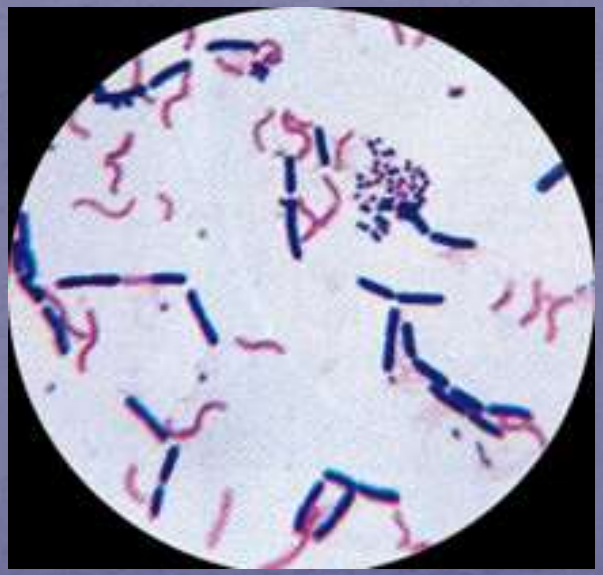
بسم الله الرحمن الرحيم

140 Micro

Lab 7: Gram Stain

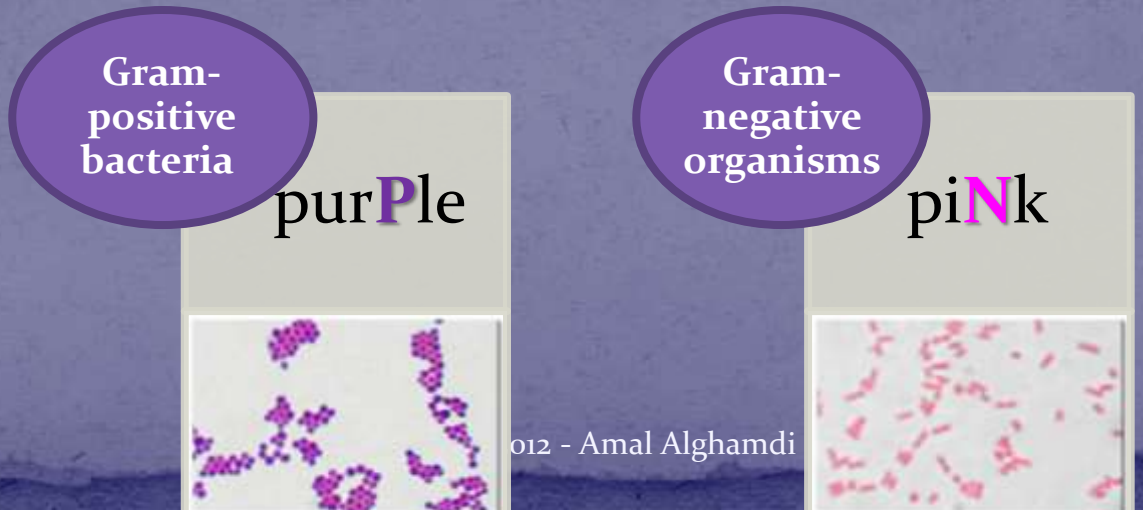
Gram stain

((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

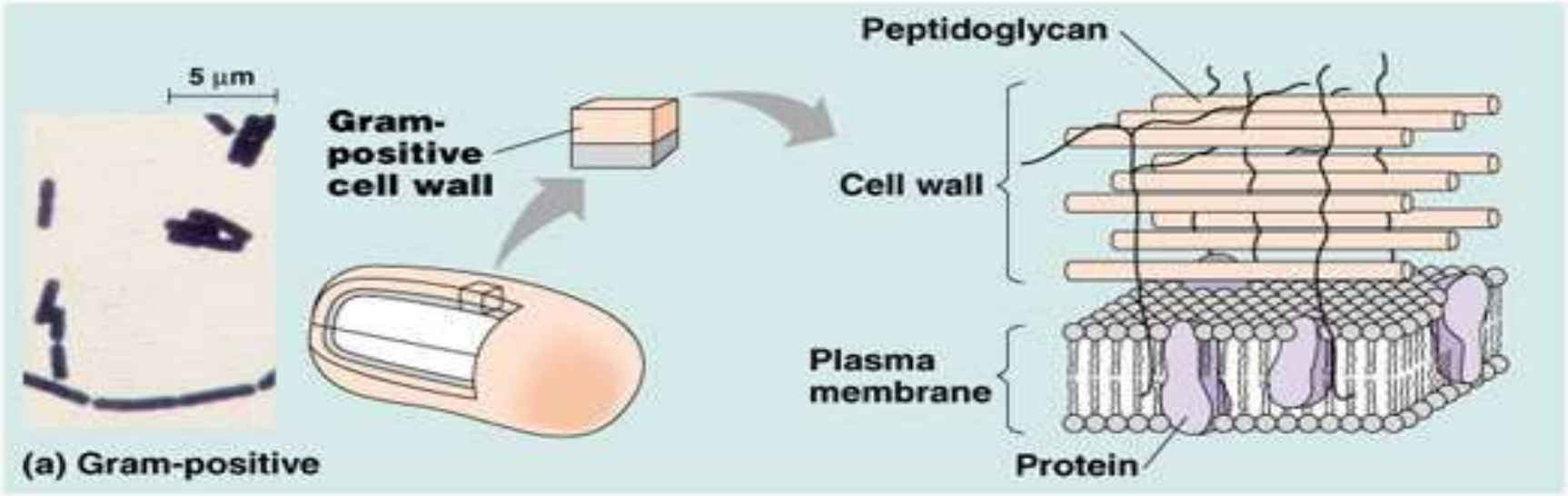
based on their cell wall structure .

- 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 decoulorization 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 :

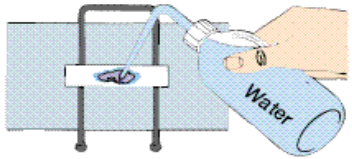
1- Fresh (24hrs)Cultures of :
Staphylococcus aureus,
Bacillus subtilis,
Escherichia coli
2- Microscopic Slides
3- Water
4-marker

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]

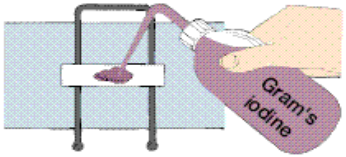
The method

Figure 8.3 Gram-stain Procedure.

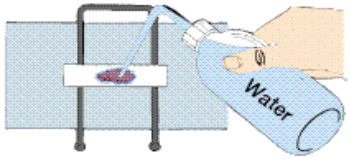
(a) Crystal violet; 30 seconds



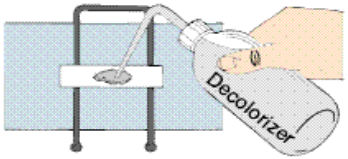
(b) Rinse for 5 seconds



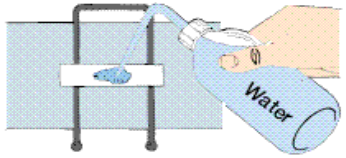
(c) Cover with Gram's iodine for 1 minute



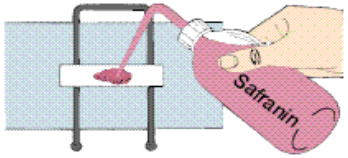
(d) Rinse with water for 5 seconds



(e) Decolorize for 15–30 seconds

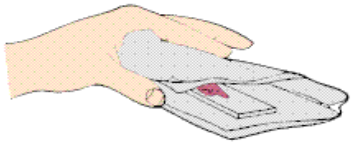


(f) Rinse with water for 5 seconds

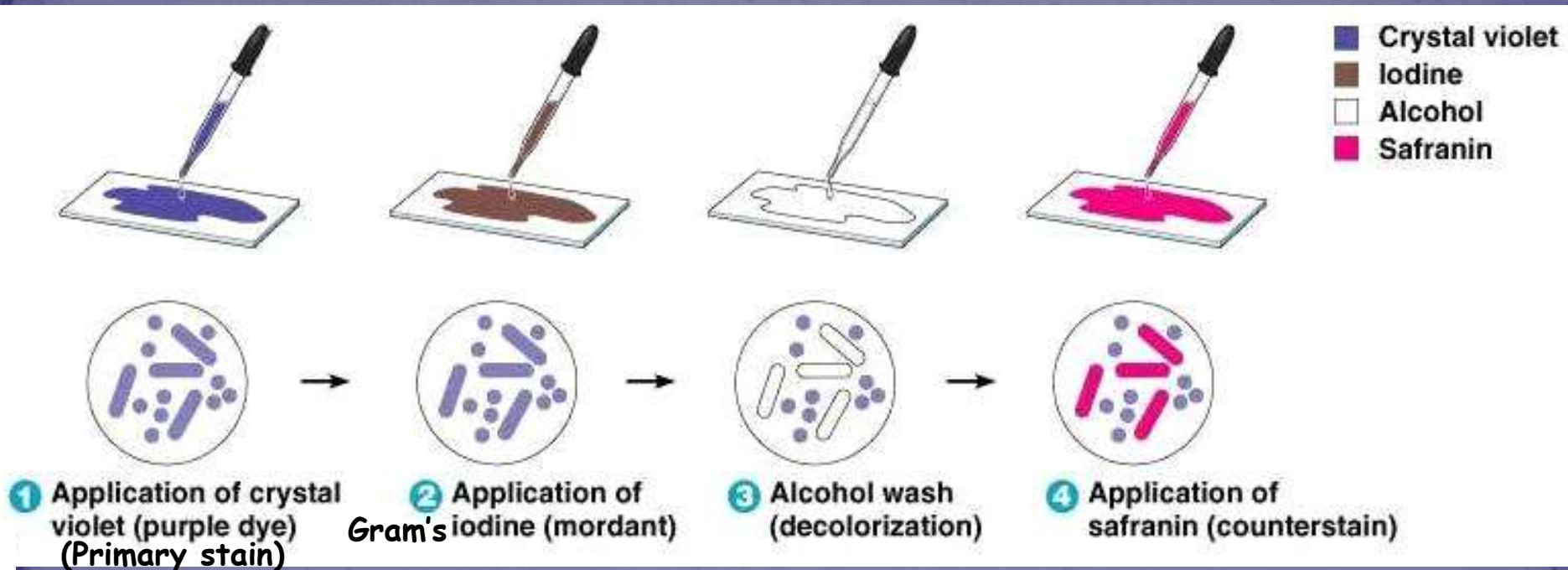


(g) Counterstain with safranin for about 60–80 seconds

(h) Rinse for 5 seconds



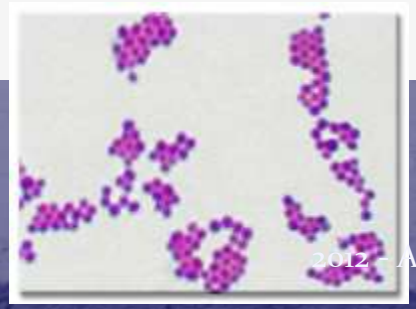
(i) Blot dry with bibulous paper

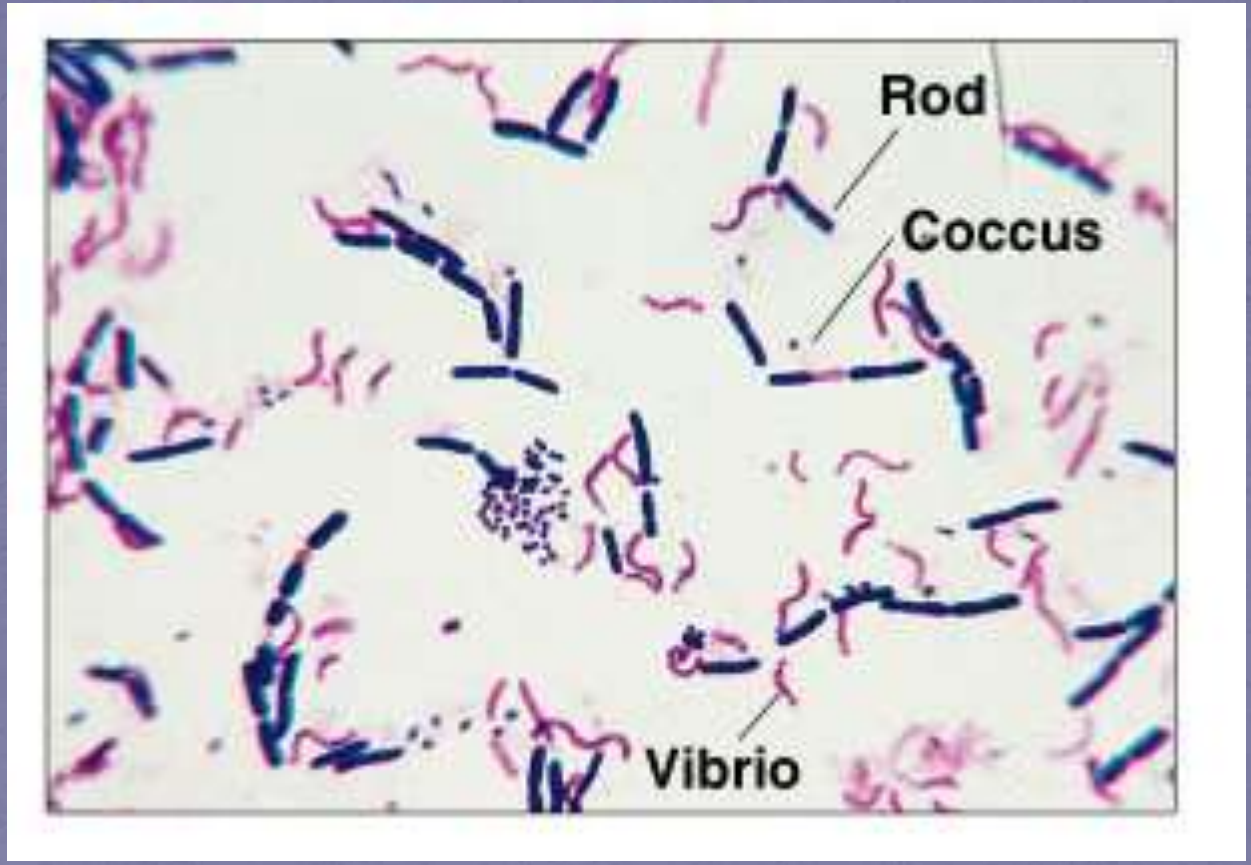


Gram's (Primary stain)

Step	Gram-positive organisms	Gram-negative organisms
1. Unstained	Clear	Clear
2. Crystal violet	Violet	Violet
3. Iodine	Violet	Violet
4. Decolorization (alcohol-acetone)	Violet	Clear
5. Safranin	Purple	Red

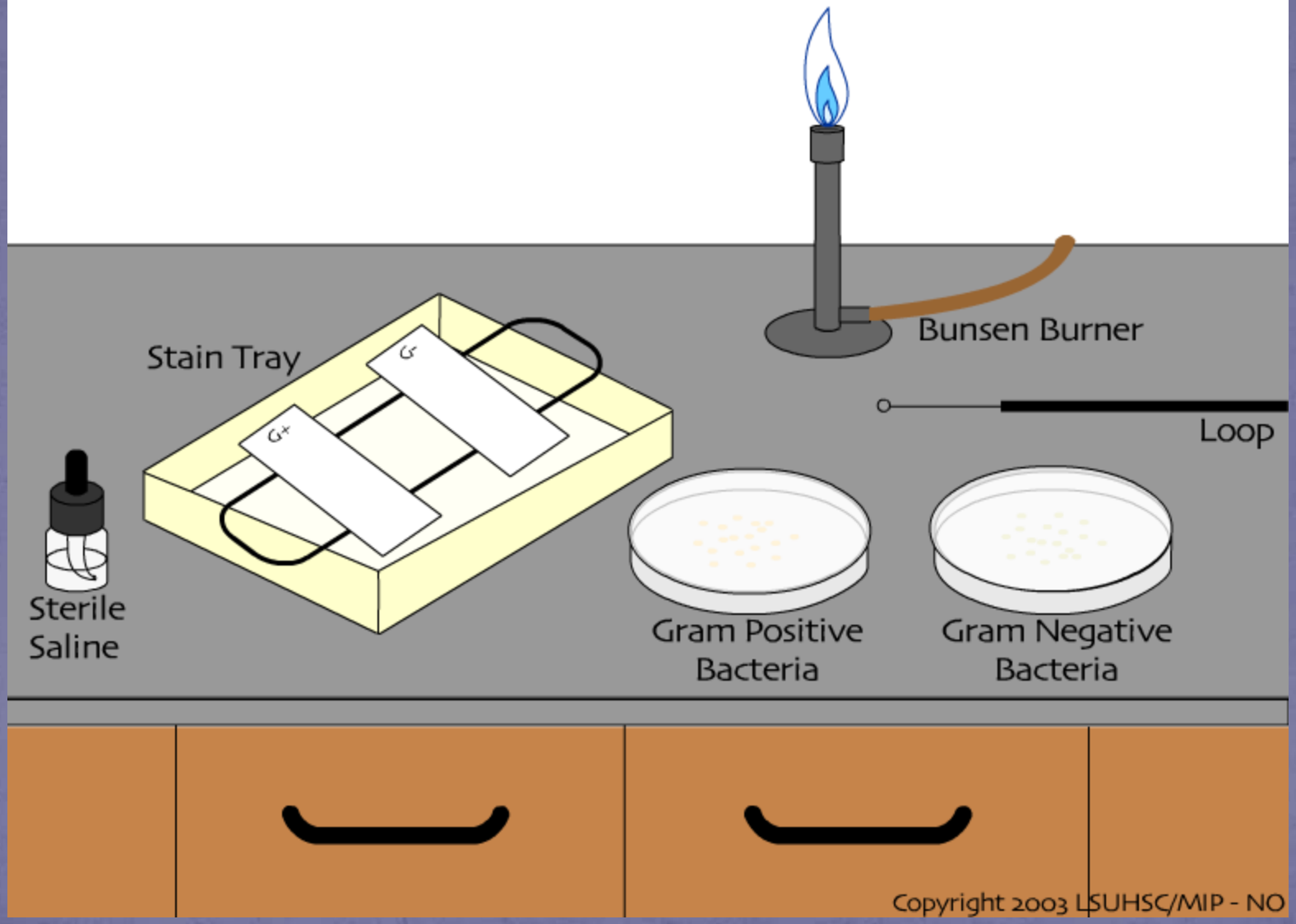
enters bacterial cell & forms iodine-crystal violet complexes



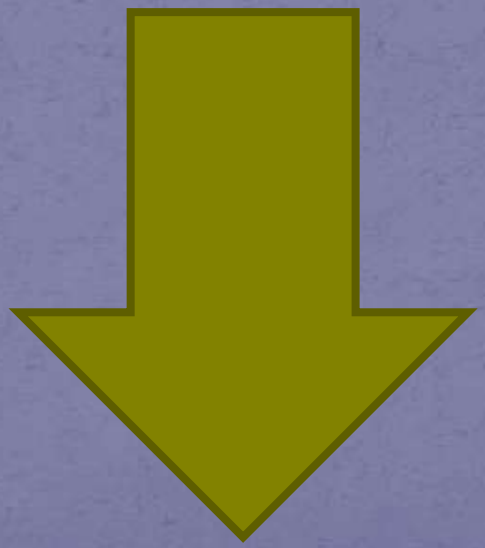

Rod
Coccus
Vibrio

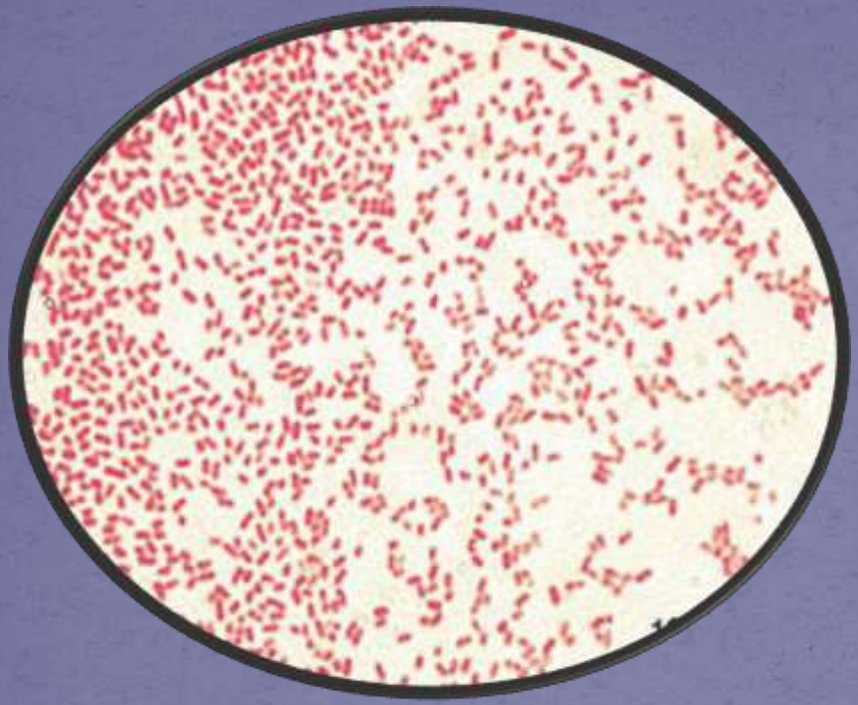
Making a Gram Stain

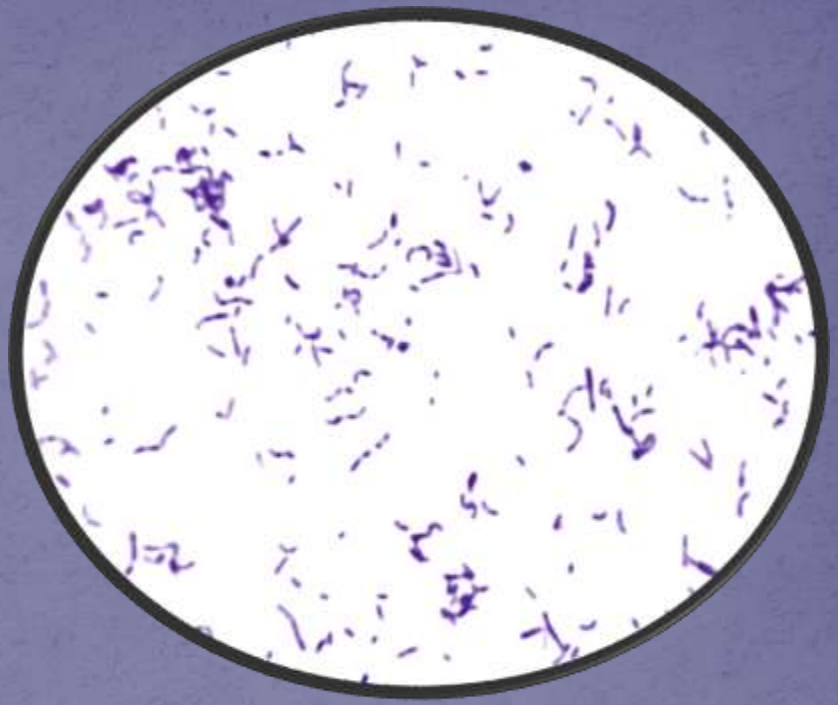
Start



The bacteria under the microscope







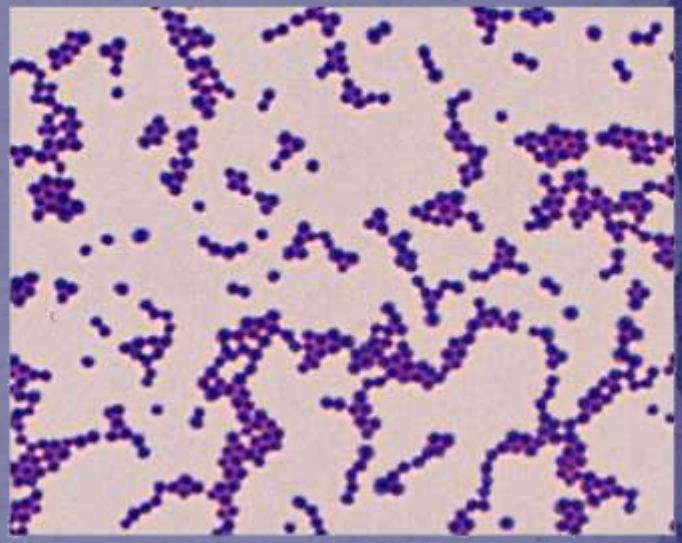
Gram –ve

Gram +ve

Results:

Shape: Cocci

Arrangment: irregular clusters



Colour: Violet

Gram's reaction: Gram's +ve

Name of microorganism: Staphylococci

Results:

Shape: Bacilli

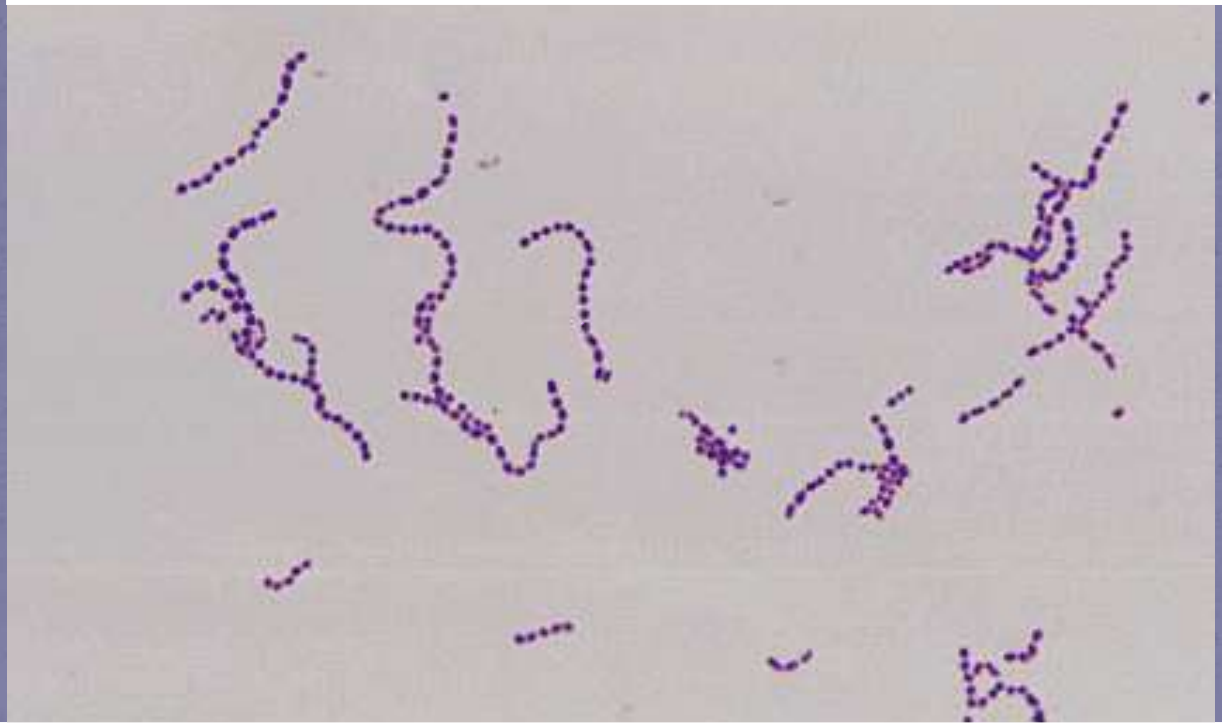
Arrangment: Chains

Colour: Violet

Gram's reaction: Gram's +ve

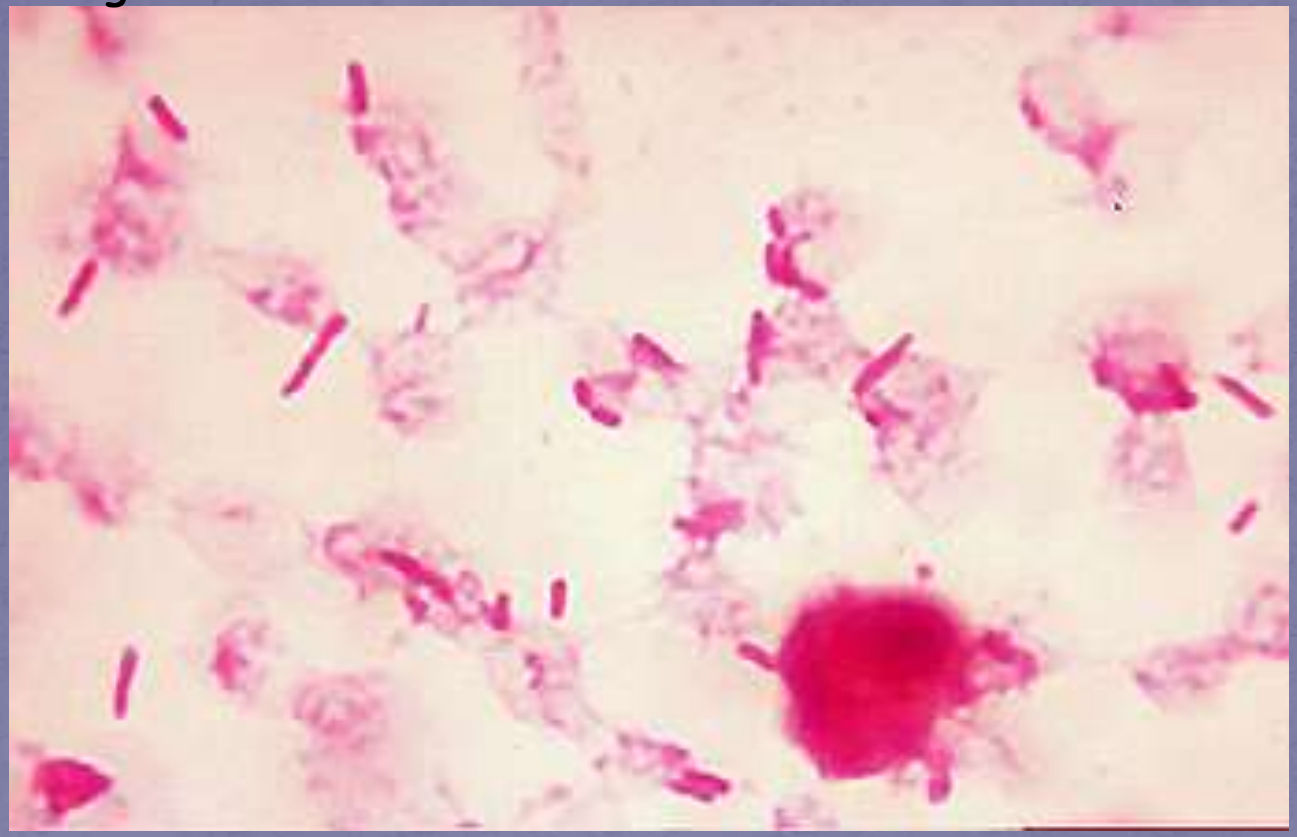
Name of microorganism: Bacillus

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

أ.شروق الشهراني
أ.أمل الغامدي