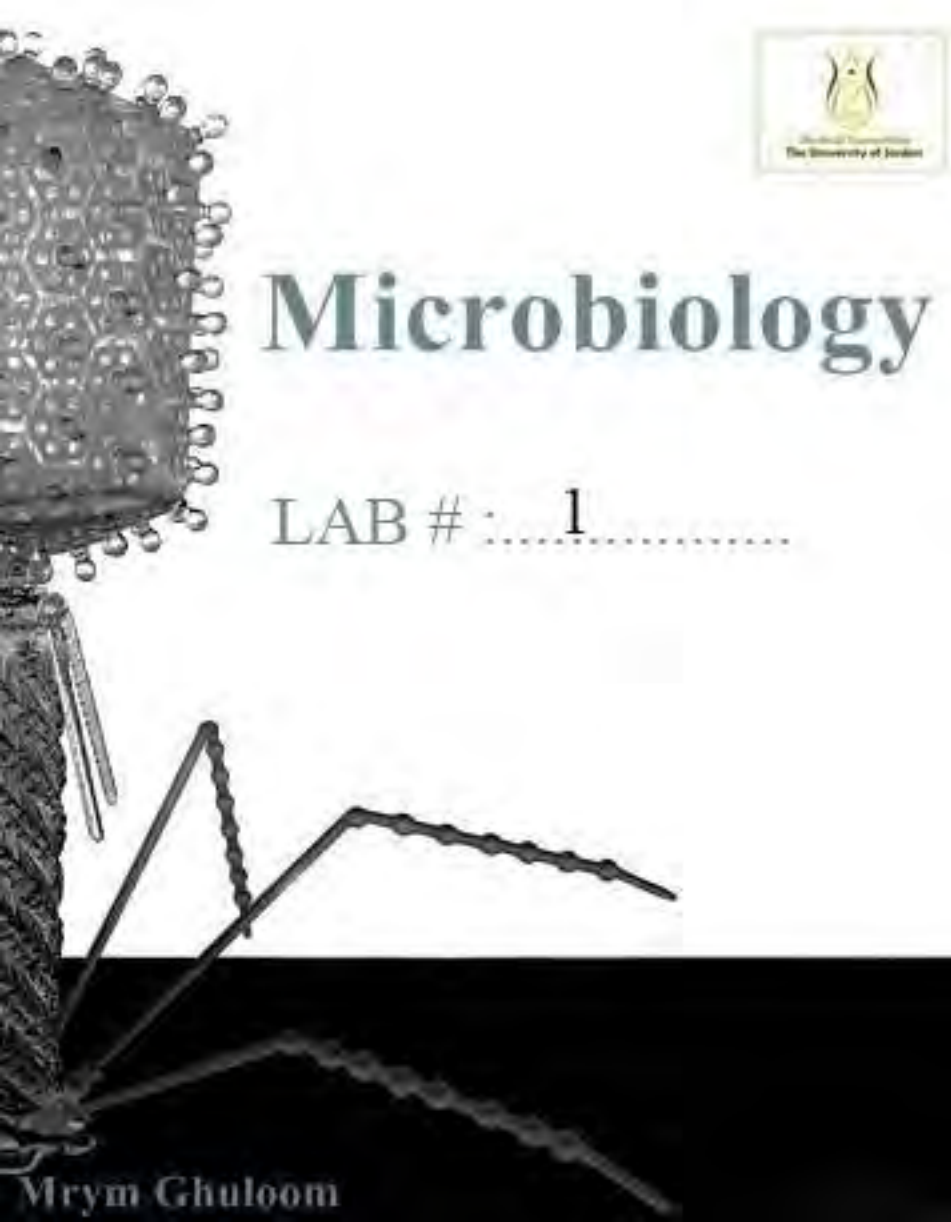


Microbiology

LAB # : 1

Mrym Ghuloom

GRAM STAINING

INTRODUCTION:

The Gram stain is a **DIFFERENTIAL STAIN** which allows most bacteria to be divided into two groups, Gram-positive bacteria and Gram-negative bacteria. The technique is based on the fact that the Gram positive cell wall has a stronger attraction for crystal violet when Gram's iodine is applied than does the Gram negative cell wall. Gram's iodine is known as a **MORDANT**. It is able to form a complex with the crystal violet that is attached more tightly to the Gram-positive cell wall than to the Gram-negative cell wall.

This complex can easily be washed away from the Gram-negative cell wall with ethyl alcohol. Gram-positive bacteria, however, are able to retain the crystal violet and therefore will remain purple after **DECOLORIZING** with alcohol. Since Gram-negative bacteria will be colorless after decolorizing with alcohol, **COUNTERSTAINING** with safranin will make them appear pink.

The Gram stain is probably the most commonly used staining procedure in microbiology. It is extremely useful in identifying bacteria. It is important that you understand the color changes that occur at each step in the Gram stain. It is also important that you understand the function of each reagent used in this procedure. It takes some practice and patience to be able to reliably Gram stain.

MATERIALS:

1. 2 microscope slides Gram stain reagents (crystal violet, Gram's iodine, 95%.
   ethyl alcohol, and safranin)
2. Fresh cultures of ***S. epidermidis*** **and** ***E.coli*** **mixture**
3. Fresh cultures of ***Bacillus subtilis and Diphteroid bacilli***

PROCEDURE:

SMEAR PREPARATIONS: Remember to label the slides.

1- **S. epidermidis and E.coli mixture:**
2- **B. subtilis and Diphteroid bacilli mixture:**

Prepare smear using aseptic technique.SEE PINK EXERCISE for SMEAR PREPARATION!! **THE FIRST LOOPFUL OF ORGANISM IS NOT SMEARED OUT UNTIL THE SECOND ORGANISM HAS BEEN ADDED**. The two organisms are then smeared out together. After air drying and heat fixing the Gram staining procedure is followed.

Prepare smears using aseptic technique. These organisms are growing on TSA slants. A loopful of distilled water is first placed on each slide. Bacteria are obtained from the slants using a sterile **needle** and proper aseptic technique. SEE PINK EXERCISE for SMEAR PREPARATION!! After air drying and heat fixing the Gram staining procedure is followed.

GRAM STAINING PROCEDURE:

1. **Cover smear with CRYSTAL VIOLET** for 20 seconds.

(PRIMARY STAIN)

2. **Gently rinse** off the stain with water and shake off the excess.

3. **Cover with GRAM'S IODINE** for one minute (MORDANT)

4. **Pour off** the Gram's iodine.

5. **Run 95% ETHYL ALCOHOL down the slide** until the solvent runs clear (about 10-20 seconds). **THIS STEP IS CRITICAL! THICK SMEARS REQUIRE MORE TIME**

THAN THIN ONES (DECOLORIZING AGENT)

6. **Rinse with water** to stop the action of the alcohol.

7. **Cover with SAFRANIN** for 20 seconds (COUNTER STAIN).

8. **Gently rinse** off the stain with water. Blot with filter paper and clean off the bottom of the slide with 95% alcohol.

HELPFUL SUGGESTIONS to perform good smears

a) DO NOT make your smears too thick!

b) Be very careful when you decolorize.

c) Be sure your cultures are young, preferably 18-24 hours old. Older cultures tend to lose the ability to retain stains.

RESULTS:

Observe your smears in the microscope using oil immersion lens as follow:

Staphylococcus Gram-positive cluster of cocci

E.coli Gram-negative bacilli

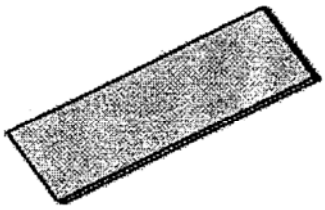
Bacillus Gram-positive, spore-forming bacilli

Diphteroid Gram-positive pleomorphic small cocci to bacilli

GRAM STAINING

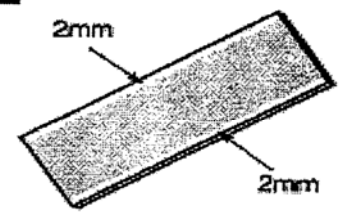
Flow Through Procedure

1



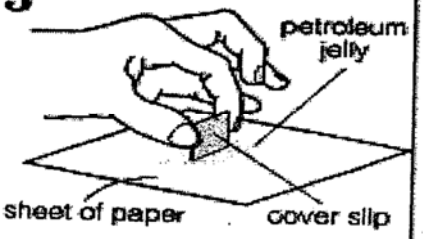
Wipe bottom of biofilm slide clean

2



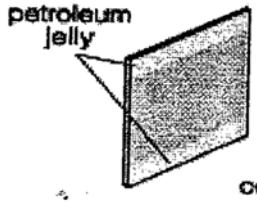
Clean top edges of slide about 2mm

3



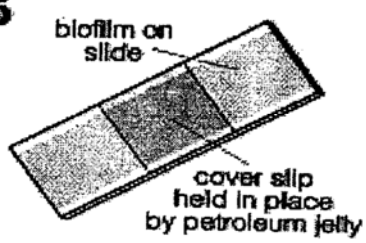
Build up a ridge of petroleum jelly on the top and bottom of a cover slip

4



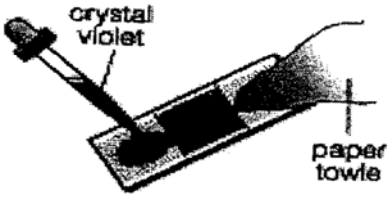
Cover slip with petroleum jelly

5



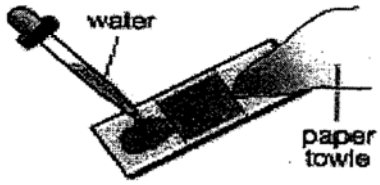
Biofilm on slide with cover slip

6



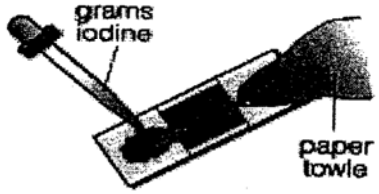
Add crystal violet-wait 30 sec.

7



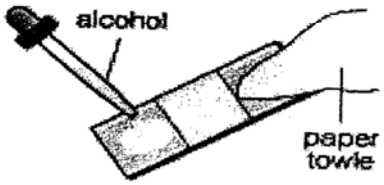
Wash with water

8



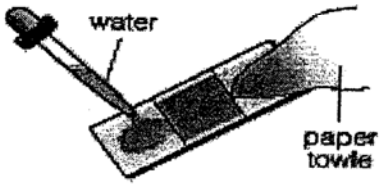
Add Grams Iodine-wait 1.5 min.

9



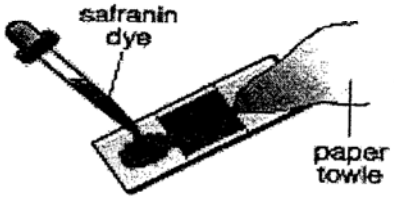
Decolorize with alcohol

10



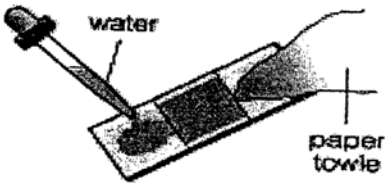
Wash with water

11



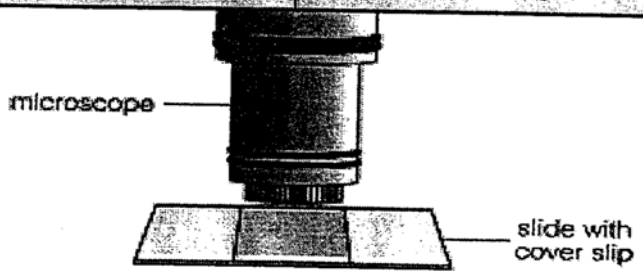
Stain with Safranin dye-wait 30 sec.

12

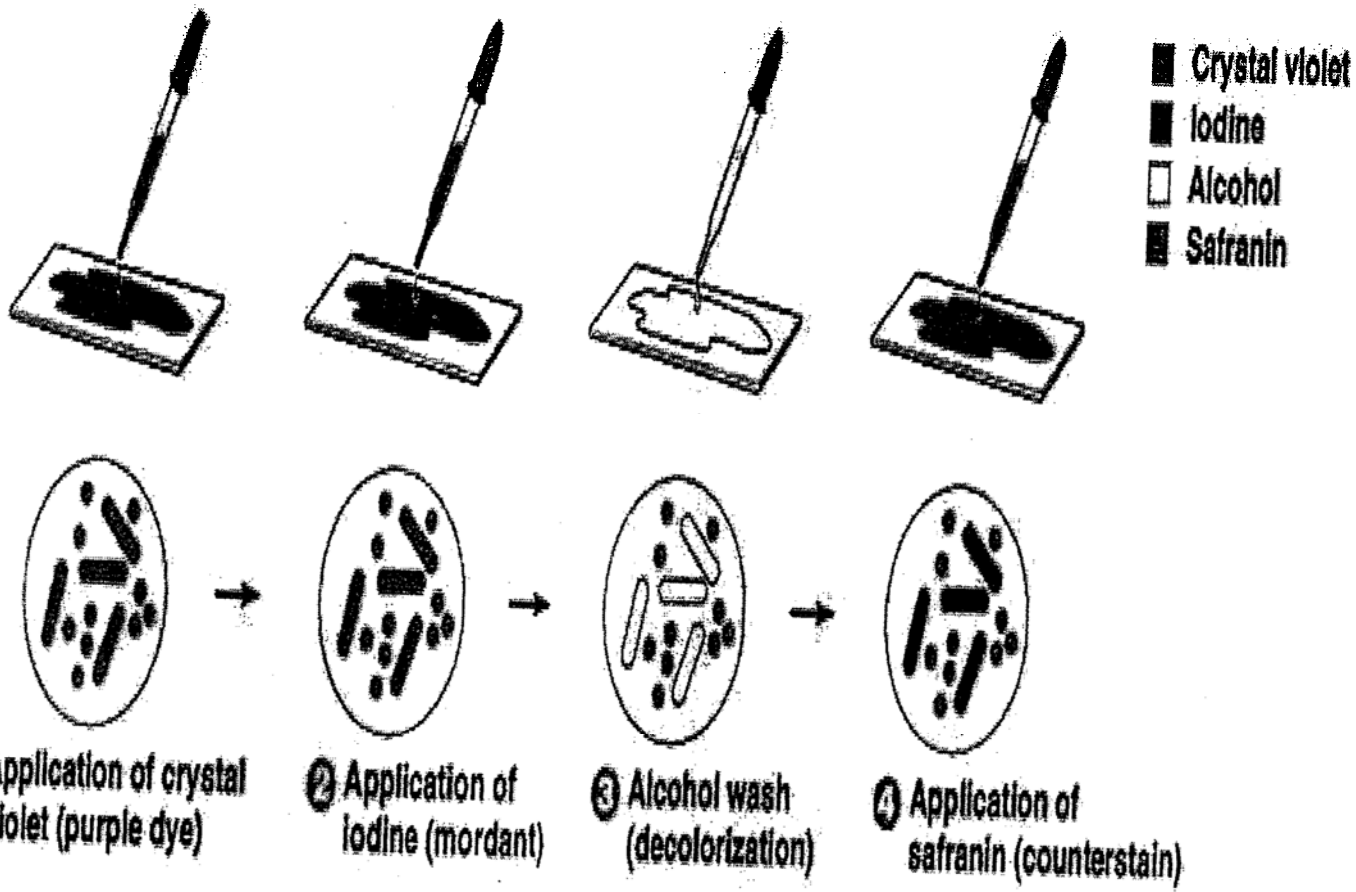


Wash with water

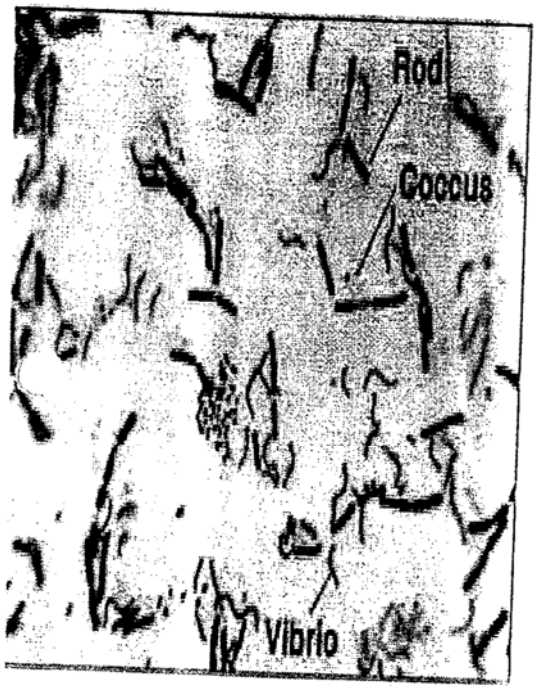
13



Examine under oil immersion through the cover slip



(a)



b)