

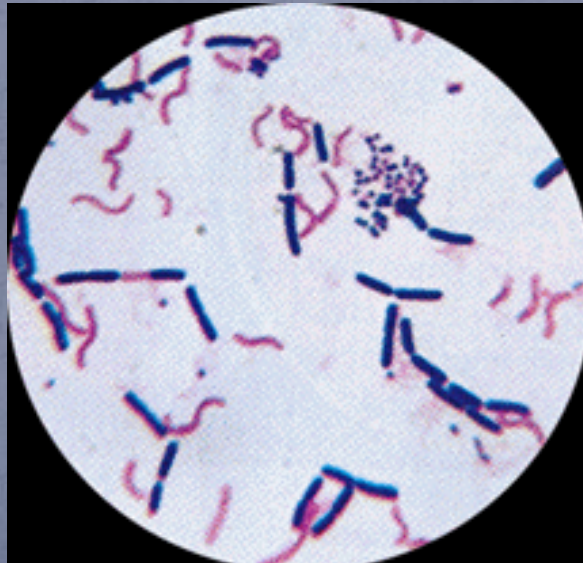
بسم الله الرحمن الرحيم

140 Micro

Lab 7: Gram stain

Gram stain

((صبغة جرام))



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

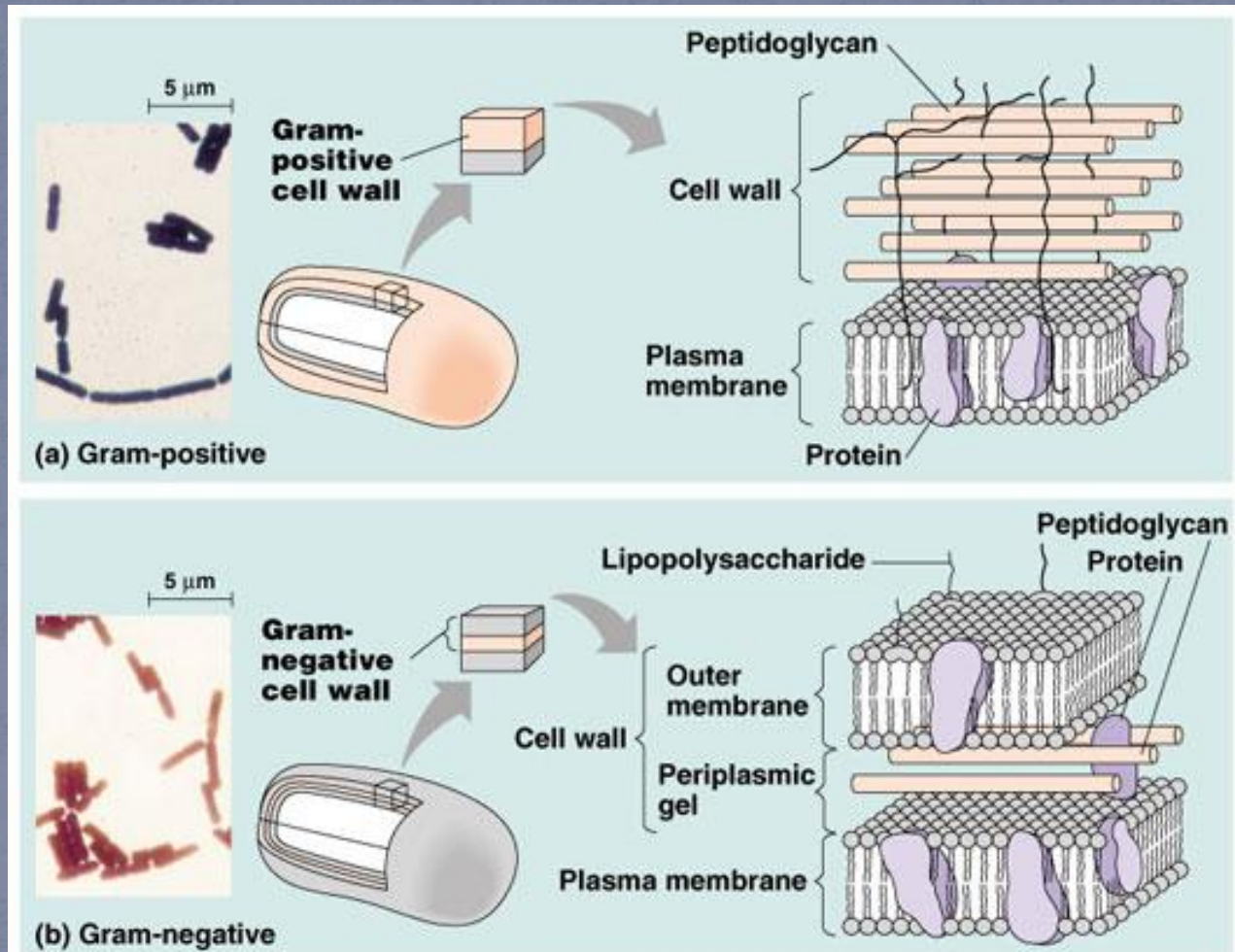
The gram stain is called a

differential stain

because it stain cell differently

based on their cell wall structure .

The cell wall structure



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.

The material :

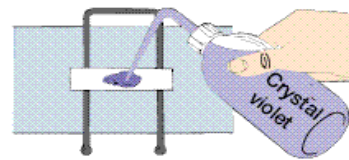
Cultures of : *Staphylococcus aureus*,
Bacillus subtilis,
E.coli

1. Crystal violet .
2. Iodine solution .
3. Alcohol 95% .
4. Safranin .
5. Water .

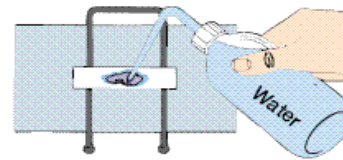


The method

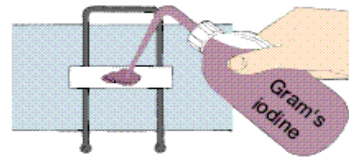
Figure 8.3 Gram-stain Procedure.



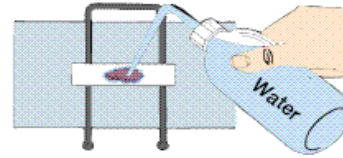
(a) Crystal violet; 30 seconds



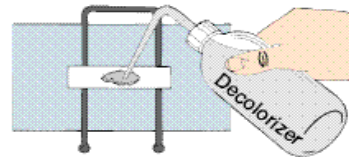
(b) Rinse for 5 seconds



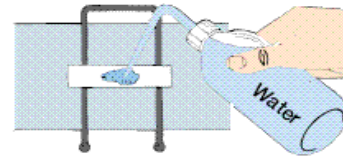
(c) Cover with Gram's iodine
for 1 minute



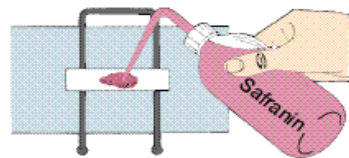
(d) Rinse with water for
5 seconds



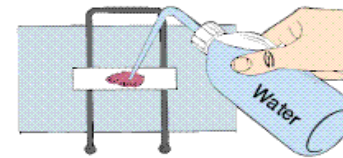
(e) Decolorize for 15–30
seconds



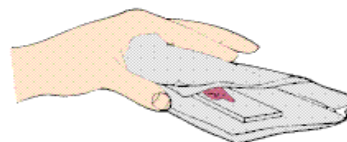
(f) Rinse with water for
5 seconds



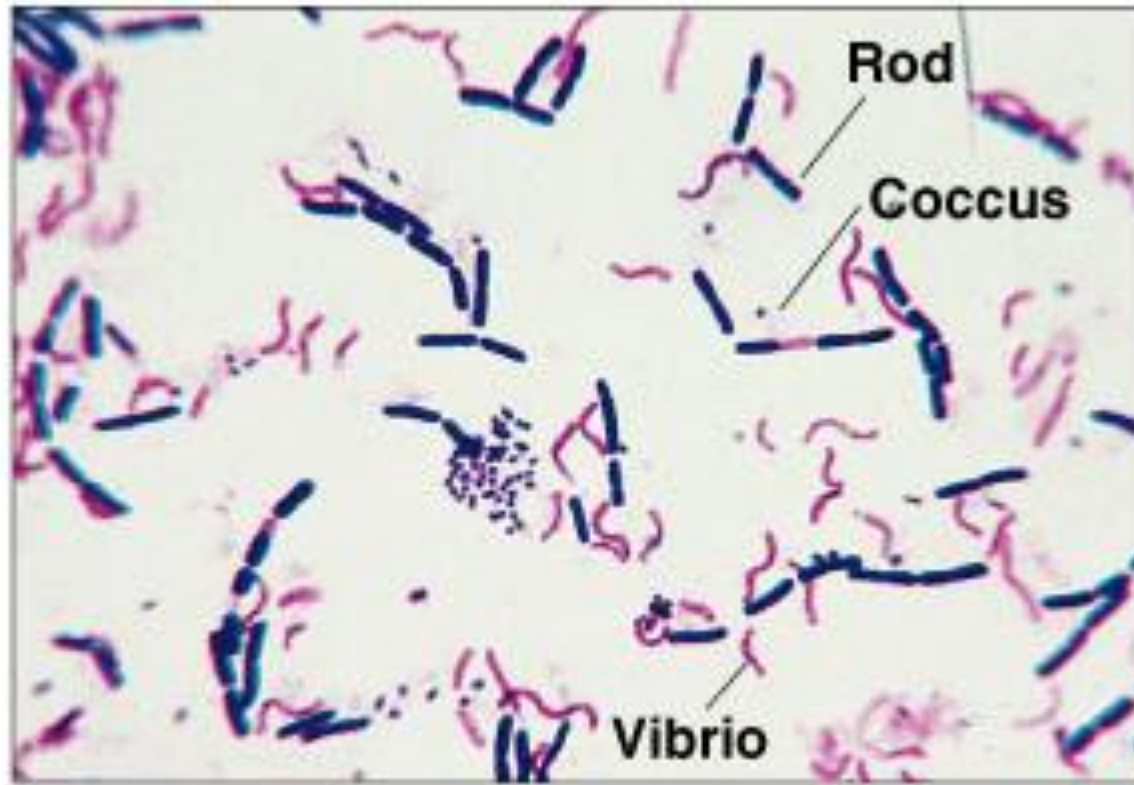
(g) Counterstain with safranin
for about 60–80 seconds



(h) Rinse for 5 seconds

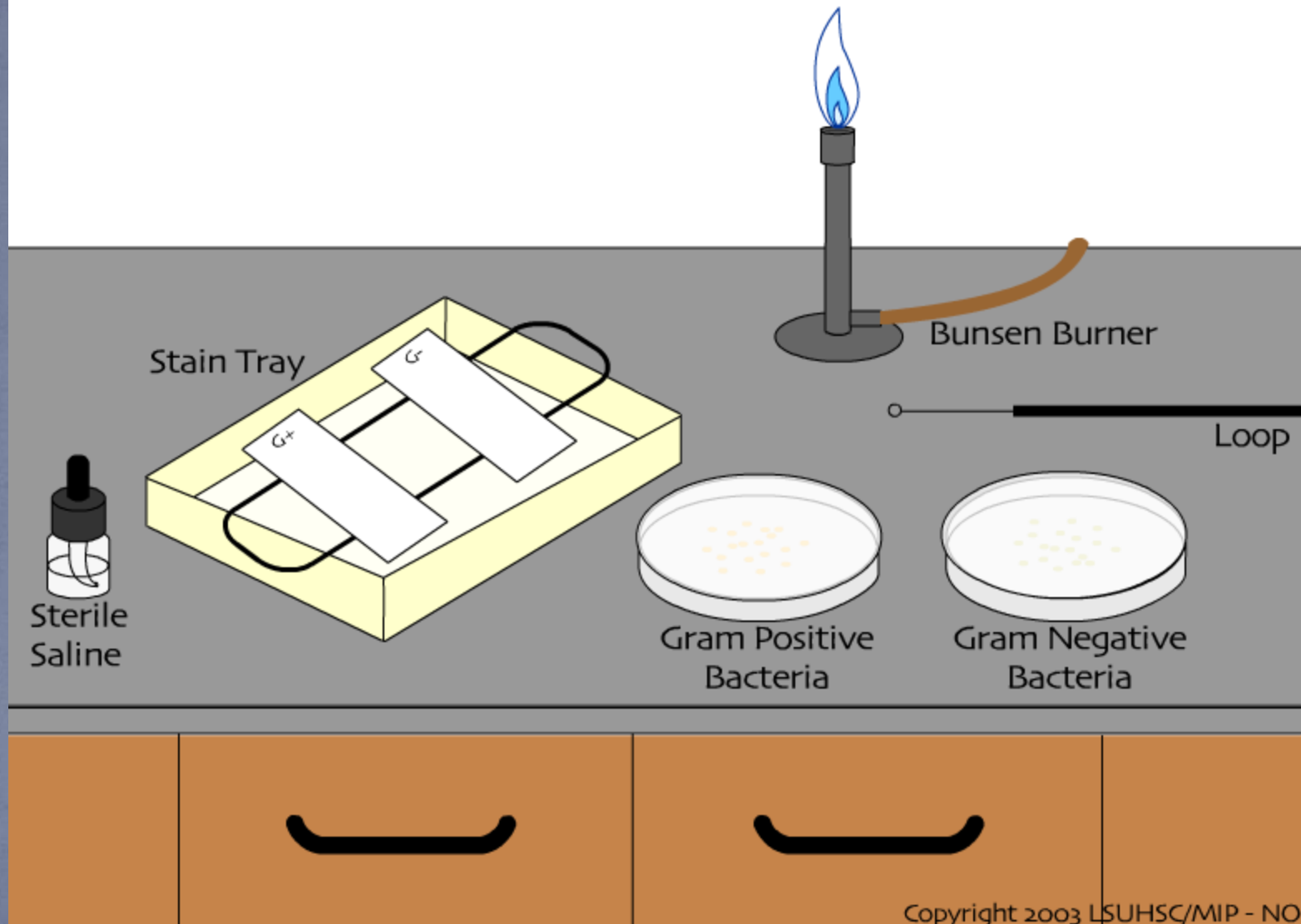


(i) Blot dry with bibulous paper



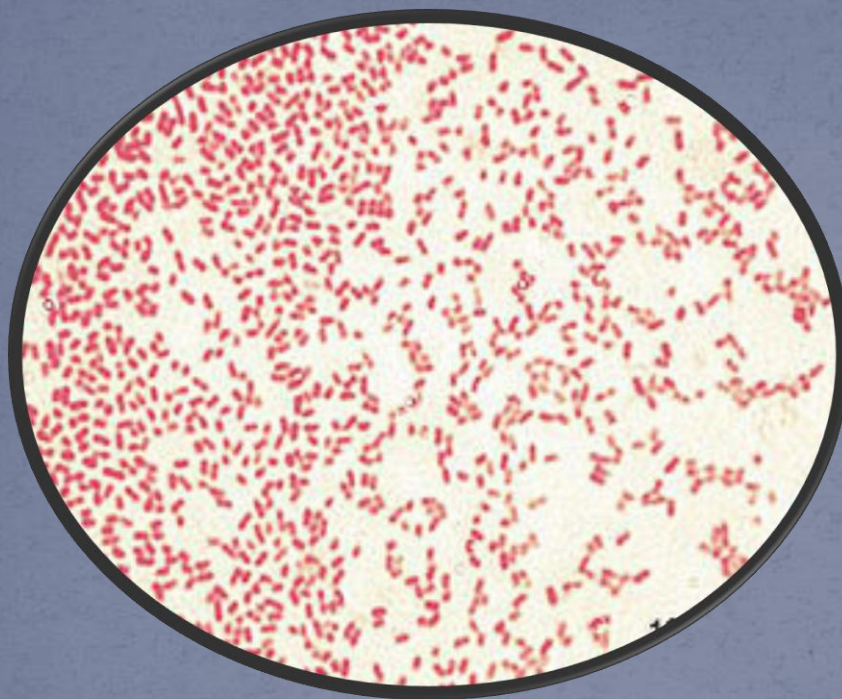
Making a Gram Stain

Start 

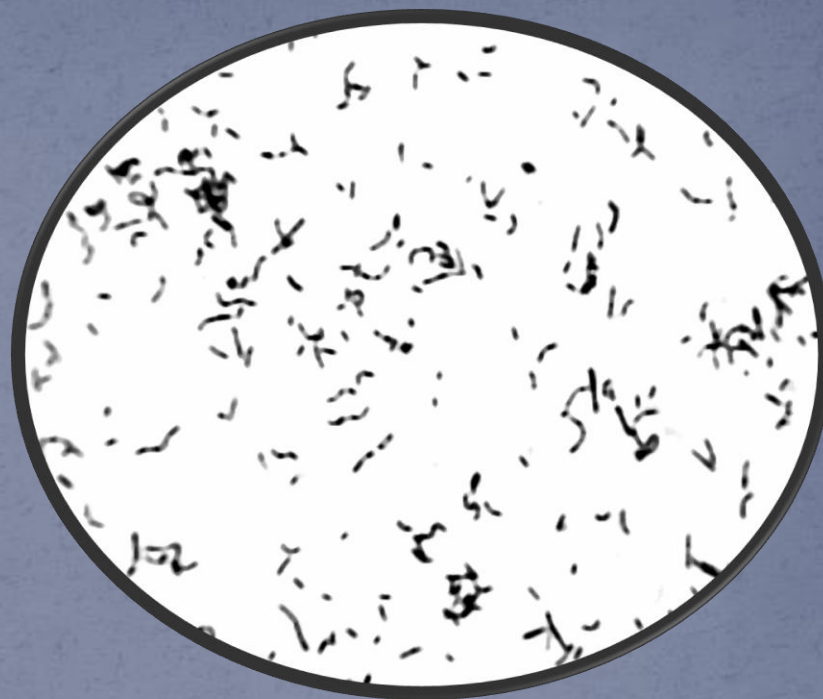


The bacteria under the microscope





Gram -ve



Gram +ve

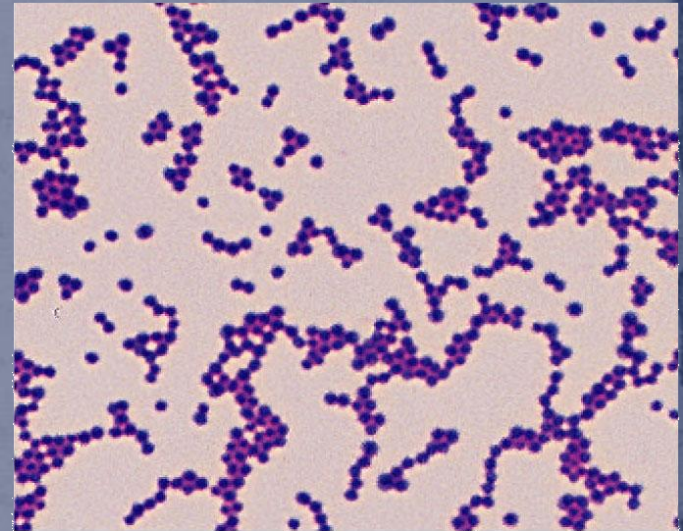
Results:

Shape: **Cocci**

Arrangement: **irregular clusters**

Colour: **Violet**

Gram's reaction: **Gram's +ve**



Name of microorganism: **Staphylococci**

Results:

Shape: **Bacilli**

Arrangement: **Chains**

Colour: **Violet**

Gram's reaction: **Gram's +ve**

Name of microorganism: **Bacillus**



Thank you

أشروق الشهراني