation
Crystal violet
Iodine treatment
Decolorization
Counter stain
safranin

Morphology of bacteria:

The basis for staining is to study the morphology and structure of bacteria.

Bacteria primarily have distinct shapes;

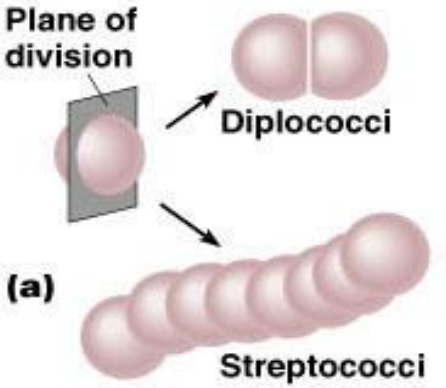
spherical (**coccus/cooci**) and
rod shaped (**bacillus/bacilli**)
filamentous.

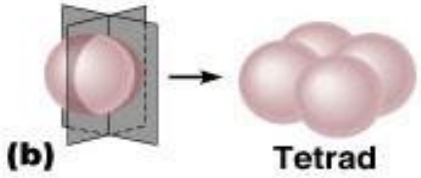
Based on the planes of divisions seen in the organism bacteria may also have specific arrangements of the cells.

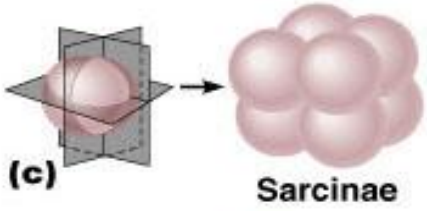
Diplococci are formed when the plane of division is vertical and the resultant two coccal cells do not completely separate from each other.

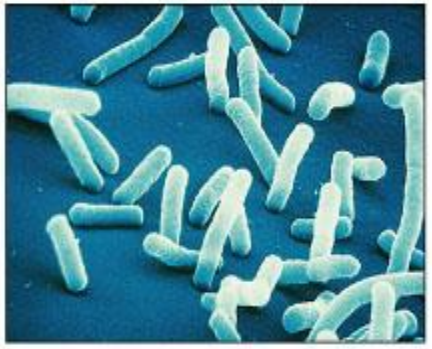
If the cells divide in the vertical plane continually and the cells do not separate it results in a chain of coccal cells called a **streptococci/streptobacilli.**

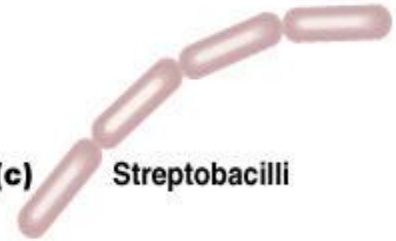
Other arrangements include: **tetrad** (4), **sarcina** (8), **staphylococcus** (irregular clusters).

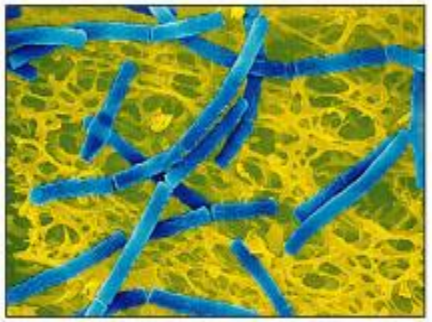


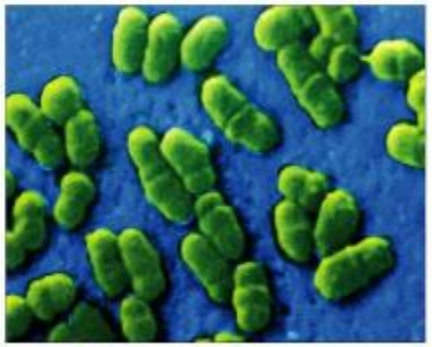




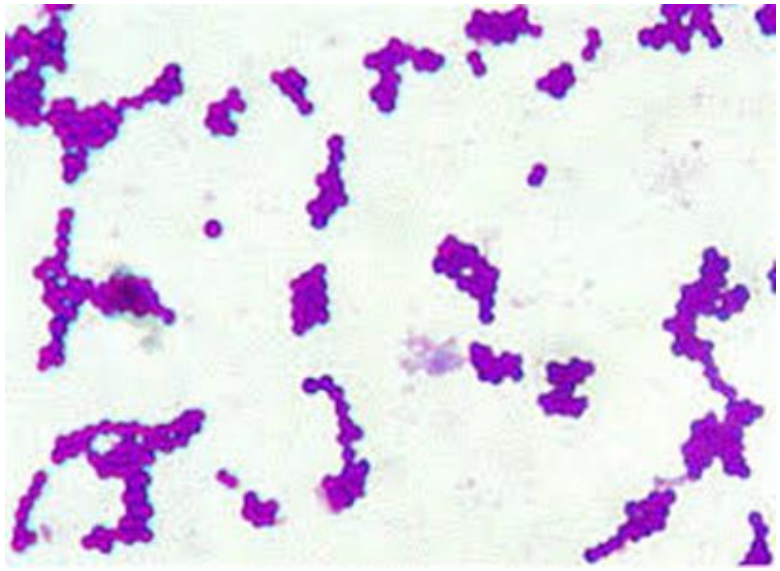


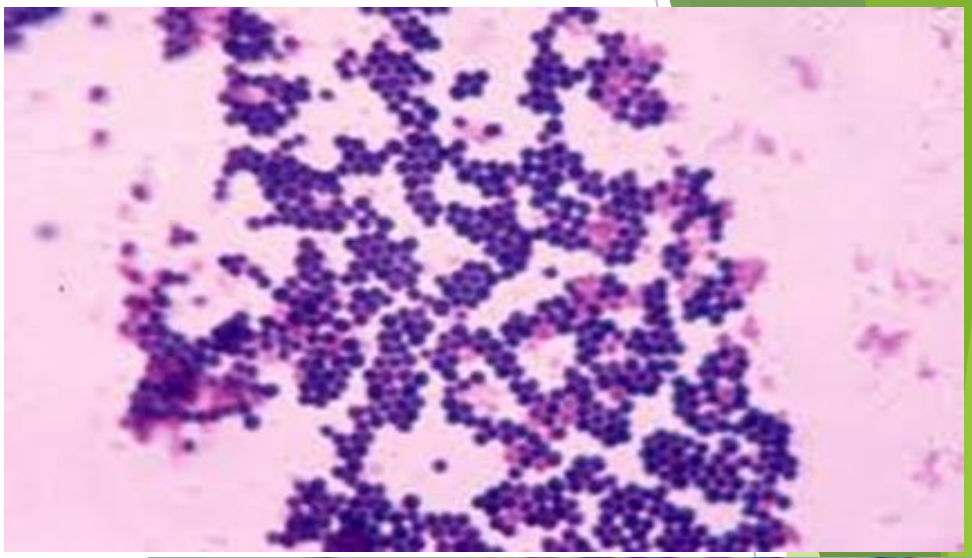


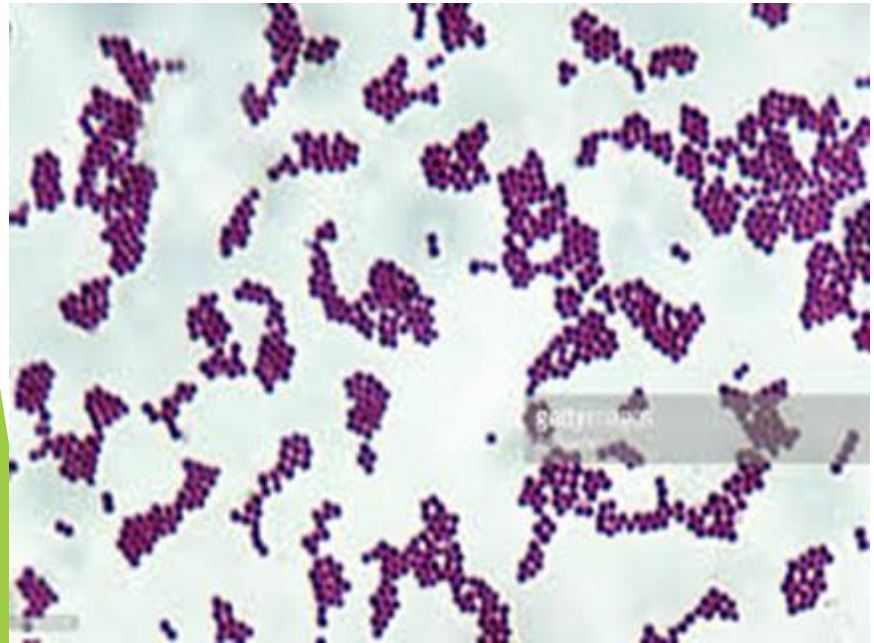


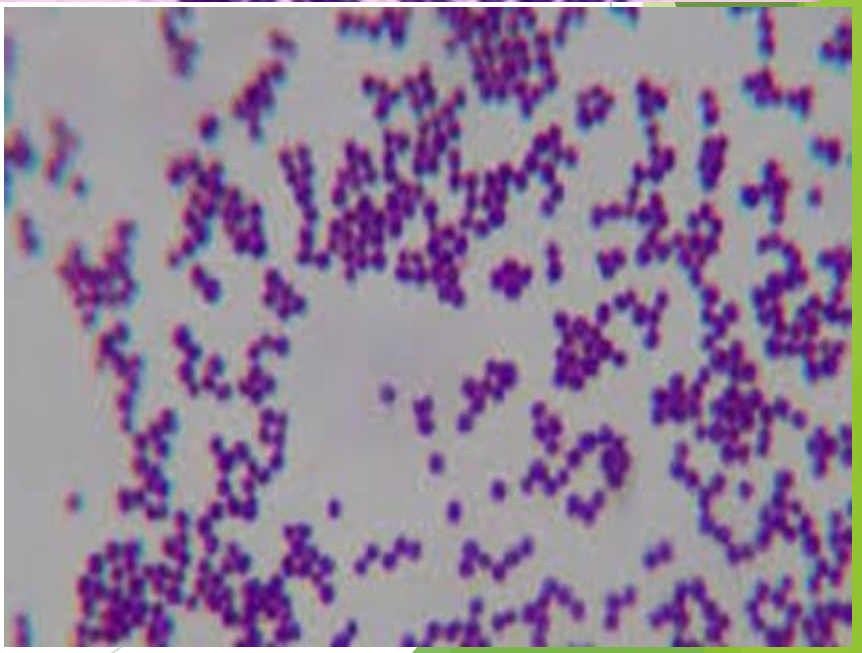


G+ cocci

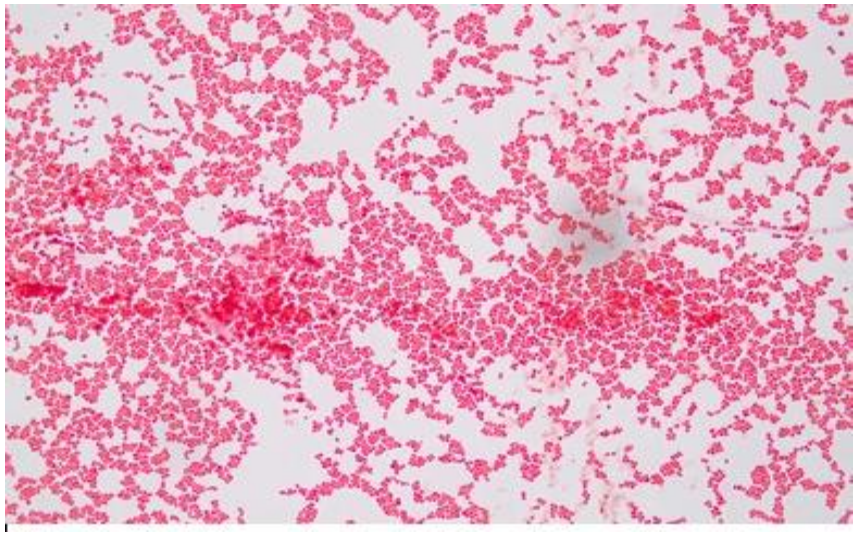


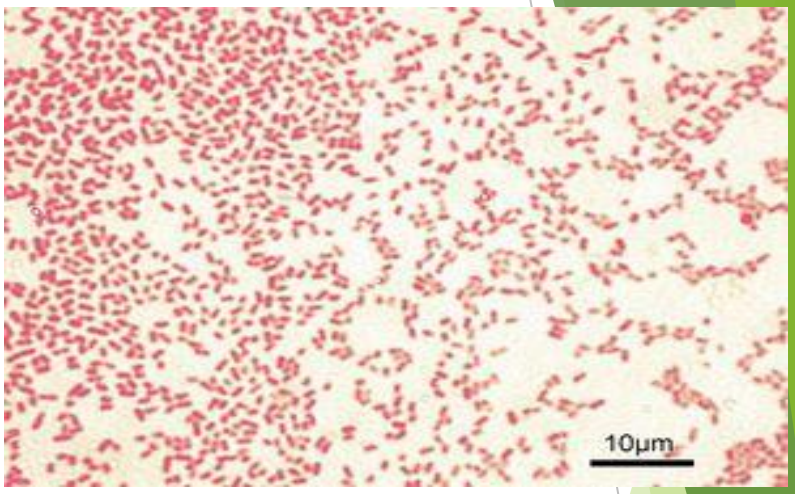


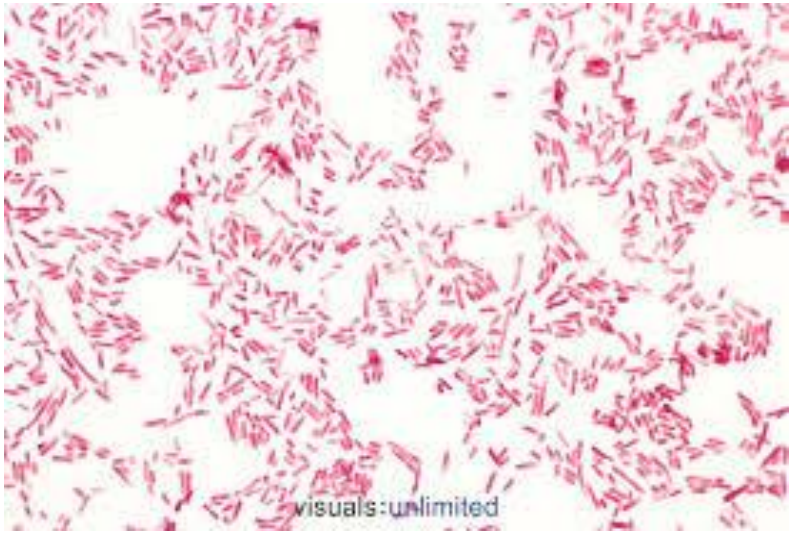


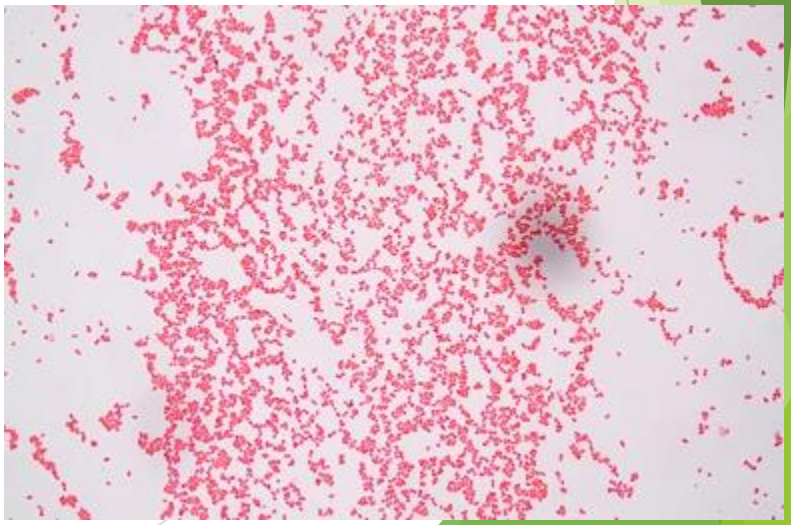


G- cocci







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