

LAB 7: Compound Staining “Gram Staining”.



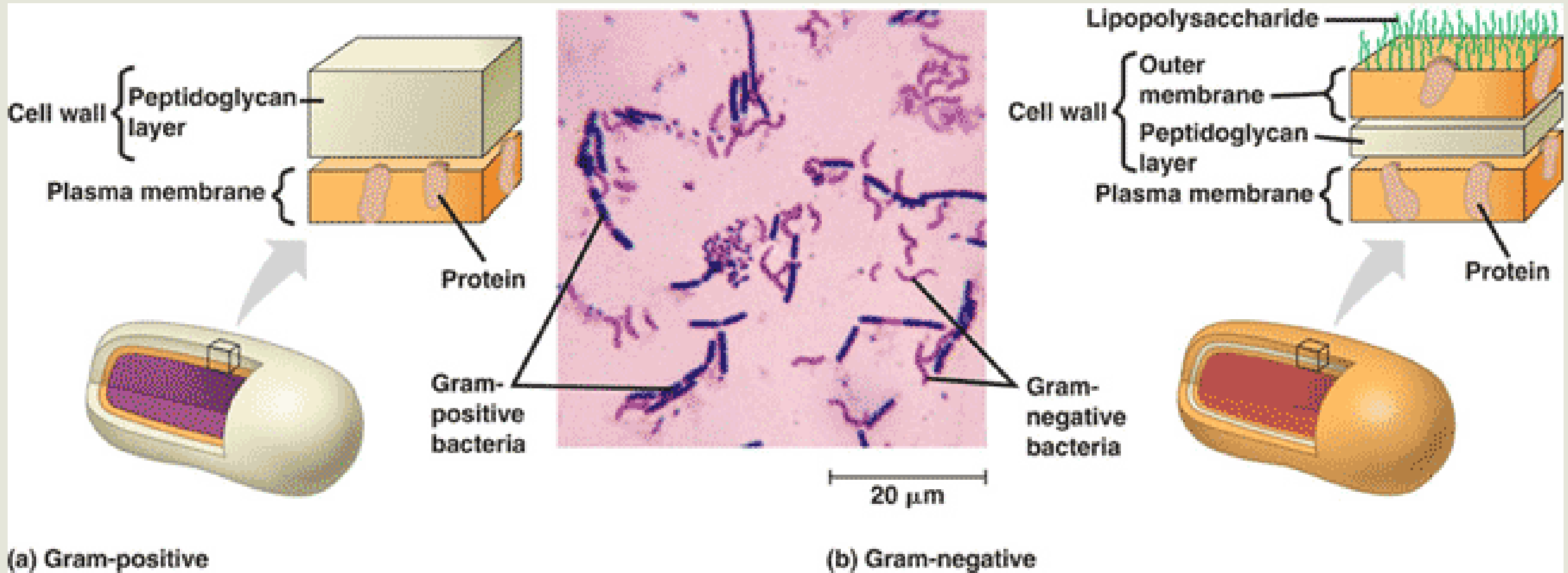
140 MBio
Practical

Aim:

It is called a differential stain because it stains bacterial cell walls differently based on the bacterial cell wall structure .

It is used to differentiate between **gram-positive** and **gram-negative** bacteria, which have distinct and consistent differences in their cell walls.

The Bacterial Cell Wall Structure



Hypothesis:

Gram-positive bacteria

Have a **thick peptidoglycan layer** surrounds the cell.

The stain gets trapped into this layer and the bacteria turned purple.

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 decolorization with Alcohol.

Therefore, cells will be counterstained with **safranin** and turned red.

Material:

Cultures of : Staphylococcus aureus,
Pseudomonas sp.
Bacillus subtilis,
E. coli

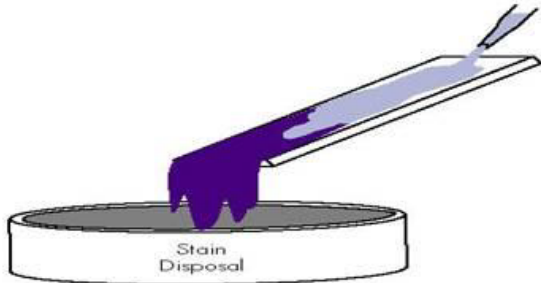
1. Basic Stain “Crystal violet” .
2. Mordant “Iodine solution” .
3. Decolorizer “Alcohol 95%” .
4. Counter stain “Safranin” .
5. Water .



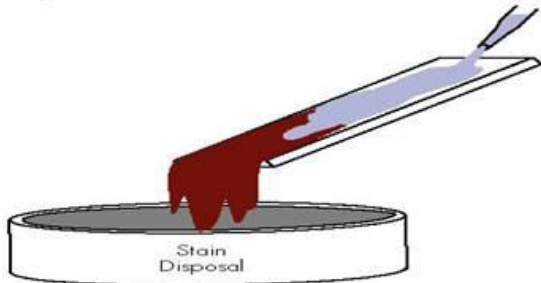
Method:



1. Begin with a heat-fixed emulsion.



3. Grasp the slide with a slide holder.
Gently rinse the slide with distilled water.



5. Grasp the slide with a slide holder.
Gently rinse the slide with distilled water.



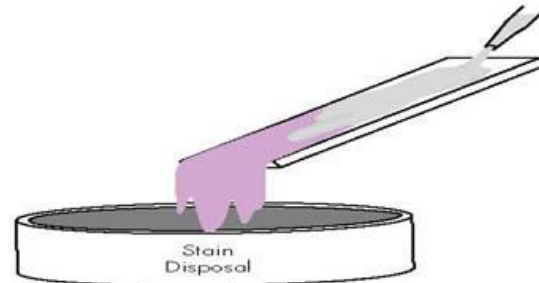
7. Counterstain with Safranin stain for 1 minute.
Rinse with water.



2. Cover the smear with Crystal Violet stain for 1 minute.
Use a staining tray to catch excess stain.



4. Cover the smear with Iodine stain for 1 minute.
Use a staining tray to catch excess stain.



6. Decolorize with 95% ethanol or ethanol/acetone
until the run-off is clear.
Gently rinse the slide with distilled water.



8. Gently blot dry in a tablet of bibulous paper.
Do not rub.
Observe under oil immersion.

Videos of gram staining Procedure

The principle:

Part 1-

<https://www.youtube.com/watch?v=NWASSXDzHRs>

Part 2-

<https://www.youtube.com/watch?v=Ly6j4pZFU3A>

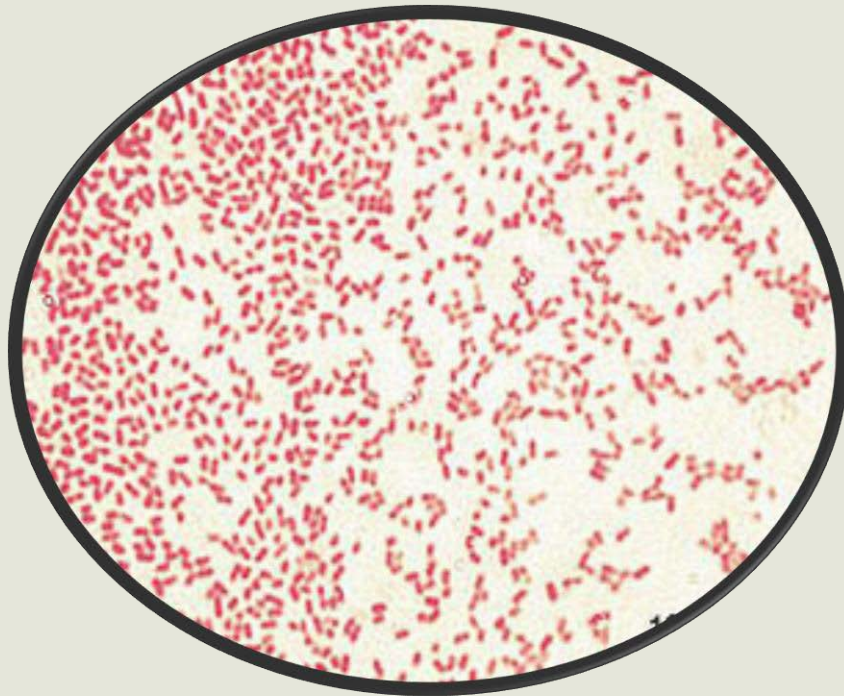
The method:

<https://www.youtube.com/watch?v=vlnIDGmgQfk>

<https://www.youtube.com/watch?v=c6d7zOIP6Vo>

Results:

The appearance of bacterial cells under light microscope.



Gram -ve



Gram +ve

Results:

Shape: A- Cocci, B- Bacilli

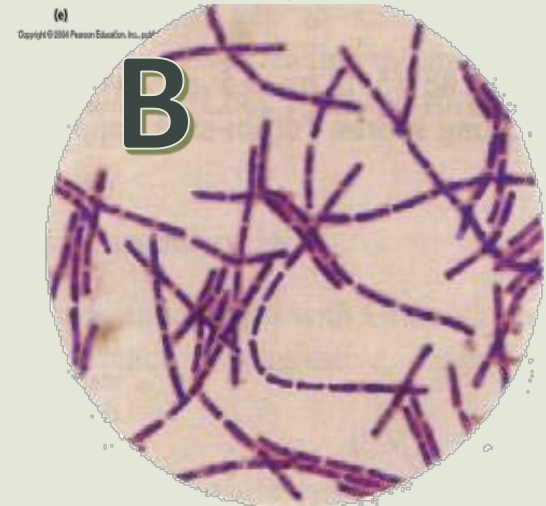
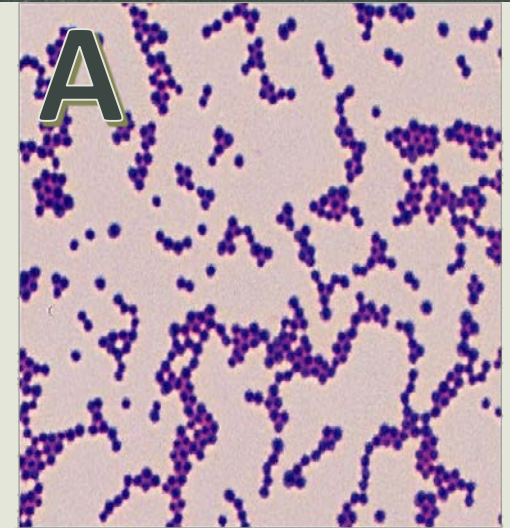
Arrangement: A- irregular clusters, B- chain

Colour: Violet

Gram's reaction: Gram's +ve

Name of microorganism:

A- Staphylococcus, B- Bacillus



Results:

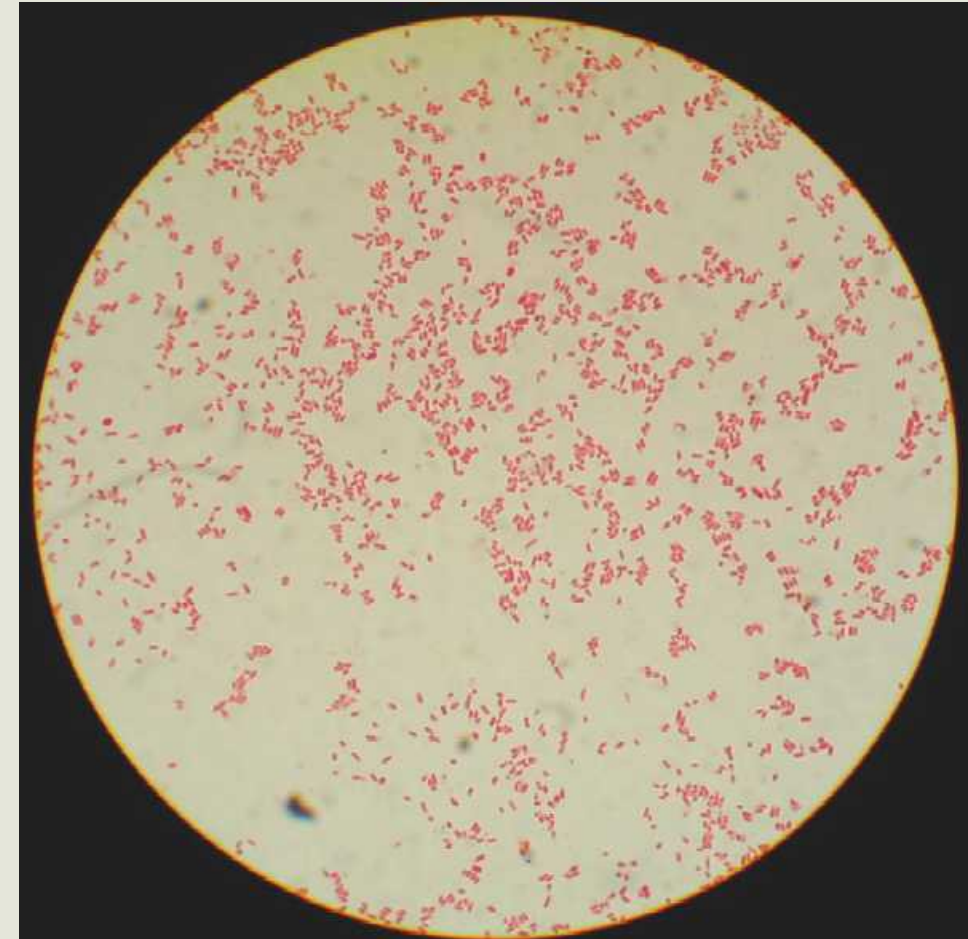
Shape: **Bacilli** (short rods)

Arrangement: **Mono-**

Color: **Red-pink**

Gram's reaction: **Gram's -ve**

Name of microorganism: **E. coli**



The background is a dark grey chalkboard with various white chalk sketches. On the left, there is a globe on a stand. Above it are some circular diagrams and a ruler. In the center, there are several books stacked. On the right, there is a microscope and some other scientific or technical drawings.

Thank you for listening!

ahamdan1@ksu.edu.sa