PRATICAL WORK (Dr BENDIMERAD Nahida)

DOUBLE COLOURING : GRAM STAINING

I-PREPARING A RUBBING

-Put a drop of a bacterial suspension on a slide

-Spread the drop without rubbing

-fix the preparation to the flame of the Bensen burner until the blade is dry

II- USE TWO DYES

- On the surface of the blade put

-Put two or three drops of the first dye, GENTIAN VIOLET. Leave for 1 minute. Remove the excess

-Apply two or three drops of lugol. Leave for 30 minutes. Remove the excess

-Add a few drops of alcohol. Leave for 30 minutes

-Rinse with distilled water

-Apply two or three drops of the second colouring agent LA FUSHINE

-Leave for 1m

-Rinse with distilled water

-Dry with absorbent paper without rubbing

-Observe with a microscope GROSSX100 with immersion (a drop of immersion oil)

II- INTERPRETATION OF RESULTS

Bacteria coloured purple are GRAM positive.

Bacteria coloured pink are GRAM negative

The coloration of the bacteria is due to the composition of the bacterial wall.

If the bacterial coat is rich in polysaccharides, the alcohol cannot penetrate to remove the first dye, so the bacteria retain their violet GRAM+ colour.

If the bacterial wall is low in polysaccharide, it contains pores and the alcohol passes through these pores, it removes the first dye and allows the second dye to pass through and the bacteria turn GRAM pink.