

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

140 Micro

Lab 7: Gram stain

Gram stain

((صبغة جرام))



The three common shapes of bacteria:

1-Coccus

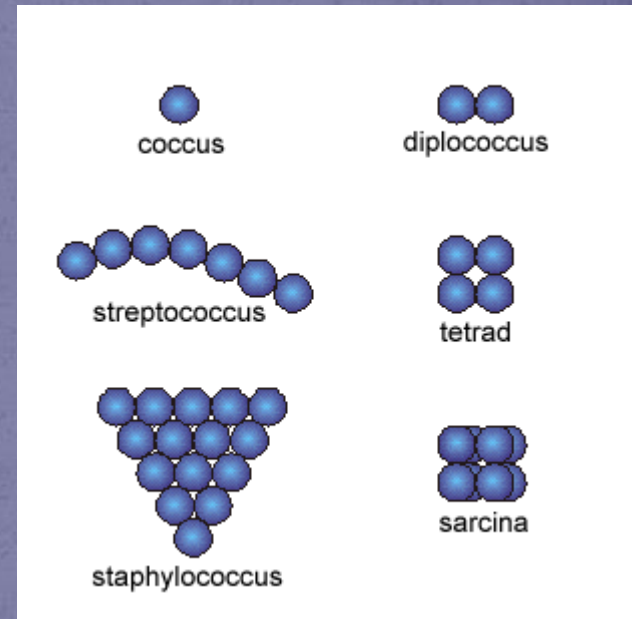
2- Bacillus

3- Spiral

1-Coccus

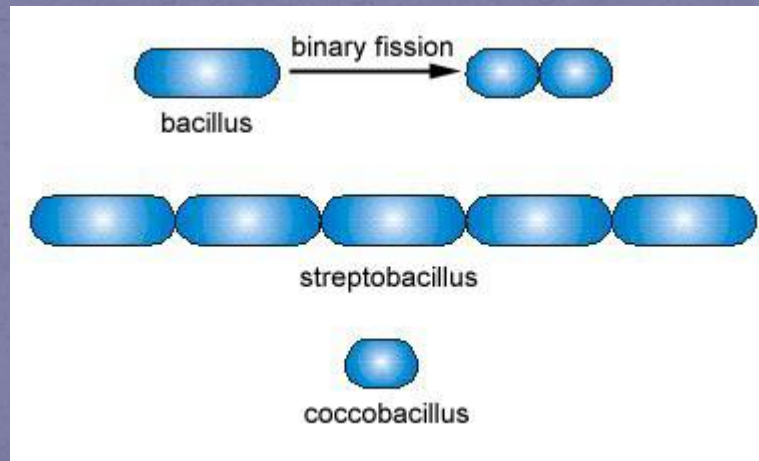
having one of the following arrangements:

- Diplococcus: a pair of cocci
- Streptococcus: a chain of cocci
- Tetrad: a square of 4 cocci
- Sarcina: a cube of 8 cocci
- Staphylococcus: cocci in irregular, often grape-like clusters



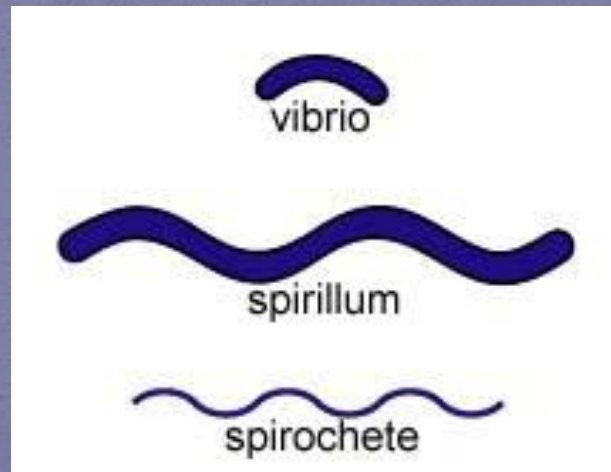
2- Bacillus

- Bacillus: a single bacillus
- Streptobacillus: bacilli in chains
- Coccobacillus: oval and similar to a coccus



3- Spiral

- Vibrio: an incomplete spiral or comma-shaped
- Spirillum: a thick, rigid spiral
- Spirochete: a thin, flexible spiral



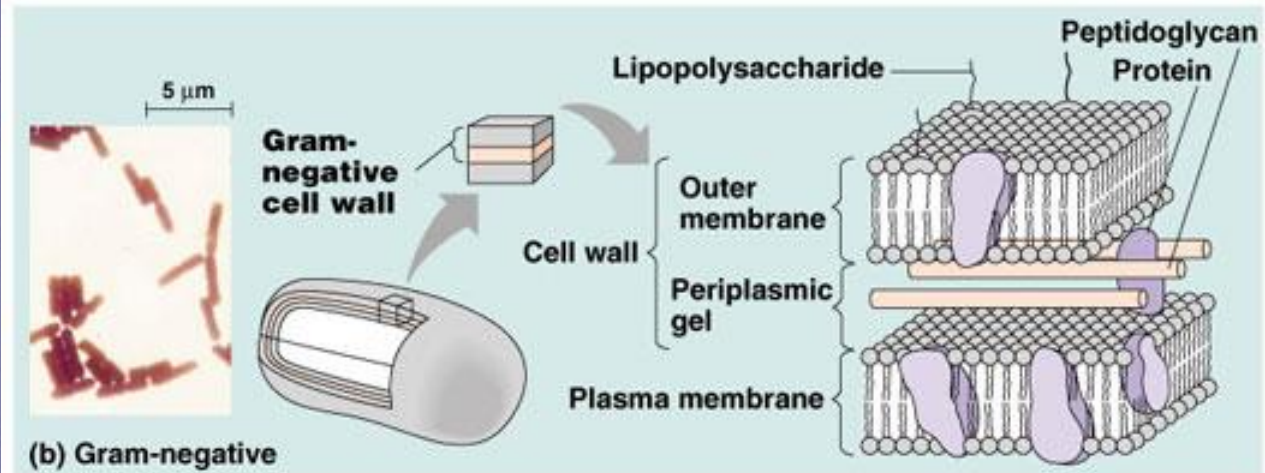
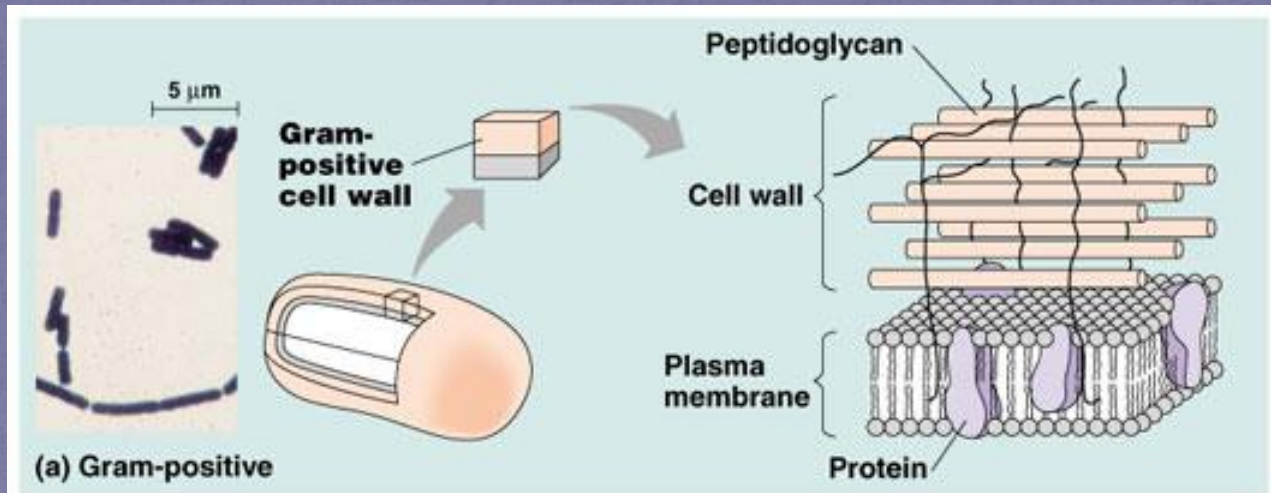
Gram stain

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

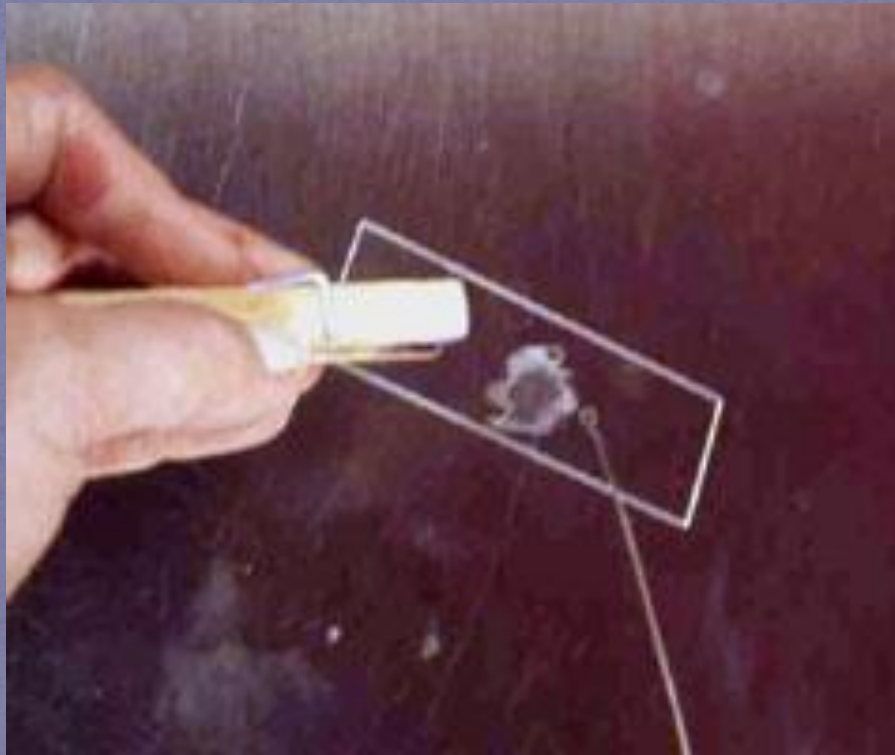
The method



1. Prepare the smear.

- place a small drop of water on a clean slide. Drag the sterile inoculating needle tip through the edge of colony.
- Gently spread the mixture into a circle to spread out.

2. Let the smear air dry completely.





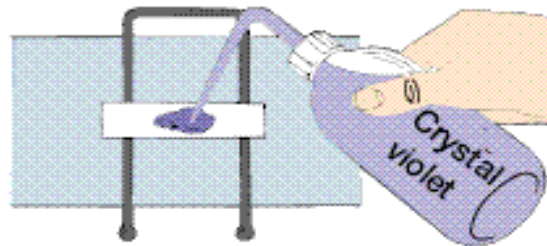
3. Heat-Fix the smear.

- Smears are heat-fixed by quickly passing the slide through a flame two or three times.
- **This causes** the microbes to **stick** to the slide and not get washed off during the staining process.

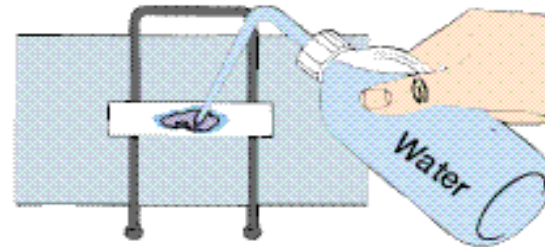
The steps of Gram stain :

Figure 8.3 Gram-stain Procedure.

1-

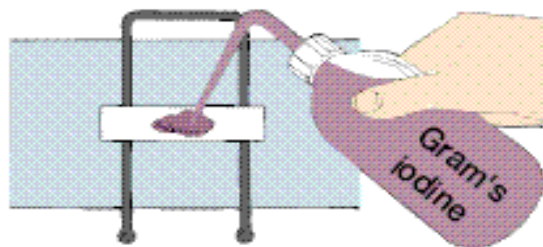


(a) Crystal violet; 30 seconds

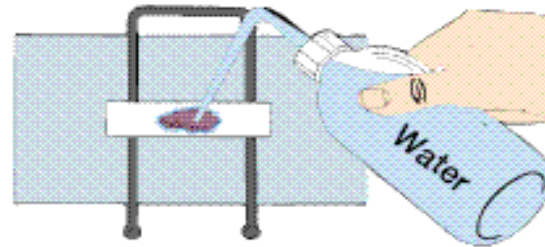


(b) Rinse for 5 seconds

2-

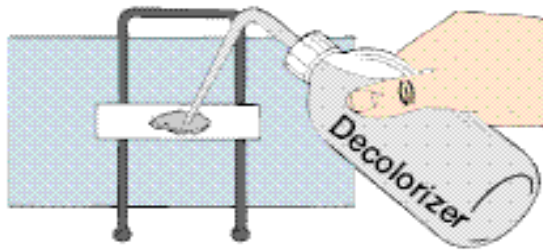


(c) Cover with Gram's iodine for 1 minute

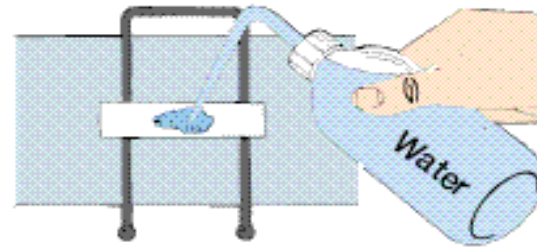


(d) Rinse with water for 5 seconds

3-

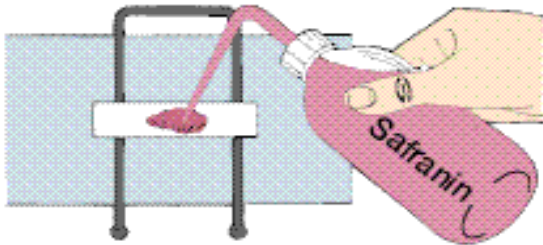


(e) Decolorize for 15–30 seconds

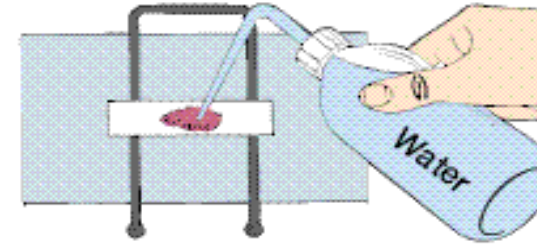


(f) Rinse with water for 5 seconds

4-

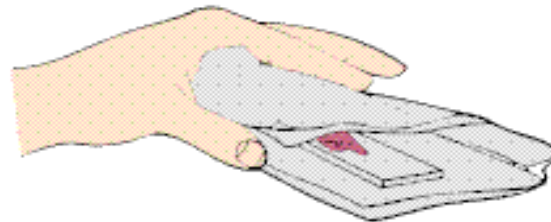


(g) Counterstain with safranin for about 60–80 seconds



(h) Rinse for 5 seconds

5-

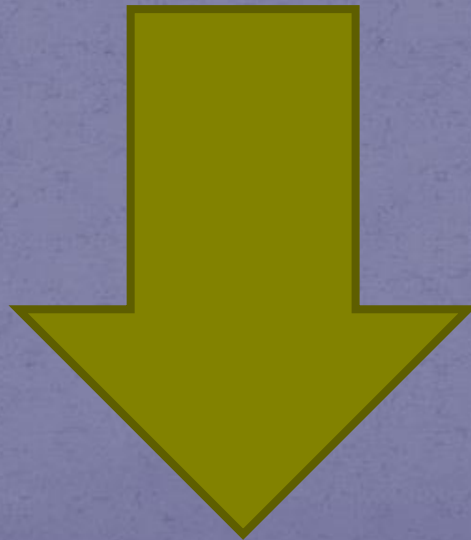


(i) Blot dry with bibulous paper

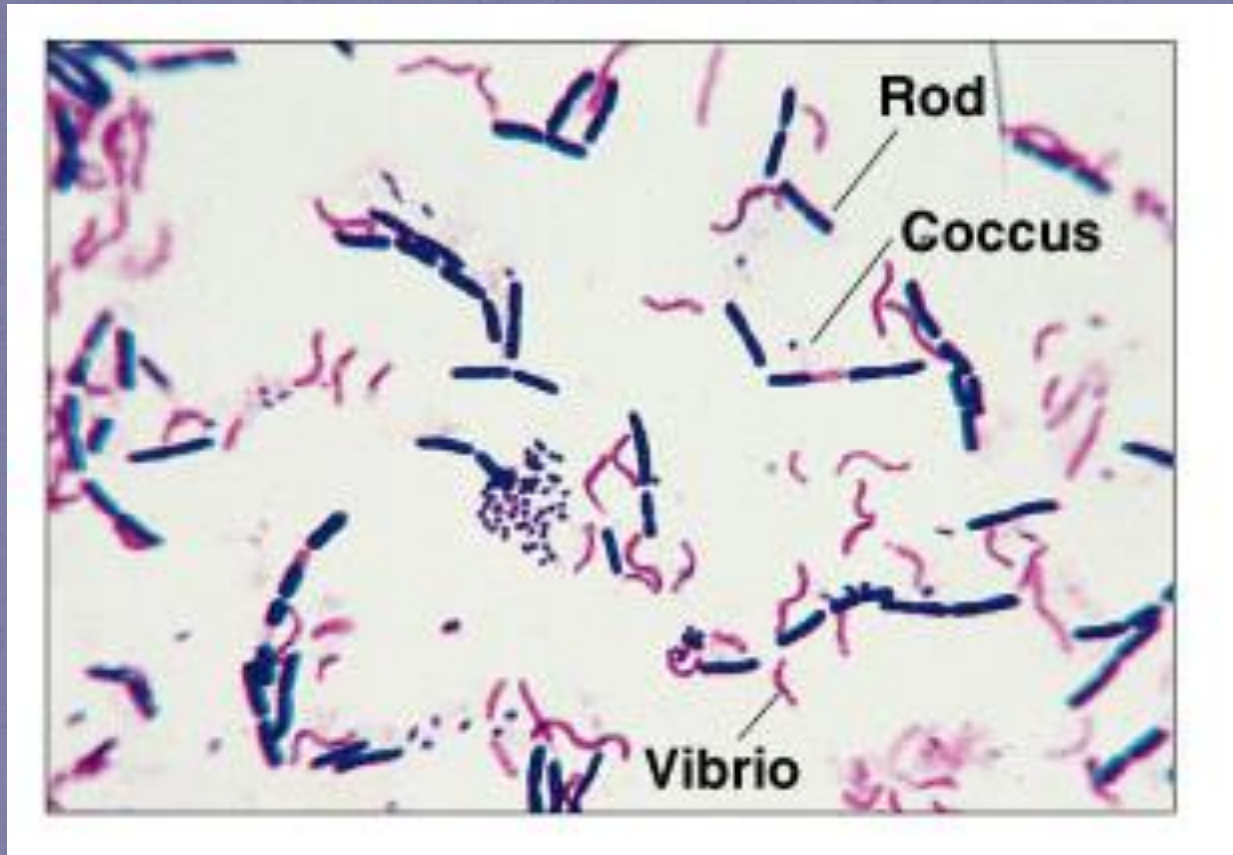
Then, place a drop of oil directly on the stained smear .Turn the oil lens into position and fine focus to observe the cells.

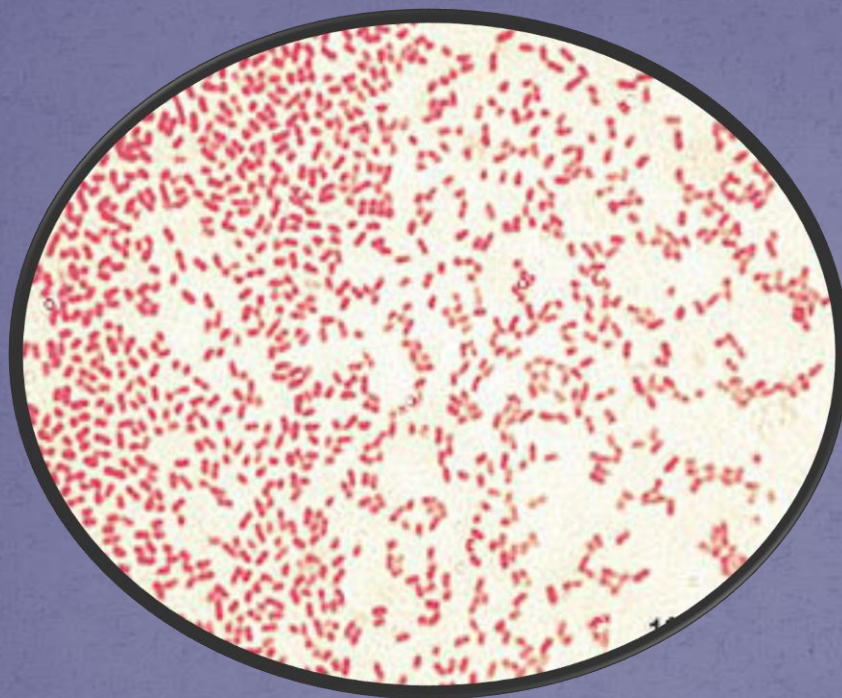


The bacteria under the microscope

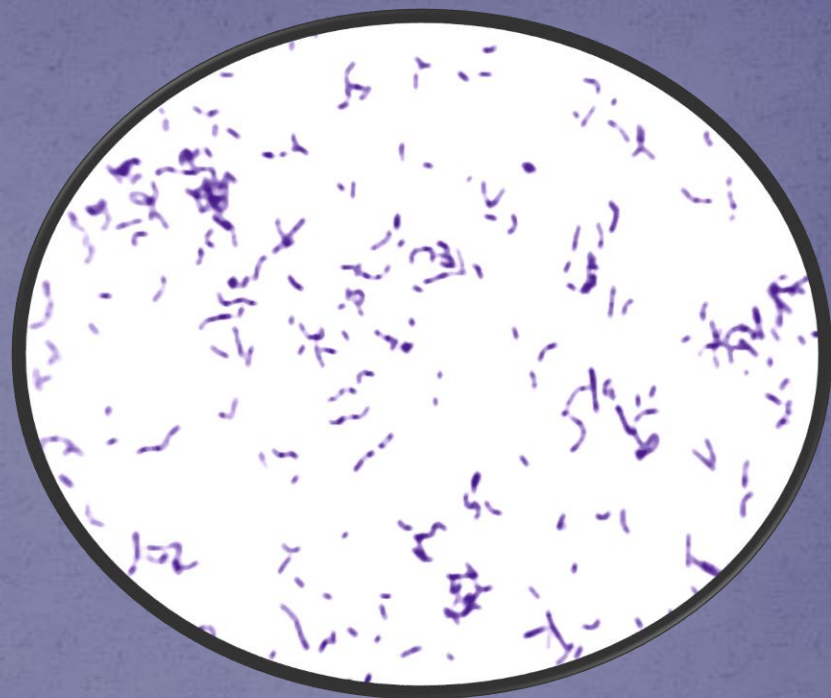


Result





Gram -ve



Gram

+ve

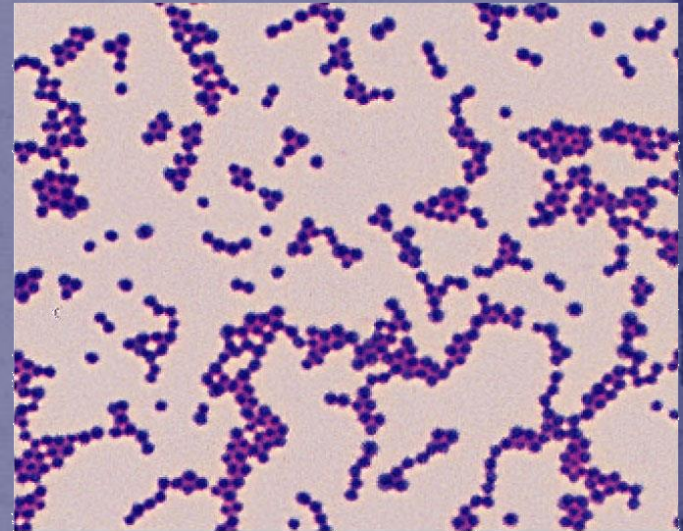
Results:

Shape: **Cocci**

Arrangement: **irregular clusters**

Colour: **Violet**

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



Name of microorganism: **Staphylococci**

Results:

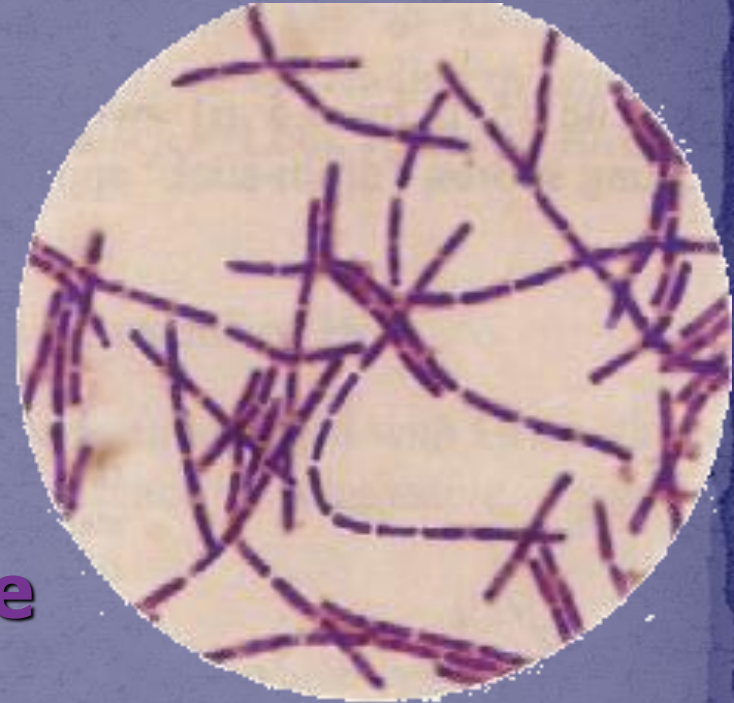
Shape: **Bacilli**

Arrangement: **Chains**

Colour: **Violet**

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

Name of microorganism: **Bacillus**



Thank you

بالتوفيق....