



بكتريا طبية 1

Differential staining of bacteria (Part 1)

Sawa University

جامعة ساوة الاهلية

College of Health and Medical

كلية التقنيات الصحية والطبية

Techniques

Department of Medical Laboratories

قسم تقنيات المختبرات الطبية

Second Stage

المرحلة / الثانية

محاضرة رقم 8

Lecture No. 8

Practical

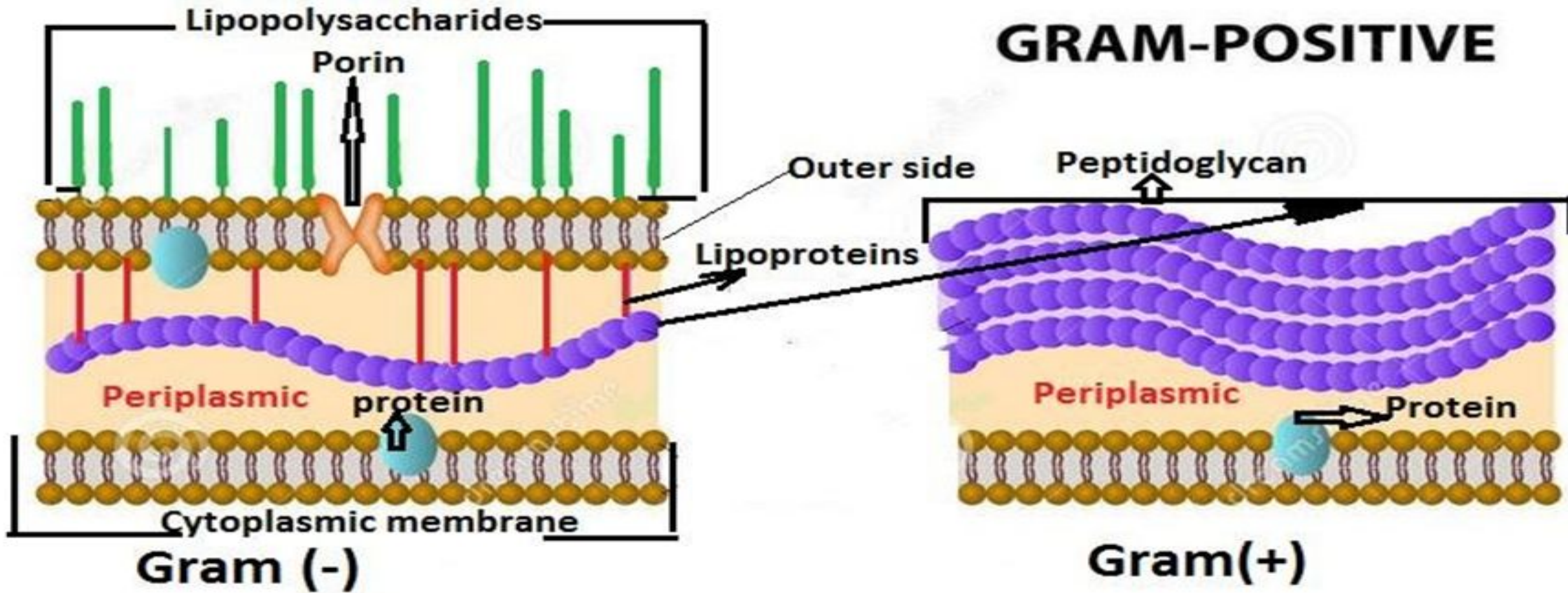
الجانب العملي

تدريسي المادة : دكتور معين حسن

Differential staining of bacteria

1- Gram stain

- Gram stain is a differential stain as it differentiates bacteria in two groups (**Gram Positive** and **Gram negative**).
- Gram stain is highly useful for identification of microorganisms as the bacteria stain differently because of structural differences in their cell walls.
- In **Gram-positive bacteria**, Crystal violet-iodine complex (CVI) formed because of crystal violet and iodine penetrating the bacterial cell does not leach out on treatment with alcohol because of thick peptidoglycan layer (Fig-1).
- In **Gram-negative bacteria**, Alcohol dissolves the outer lipopolysaccharide layer; consequently, the Crystal violet-iodine complex (CVI) leaches out through thin layer of peptidoglycan.



Fig(1): the structure of cell wall of **gram positive** vs. **gram negative** bacteria.

□ The technique use of:

1. Primary stain (**crystal violet**) that stains all bacteria purple.
 2. **iodine** combines with crystal violet to form a Crystal violet-iodine complex (CVI).
 3. Decolorizing agent (**95% alcohol or acetone**) washes out the CVI complex from some bacteria.
 4. Counter stain or secondary stain (**safranin**) to stain the decolorized bacteria.
- Gram staining gives information about its morphology, arrangement. It is also the first step in the identification of microorganisms.



□ **Requirement**

a. Gram stain set:

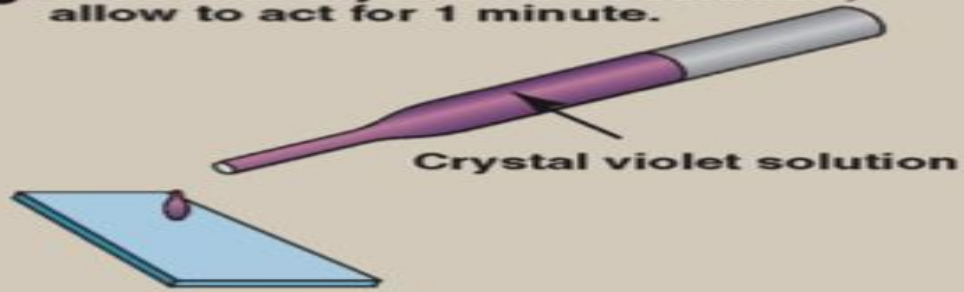
(Crystal violet, iodine, 95% ethyl alcohol or acetone, Safranine)

b. Bacterial cultures.

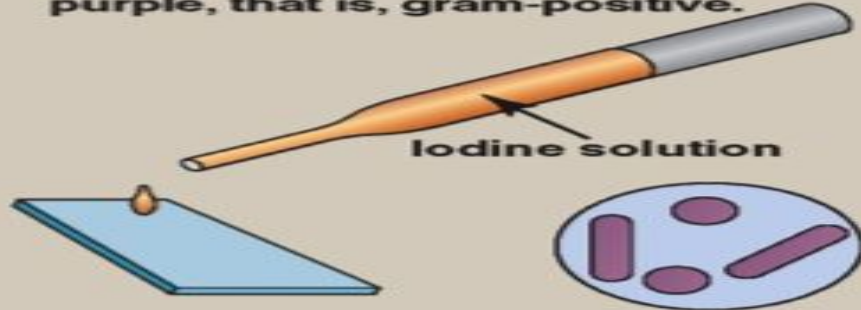
□ **Procedure**

- 1. Prepare and heat fix smears on clean glass slides.**
- 2. Place the slide on the staining rack and cover the smear with crystal violet and leave it for one minute. Wash the slide with tap water gently.**
- 3. Cover the smear with Gram's iodine for one min.**
- 4. Wash off the iodine by tilting the slide and pour water over the smear.**
- 5. Decolorize with ethyl alcohol or acetone for about 10-15 sec. or until no large amount of purple color wash out. However, do not over or under decolorize. Immediately wash with water.**
- 6. Cover the smear with safranin for 30 seconds. Wash with water. Blot dry, examine under**

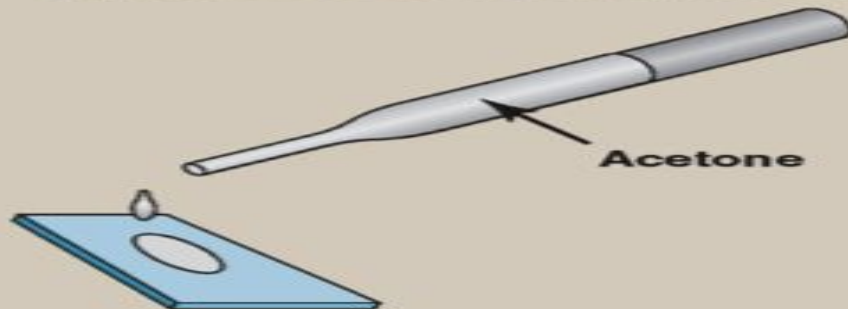
1 Heat-fix specimen to slide. Flood slide with crystal violet solution; allow to act for 1 minute.



2 Rinse the slide, then flood with iodine solution; allow iodine to act for 1 minute. Before acetone decolorization (next step), all organisms appear purple, that is, gram-positive.



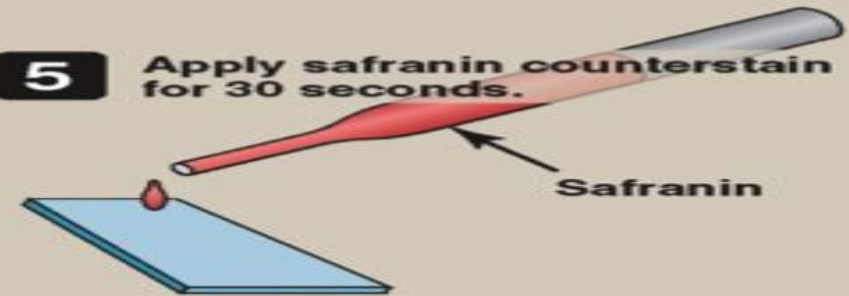
3 Rinse off excess iodine. Decolorize with acetone, approximately 5 seconds (time depends on density of specimen).



4 Wash slide immediately in water. After acetone decolorization, those organisms that are gram-negative are no longer visible.



5 Apply safranin counterstain for 30 seconds.



6 Wash in water, blot, and dry in air. Gram-negative organisms are visualized after application of the counterstain.

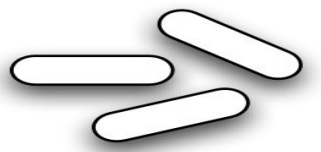
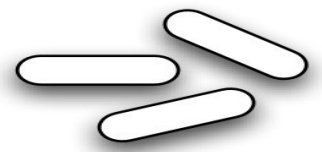


Key:  = Gram-positive violet.  = Gram-negative red.  = Colorless.

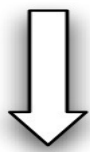
Fig: gram staining.

GRAM-POSITIVE

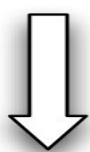
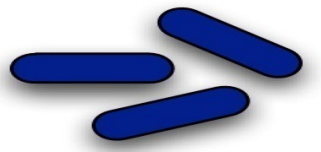
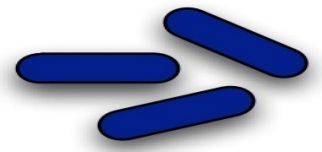
GRAM-NEGATIVE



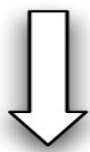
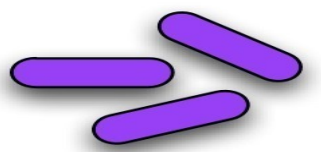
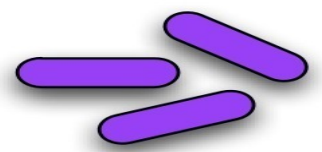
Fixation



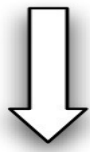
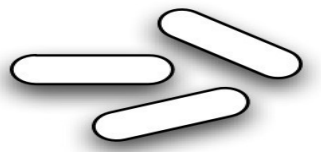
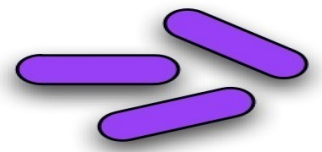
Crystal Violet



Iodine Treatment



Decolorisation



Counter stain with
Safranin

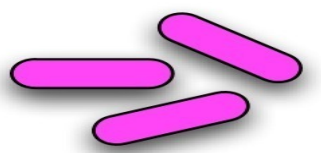
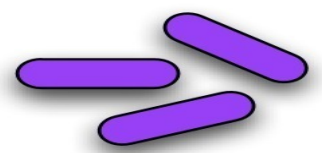


Fig: gram staining.

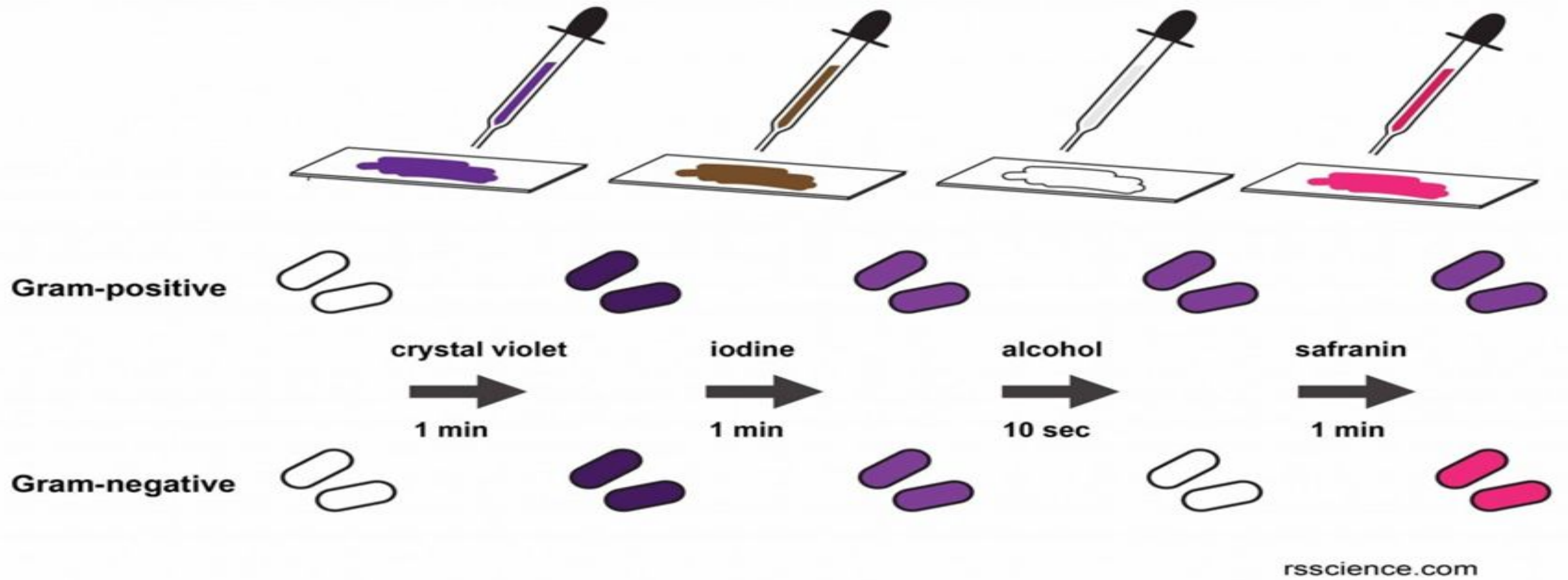
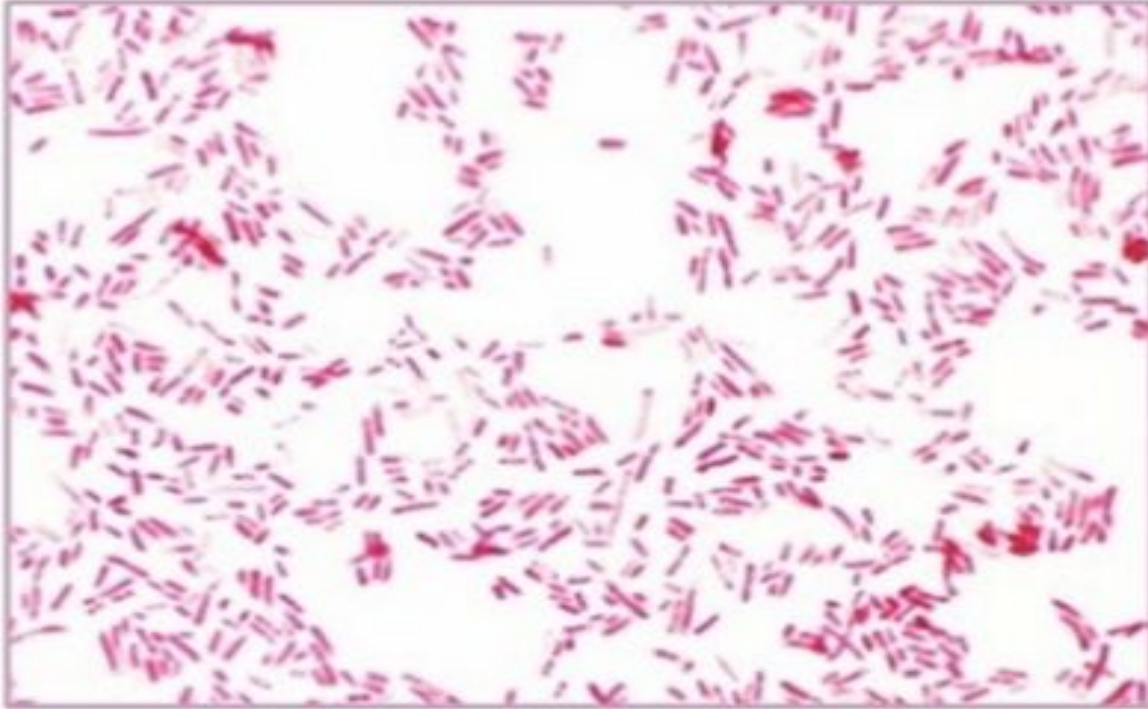
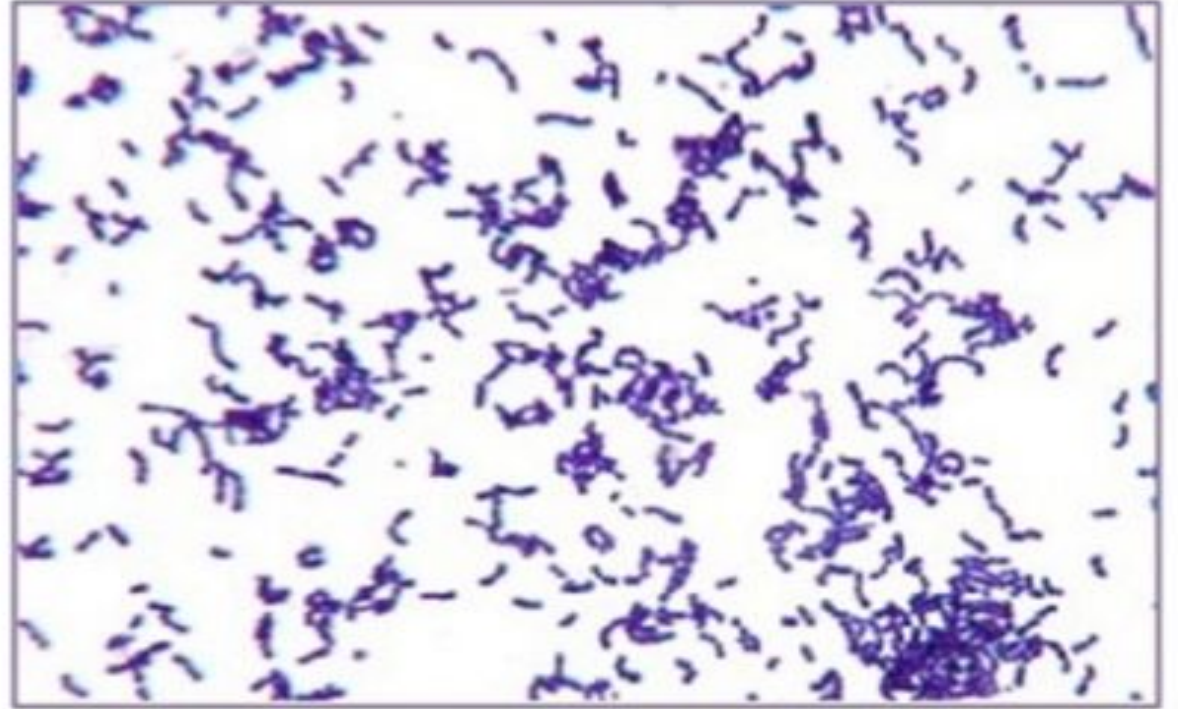


Fig: gram stain steps.

Gram staining of Bacteria



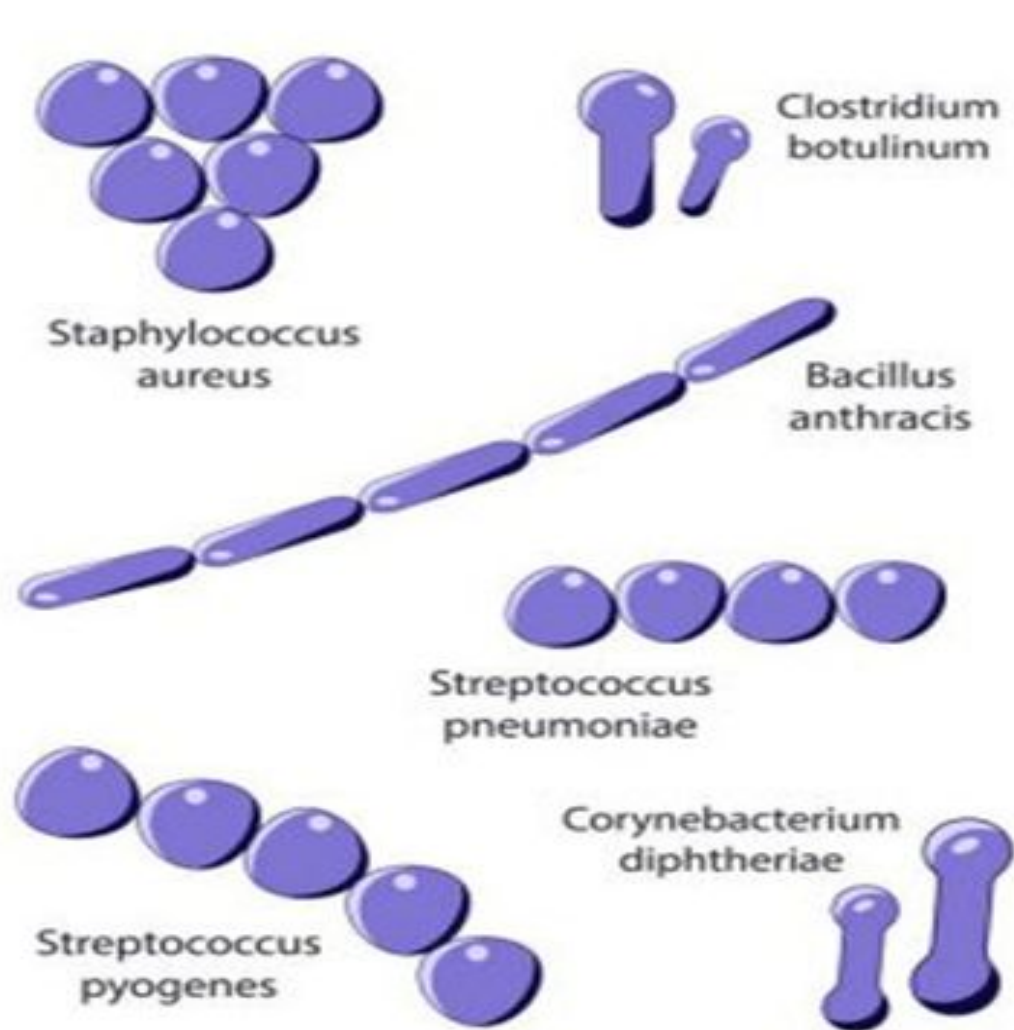
Gram-Negative Bacteria



Gram-Positive Bacteria

Fig: (4)

Gram positive bacteria



Gram negative bacteria

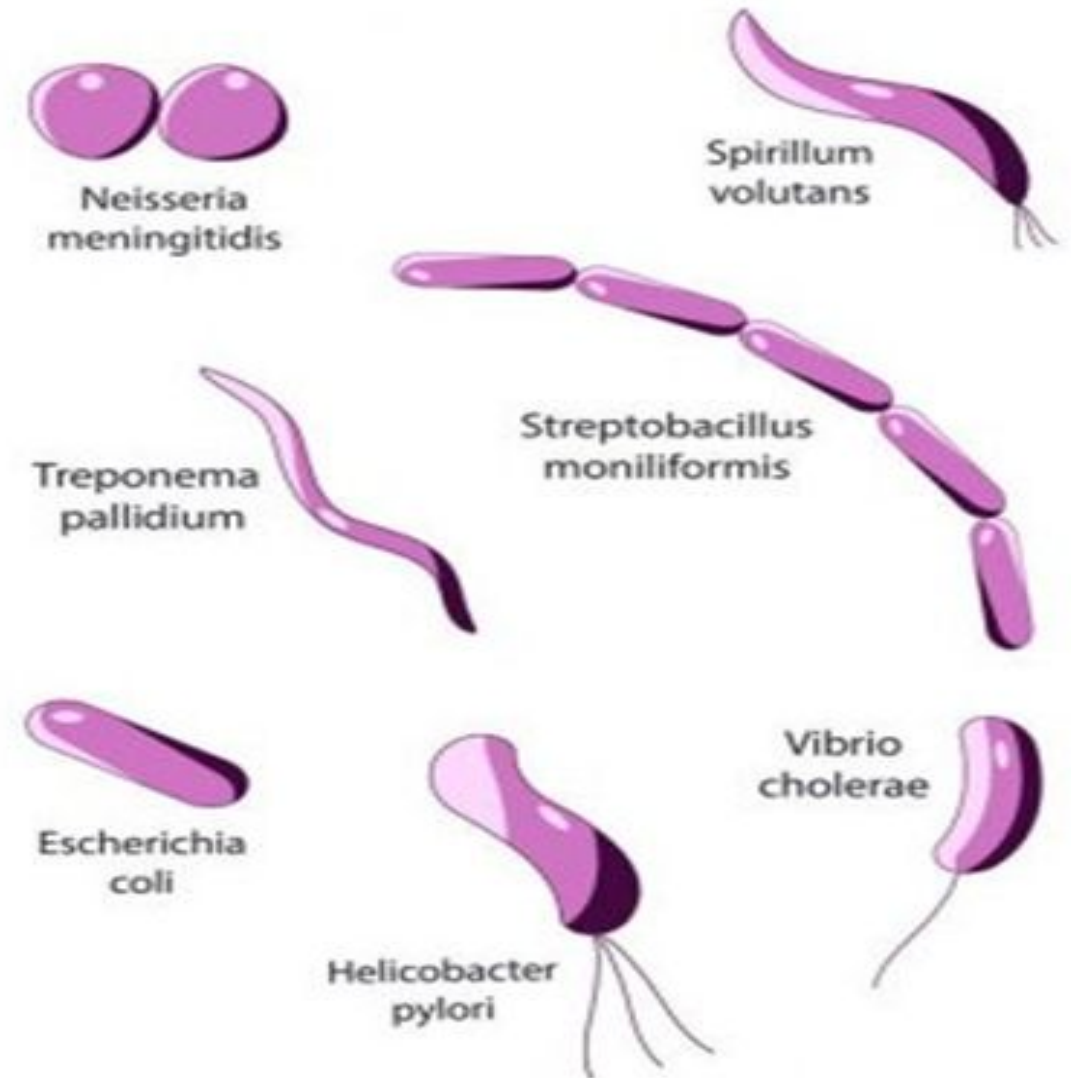


Fig: Shapes and arrangement of **gram positive** and **gram negative** bacteria.

QUIZ

Q1/ WHAT ARE THE DIFFERENTIAL STAINING OF BACTERIA?



Q2/ WHAT IS THE GRAM STAIN ?

Q3/ MENTION THE PROCEDURE OF GRAM STAIN?

Any questions?

*Thank
You*