

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

**140 Micro**

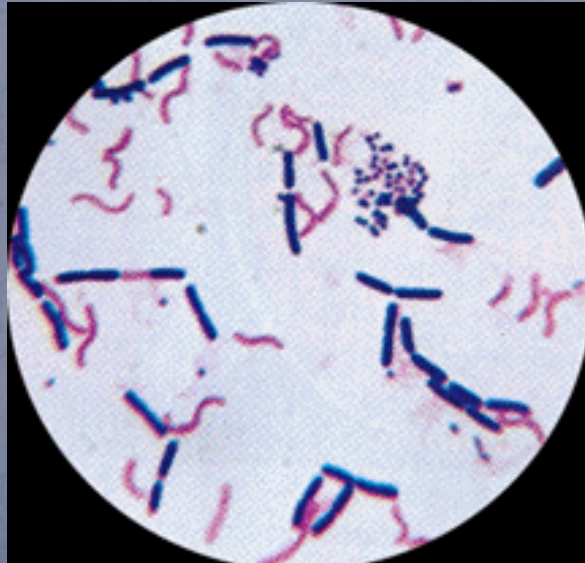
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**Lab 7: Gram stain**

# Gram stain

«صبغ جرام»

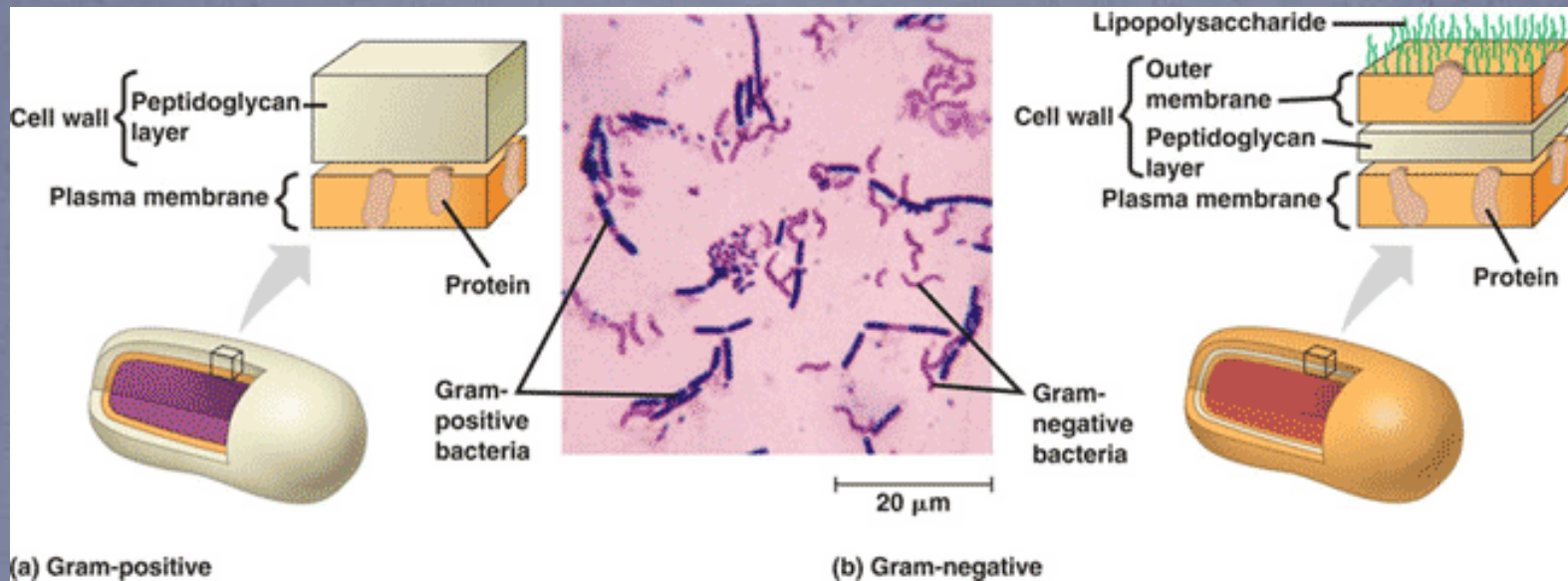
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It is used to differentiate between **gram-positive** and **gram-negative** bacteria, which have distinct and consistent differences in their cell walls.

The gram stain is called a  
**differential stain because**  
**it stains cell differently**  
**based on the bacterial 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*,  
*Pseudomonas sp.*  
*Bacillus subtilis*,  
*E.coli*

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



# The method

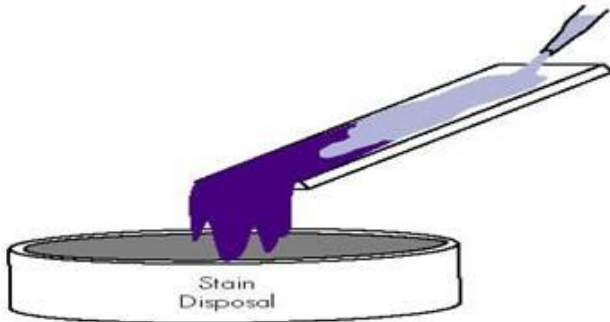




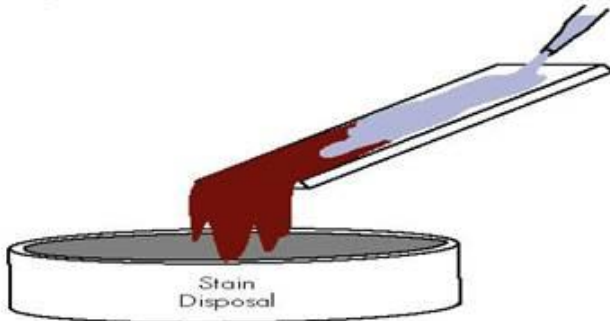
1. Begin with a heat-fixed emulsion.



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



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



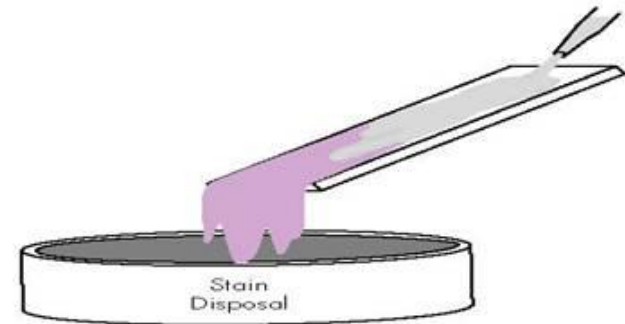
5. Grasp the slide with a slide holder.  
Gently rinse the slide with distilled water.



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



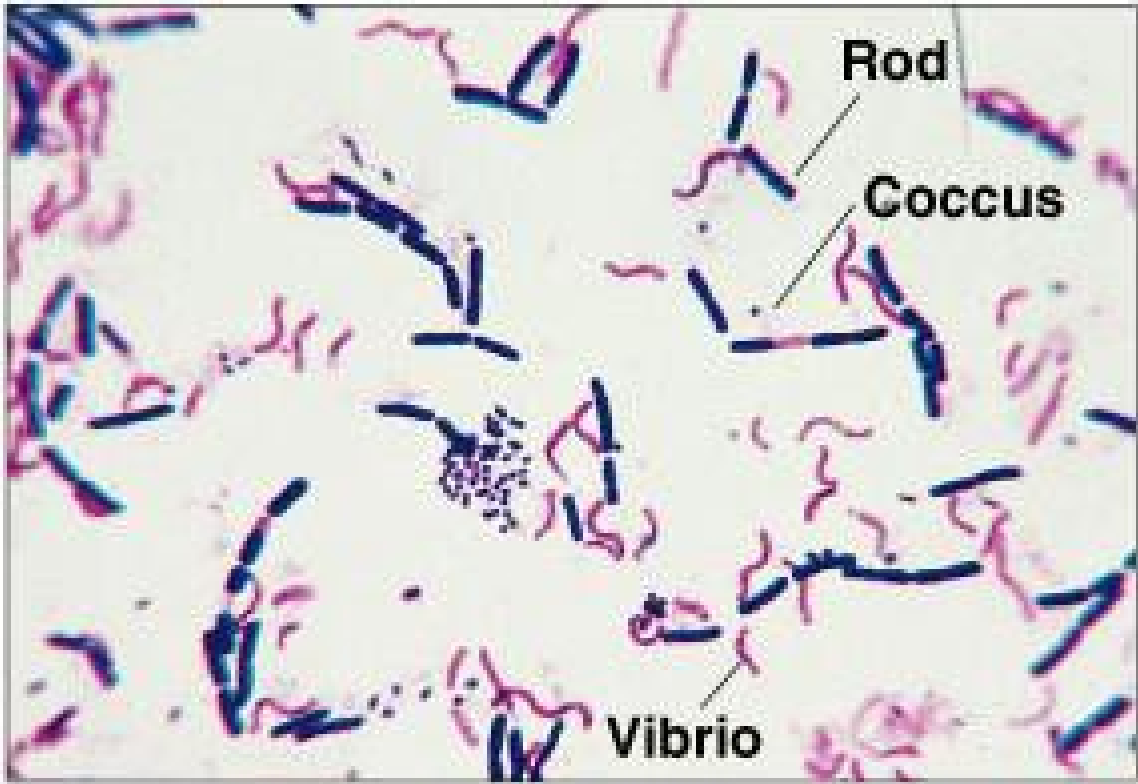
4. Cover the smear with Iodine stain for 1 minute.  
Use a staining tray to catch excess stain.



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.



# Vedios

The principle:

Part 1- <https://www.youtube.com/watch?v=NWASSXDzHRs>

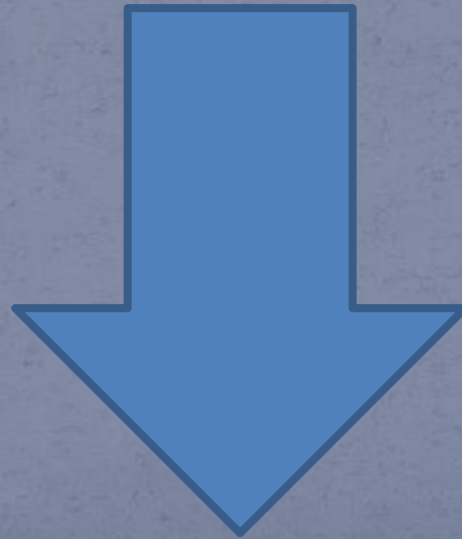
Part 2- <https://www.youtube.com/watch?v=Ly6j4pZFU3A>

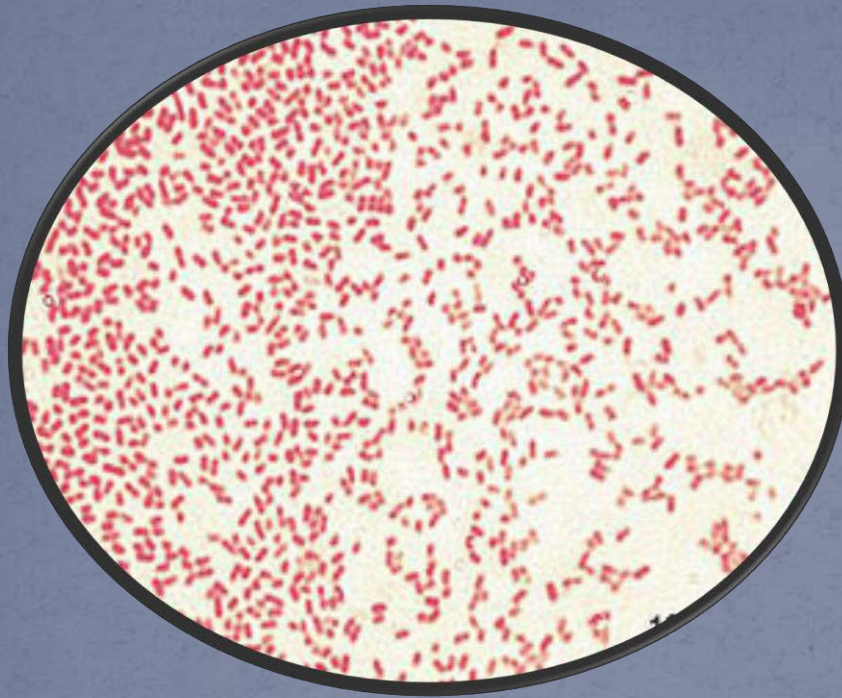
The method:

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

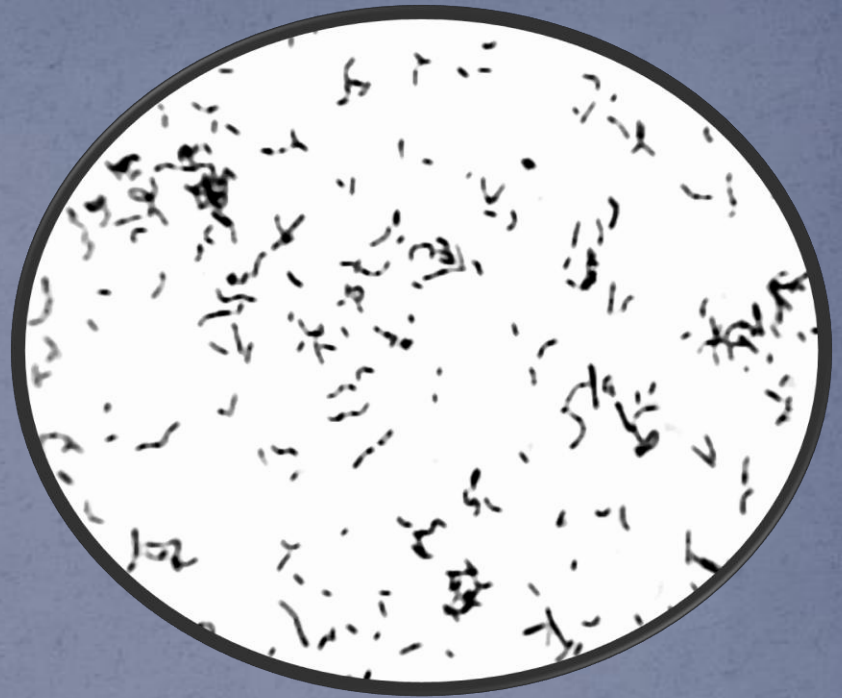
<https://www.youtube.com/watch?v=c6d7zOIP6Vo>

# The bacteria under the microscope





**Gram -ve**



**Gram +ve**

# Results:

Shape: **Cocci**

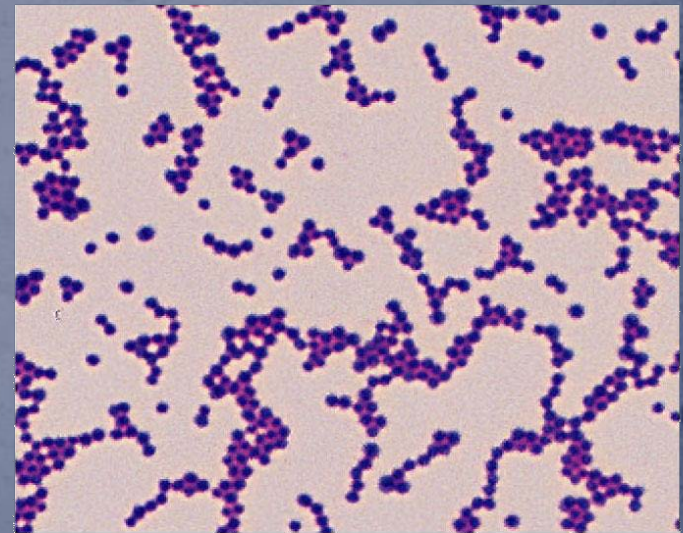
Arrangement: **irregular clusters**

Colour: **Violet**

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

Name of microorganism:

**Staphylococcus**



(e)

# Results:

Shape: **Bacilli**

Arrangement: **Chains**

Colour: **Violet**

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



Name of microorganism: **Bacillus**

Thank you

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