

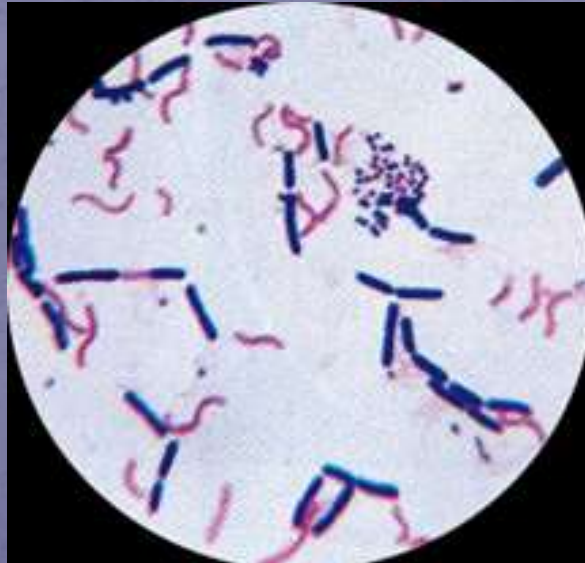
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140 Micro

Lab 7: Gram Stain

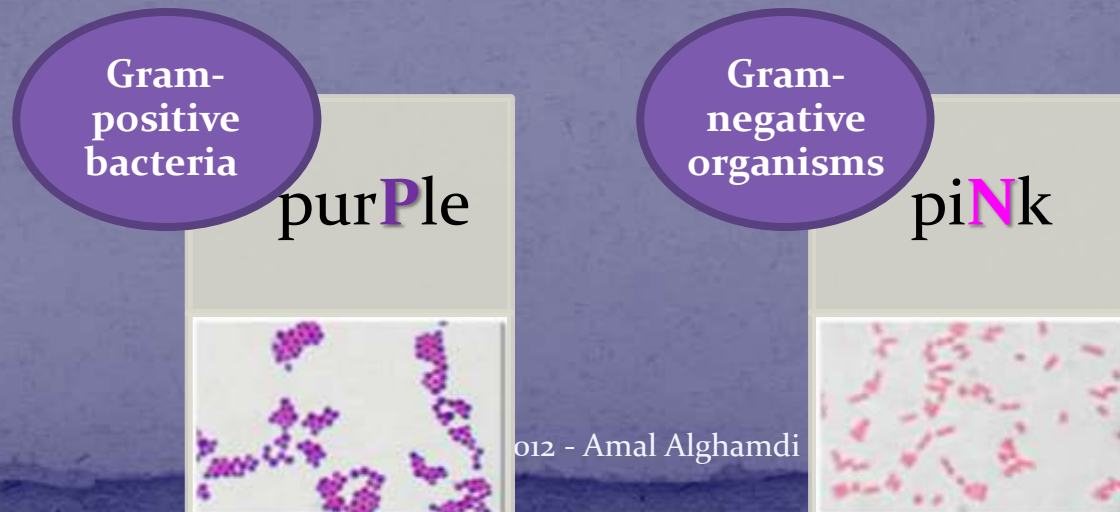
Gram stain

((صبغة جرام))



History

- Hans Christian Gram, circa 1884, was studying the etiology of respiratory disease.
- Gram's staining procedures are done millions of times daily worldwide.
- Gram's procedure divides the bacterial organisms:



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

Gram Stain as differential stain

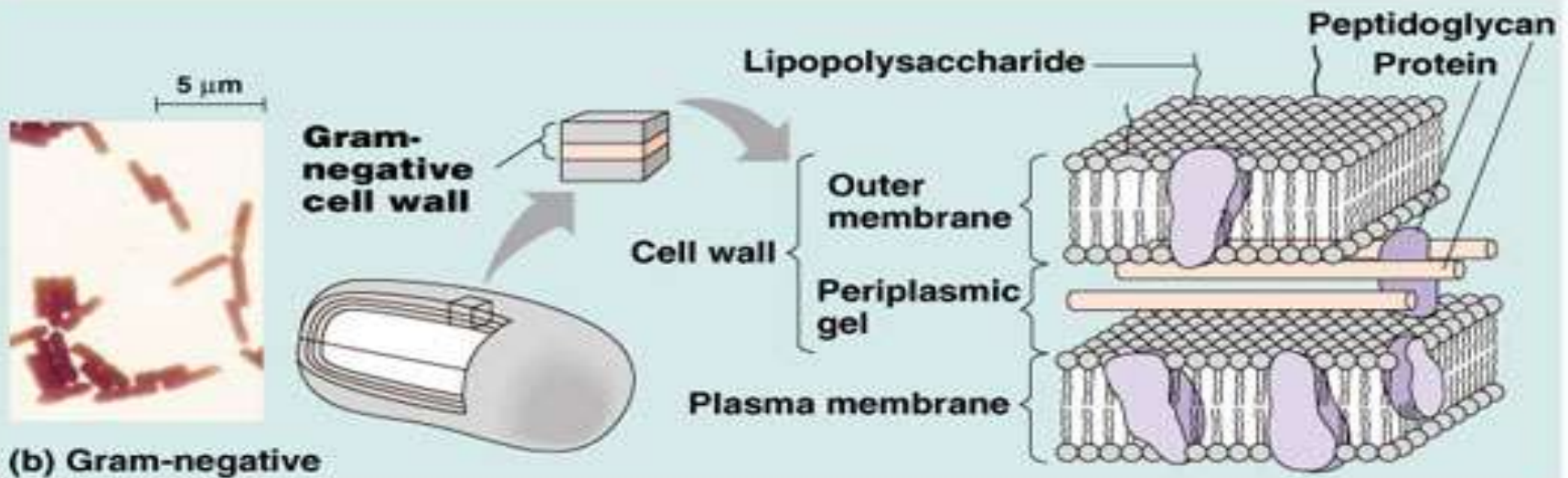
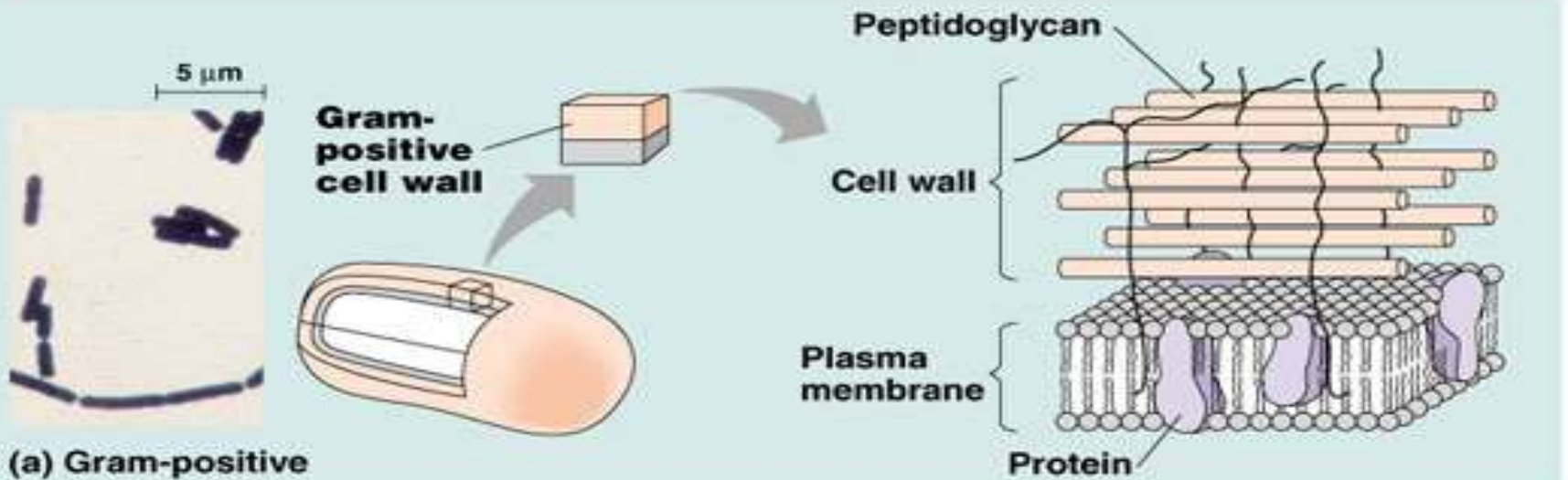
- Gram staining is normally the first step towards identifying an unknown pathogenic agent.
- **Common diseases caused by gram-positive bacteria are: wound infections, boils, diphtheria, septic sore throat, gas gangrene, scarlet fever, some pneumonias.**
- **Common diseases caused by gram-negative bacteria are: Typhoid (negative = piNk), bubonic plague, dysentery**

The gram stain is called a
differential stain

**because it stain cell differently
based on their cell wall structure .**

- A differential technique is a process that distinguishes between a variety of microbial organisms based on.
- The Gram staining technique depends upon:
 - 1-the ability of their cell wall to hold certain dyesto
 - 2-And to resist decolorization.

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.

Bacterial Cell Wall

Cell Wall Structure and Other Factors Affecting Gram Stain Results:

Gram-positive bacteria's cell walls have a distinctly different structure than that of the gram-negative bacteria cell. The gram-positive cell wall has a multitude of layers of peptidoglycan (up to 40) which resists decolorization better than the thin (often only 2 layers) gram-negative cell wall. The gram-negative cell wall also contains lipoprotein and lipopolysaccharide that can be verified through chemical analysis. The two other groupings that should be noted at this time are gram-variable and gram-non-reactive.

Other factors that can affect the gram staining procedure include the following:

- ❖ Using cells (from an old culture) that cannot resist decolorization.
- ❖ Intrusion of stain crystals into smear or clumping of stain and bacteria.
- ❖ Not allowing enough time for each stain to sit or allowing too much time for decolorizer and/or water to sit on slide.
- ❖ Using old stain reagents.
- ❖ Using thick vs. thin smears. Thin is normally much better.
- ❖ Overheating the cells during fixation.

The material :

- 1- Fresh (24hrs) Cultures of :
Staphylococcus aureus,
Bacillus subtilis,
Escherichia coli
- 2- Microscopic Slides
- 3- Water
- 4- marker



Gram's crystal violet →

[primary stain]

Gram's iodine →

[mordant - makes 1_ stain fix to cell wall]
substance that increases the reaction between the stain and the cells.

Decolorizer →
95% ethyl alcohol)

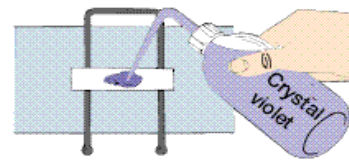
[washes stain out of cell walls with high lipid content]

Gram's safranin →

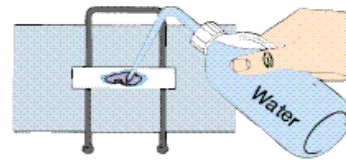
[counterstain]

The method

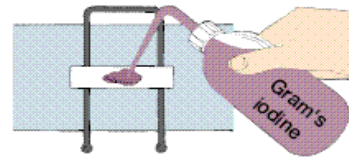
Figure 8.3 Gram-stain Procedure.



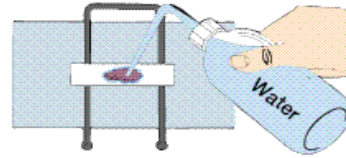
(a) Crystal violet; 30 seconds



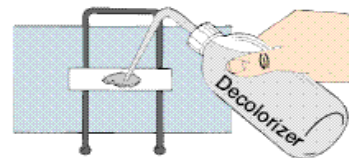
(b) Rinse for 5 seconds



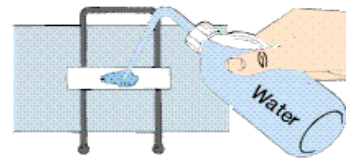
(c) Cover with Gram's iodine for 1 minute



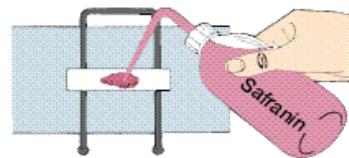
(d) Rinse with water for 5 seconds



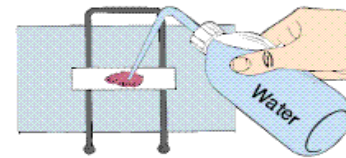
(e) Decolorize for 15–30 seconds



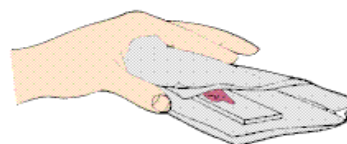
(f) Rinse with water for 5 seconds



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



(h) Rinse for 5 seconds



(i) Blot dry with bibulous paper



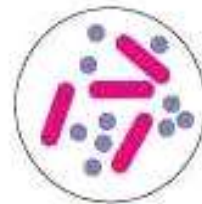
1 Application of crystal violet (purple dye) (Primary stain)



2 Application of Gram's iodine (mordant)

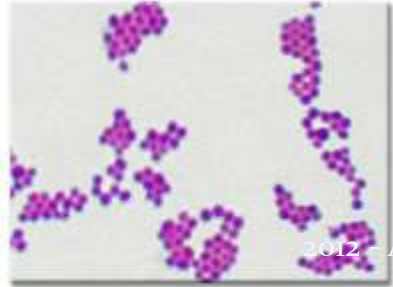



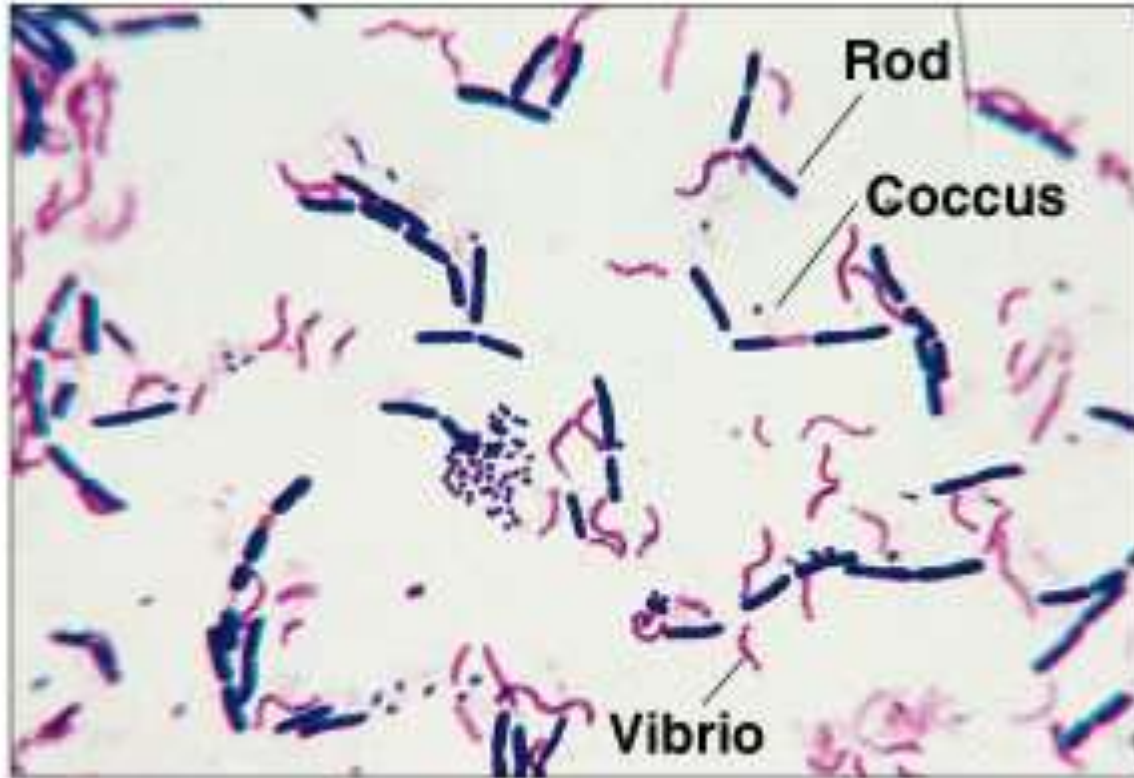
3 Alcohol wash (decolorization)



4 Application of safranin (counterstain)

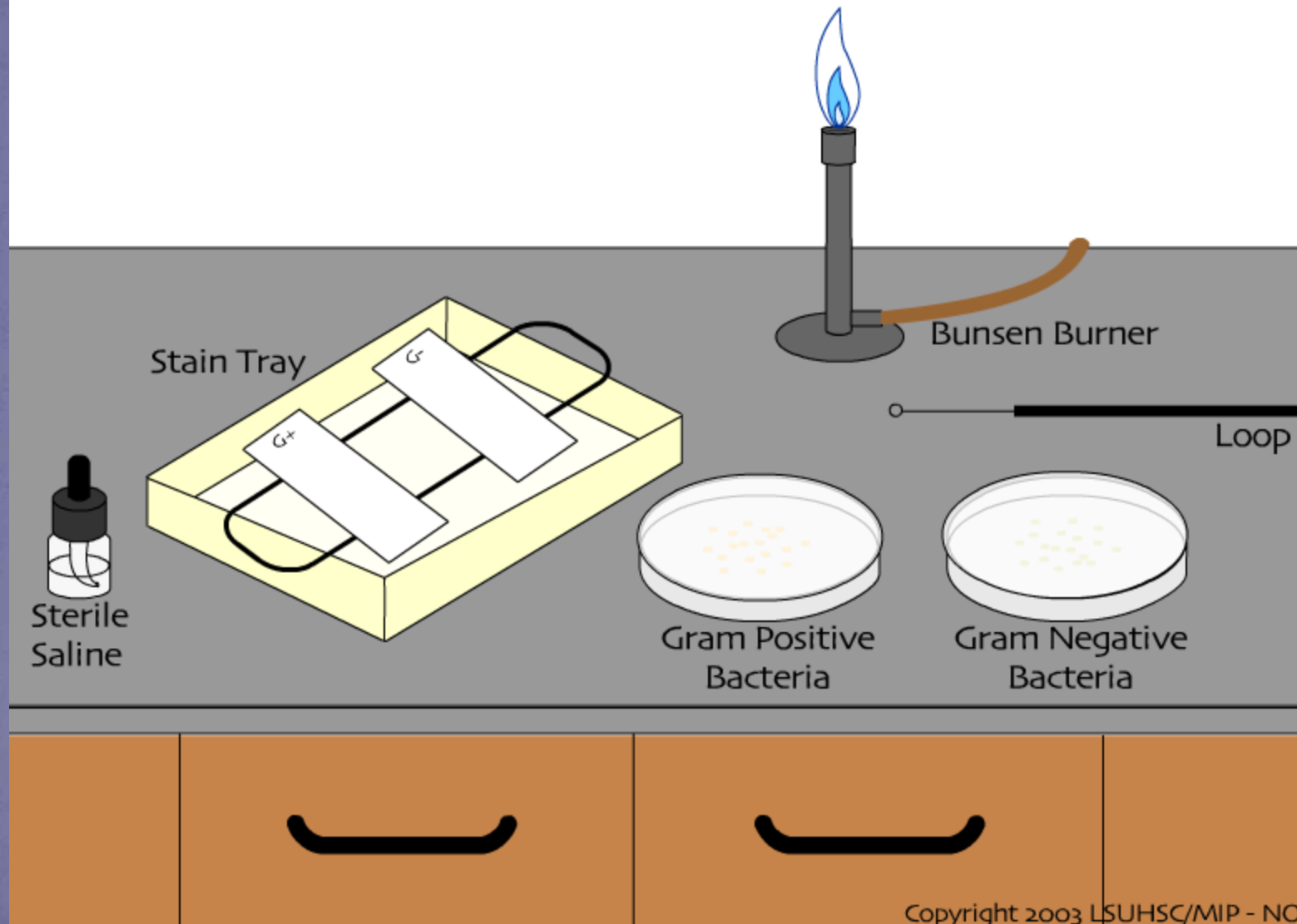
- Crystal violet
- Iodine
- Alcohol
- Safranin

Step	Gram-positive organisms	Gram-negative organisms
1. Unstained	Clear	Clear
2. Crystal violet	Violet	Violet
3. Iodine	Violet	Violet
enters bacterial cell & forms iodine-crystal violet complexes		
4. Decolorization (alcohol-acetone)	Violet	Clear
5. Safranin	Purple	Red
		

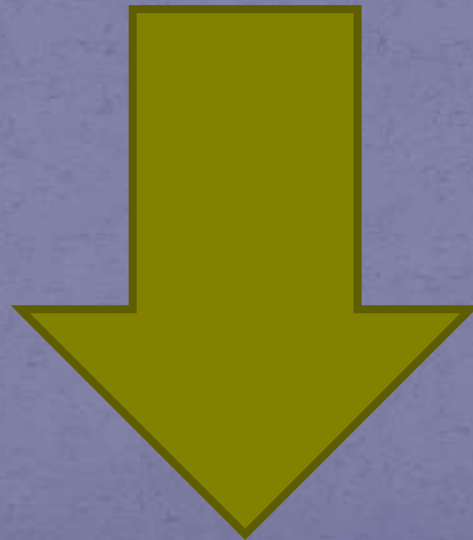


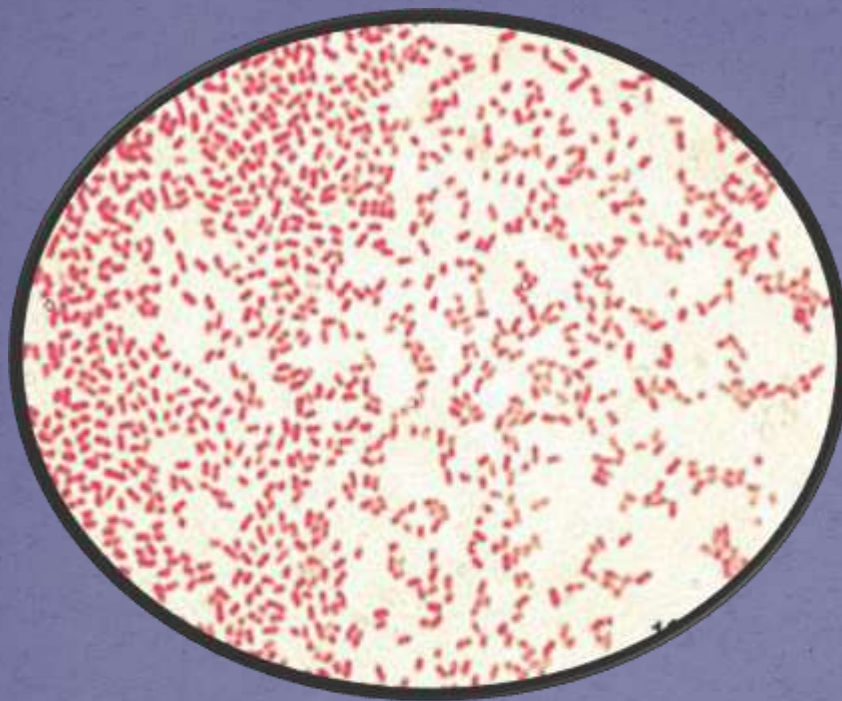
Making a Gram Stain

Start 

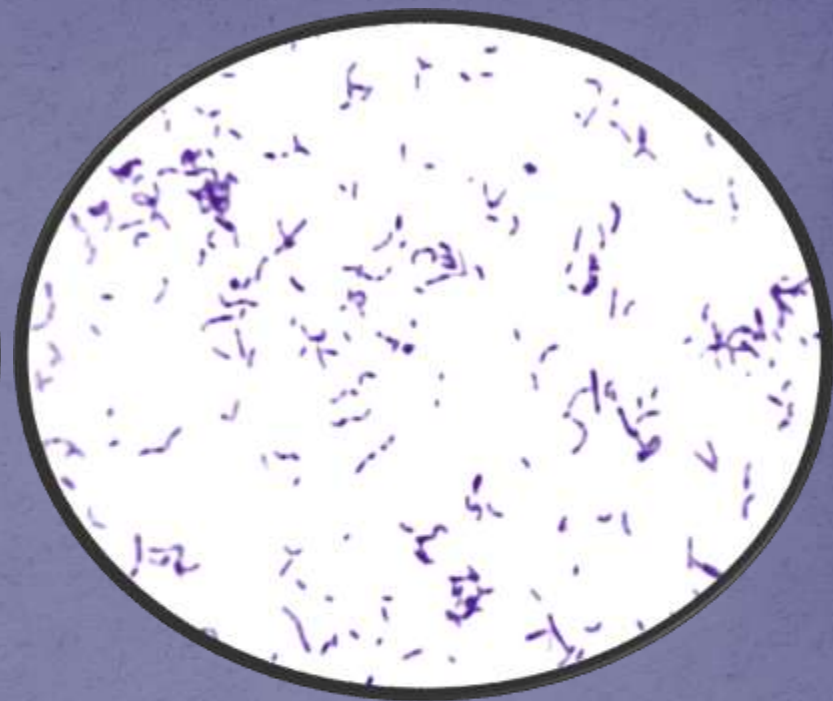


The bacteria under the microscope





Gram -ve



Gram

+ve

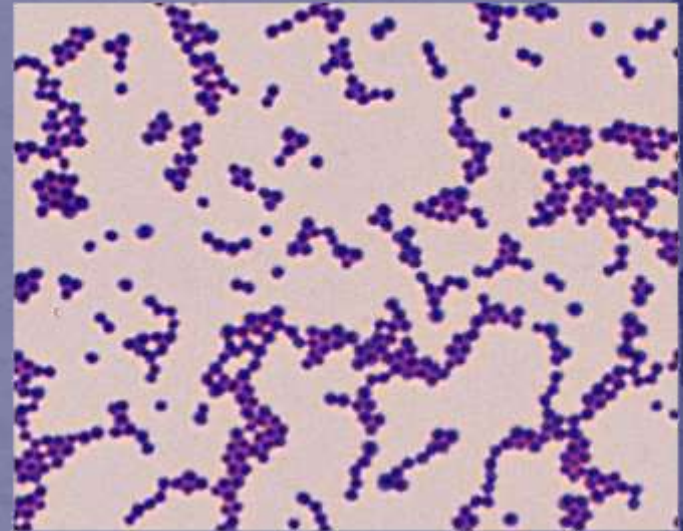
Results:

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**

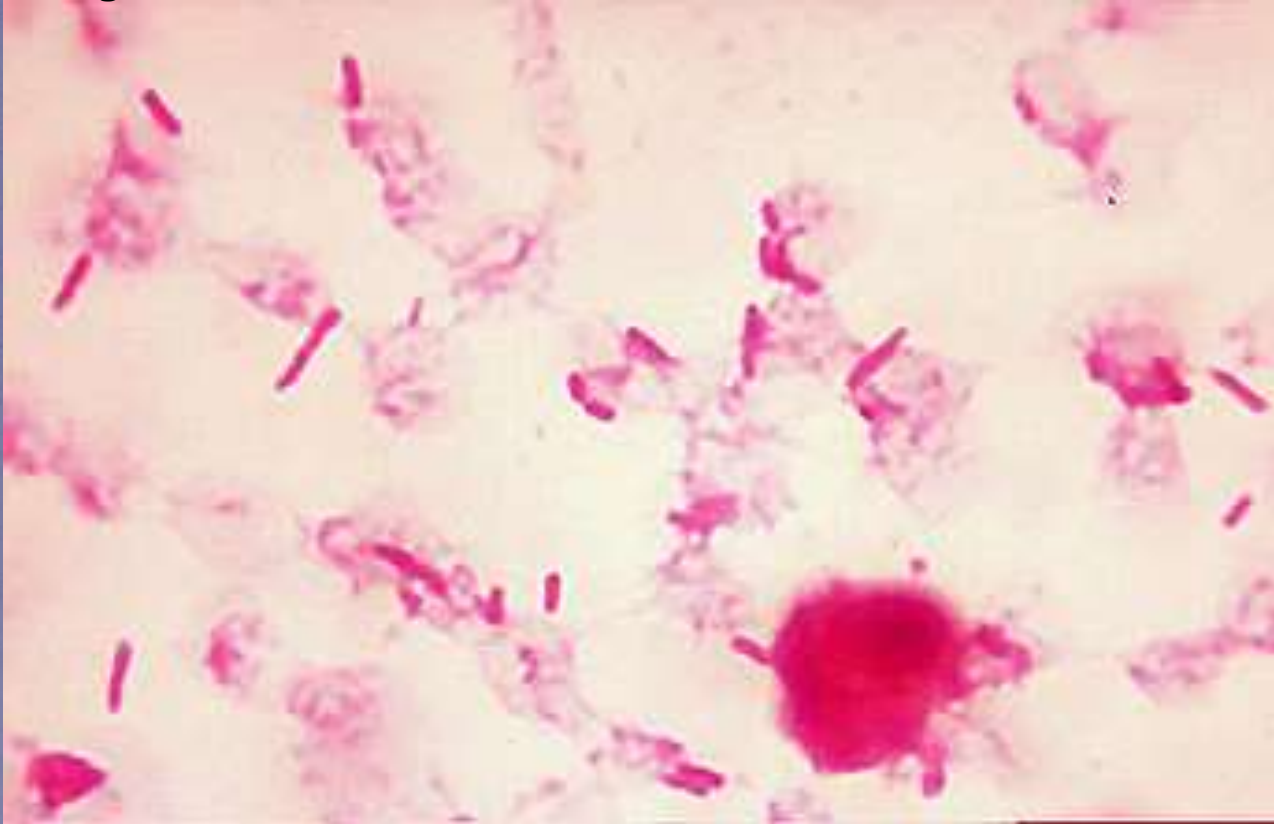


What is the *Gram* stain reaction, cell morphology, and cell arrangement seen here?



Answer: Gram-positive streptococcus

What is the *Gram* stain reaction, cell morphology, and cell arrangement seen here?



Answer: Gram-negative bacilli

Thank you

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أ.أمل الغامدي