II. LABORATORY SESSIONS

1ST SEMESTER 2016/2017

ITEM	TOPIC
1.	Introduction-Safety Rules-Simple Stains- gram Stain
2.	Cultivation Of Bacteria, Collection Of Clinical Specimens (Throat, Sputum, Blood,CSF,Urine)
3.	Antimicrobial Susceptibility Testing.
4.	Staphylococcus,Streptococcus,Bacillus,Diphtheroids, & Neisseriae
5.	E.coli,Pseudomonas,Klebsiella,&Proteus.

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GOODLUCK.

Gram Stain Procedure:

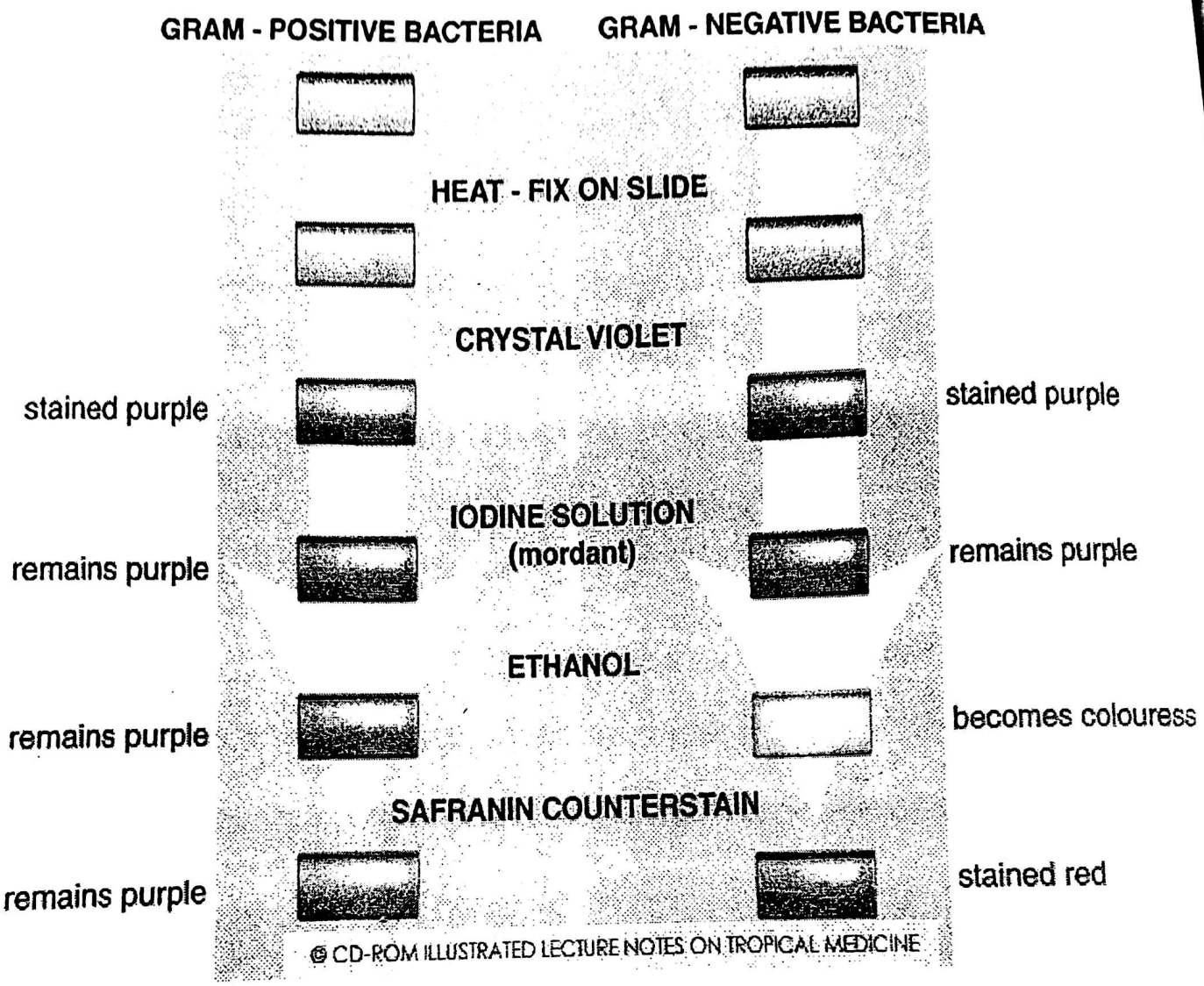
Procedure:

A. Slant Cultures

1. Prepare and heat-fix smears.
2. Prepare the smears of S. epidermidis and N. sicca on a second slide. Heat-fix.
3. Stain the slides as follows:
   a. Flood the crystal violet for one minute.
   b. Pour off excess dye and wash gently in tap water and drain the slide against a paper towel.
   c. Expose the smears to Gram's iodine for one minute by washing with iodine, then adding more iodine and leaving it on the smear until the minute is over.
   d. Wash with tap water and drain carefully. (Do not blot.)
   e. Wash with 95% alcohol for 30 seconds.
   f. Wash with tap water at the end of the 30 seconds to stop the decolorization. Drain.
   g. Counterstain with 0.25% safranin for 30 seconds.
   h. Wash, drain, blot, and examine under oil.
   i. Draw the cells showing morphology, grouping, and relative sizes. Color a few of the cells of each bacterial species to show the Gram reaction.
   j. Save these slides and the ones from parts B & C of this exercise to use at the next lab period.

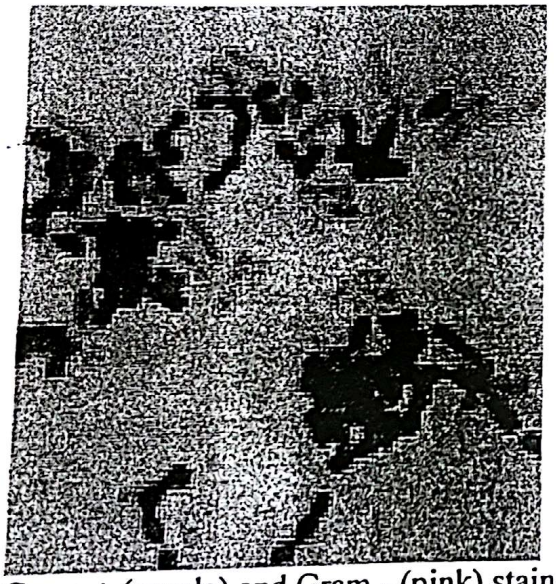
B. Broth Cultures

1. Because the smear made from the broth will be a thin smear and nearly invisible to the naked eye even after staining, it may be advisable to draw a ring with a felt open on the under side of the slide to mark the area in which the broth smear will be made. Also, when making a smear from broth do not add a drop of water to the slide.
2. Hear-fix the smears, Gram stain them with the above procedure, and examine them. When focusing the broth smear use the technique suggested for thin smears.
3. Compare the appearance of the cells in the two smears.

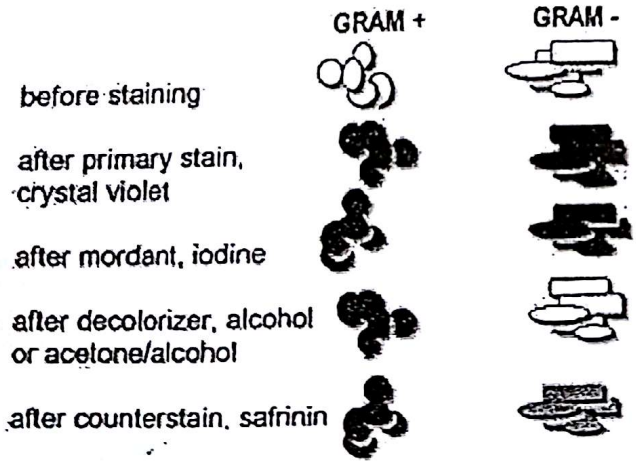


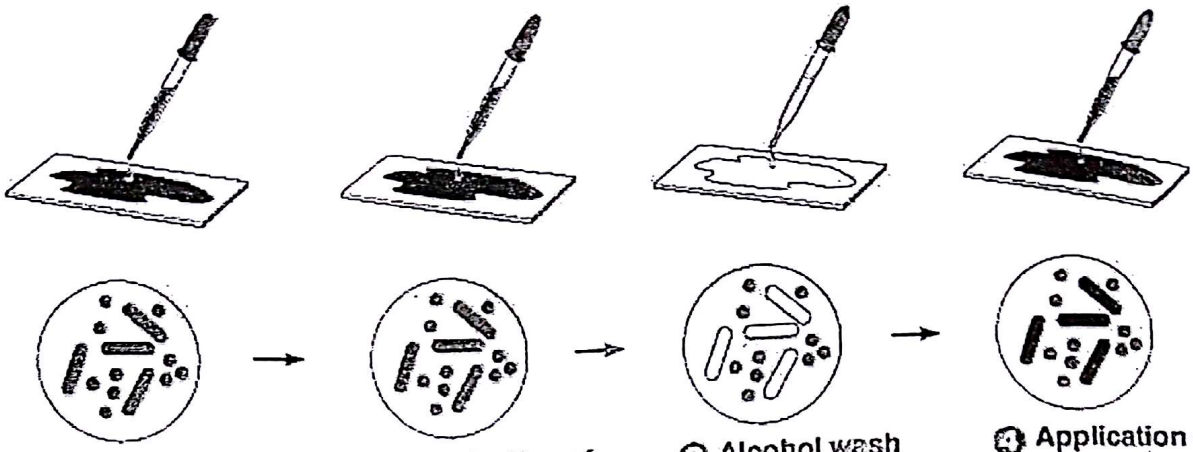
Gram Stain Procedure:

1. Flood the slide with **Crystal Violet** (the *primary stain*).
2. After 1 minute, rinse the slide with water.
3. Flood the slide with **Iodine** (Iodine is a *mordant* that binds with Crystal violet and is then unable to exit the Gram+ peptidoglycan cell wall.)
4. After 1 minute, rinse the slide with water.
5. Flood the slide with **Acetone Alcohol.** (Alcohol is a *decolorizer* that will remove the stain from the Gram-negative cells.)
6. After 10 or 15 seconds, rinse the slide with water. (Do not leave the decolorizer on too long or it may remove stain from the Gram-positive cells as well.)
7. Flood slide with **Safrinin** (the *counterstain*).
8. After 1 minute, rinse the slide with water.
9. Gently blot the slide dry. It is now ready to be viewed under oil immersion (1000x TM) with a bright-field compound microscope



Gram + (purple) and Gram - (pink) stained cells.





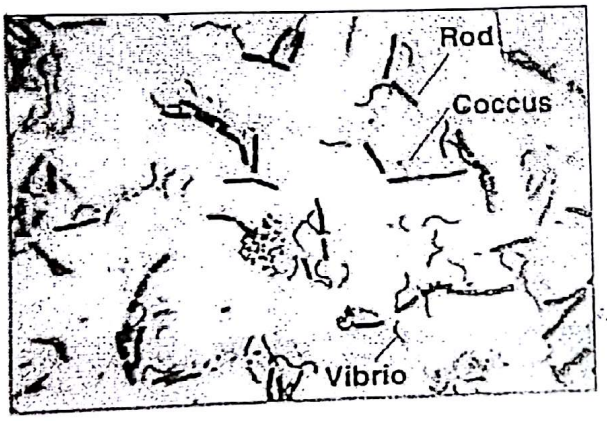
1 Application of crystal violet (purple dye)

2 Application of iodine (mordant)

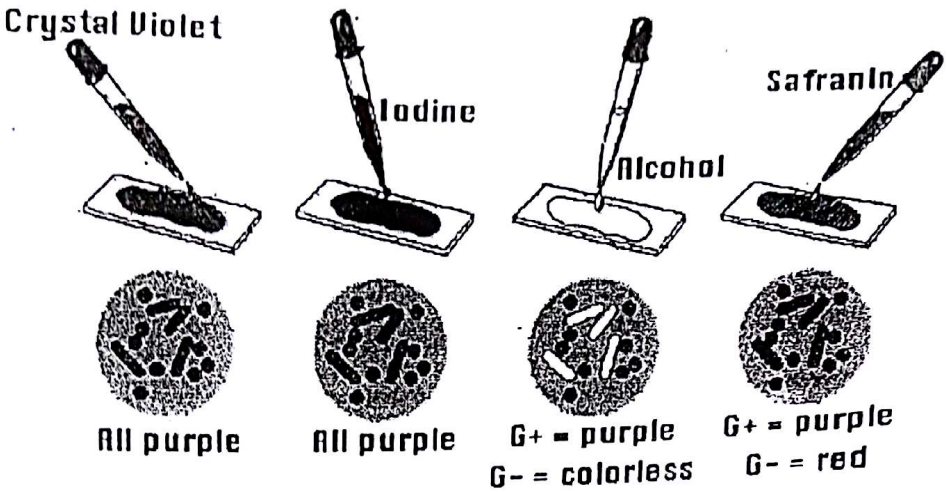
3 Alcohol wash (decolorization)

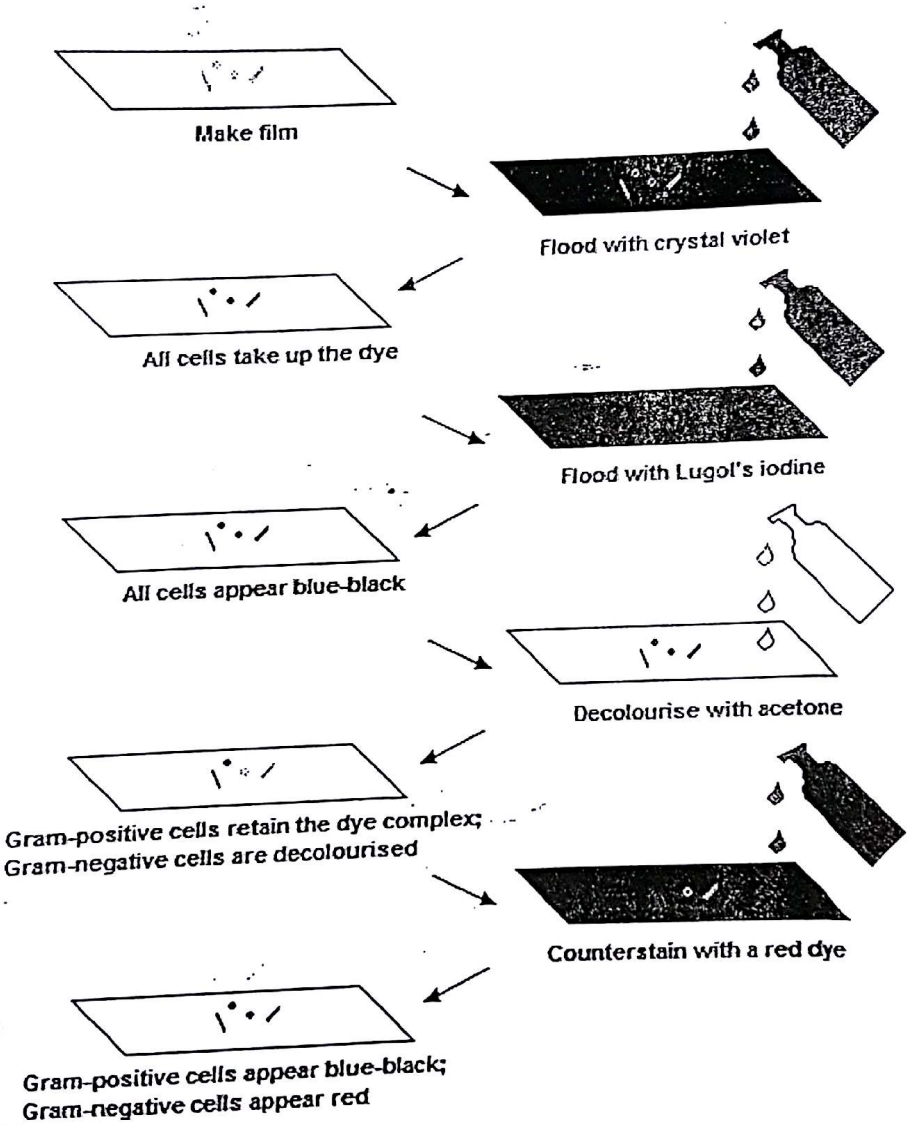
4 Application of safranin (counter

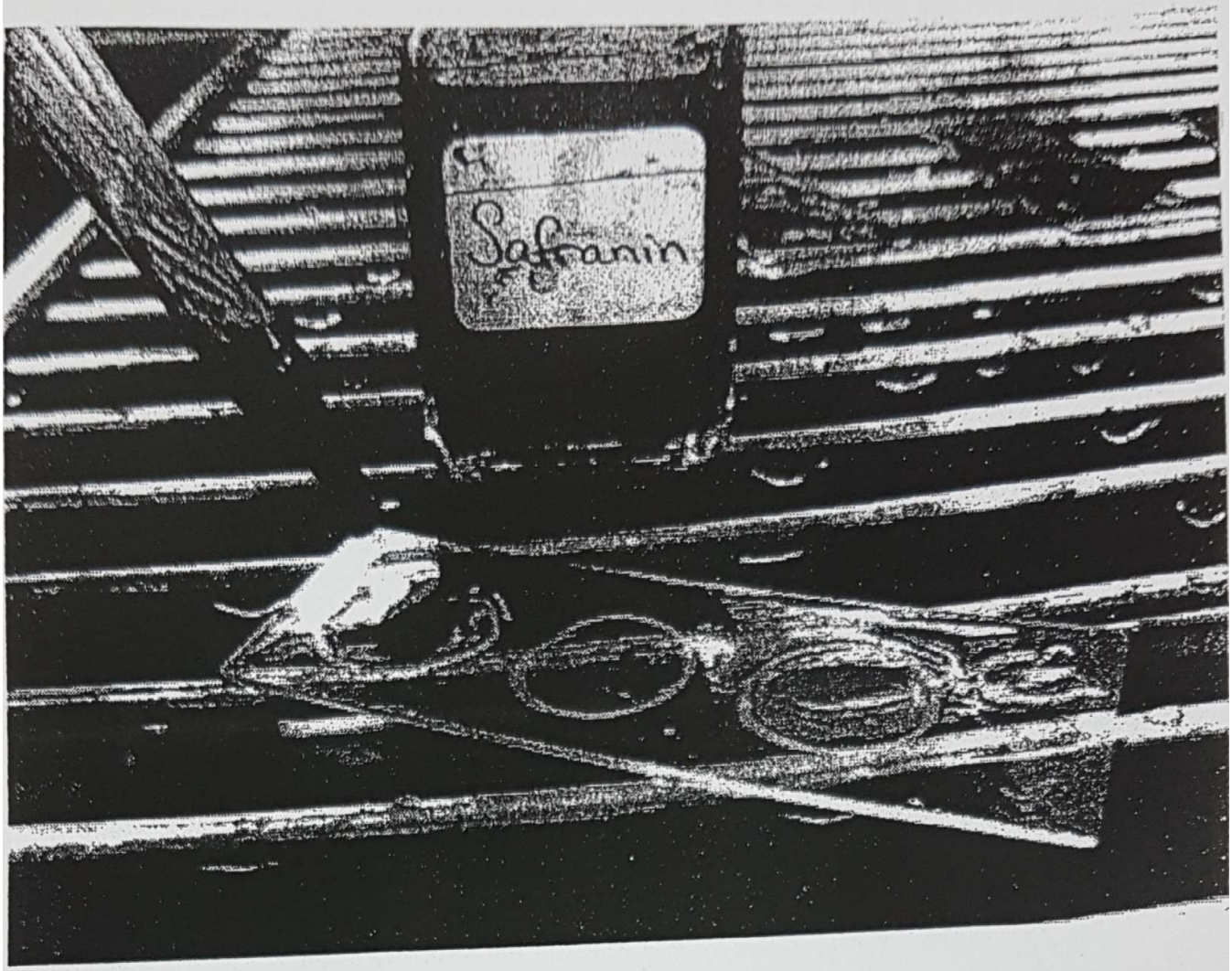
(a)

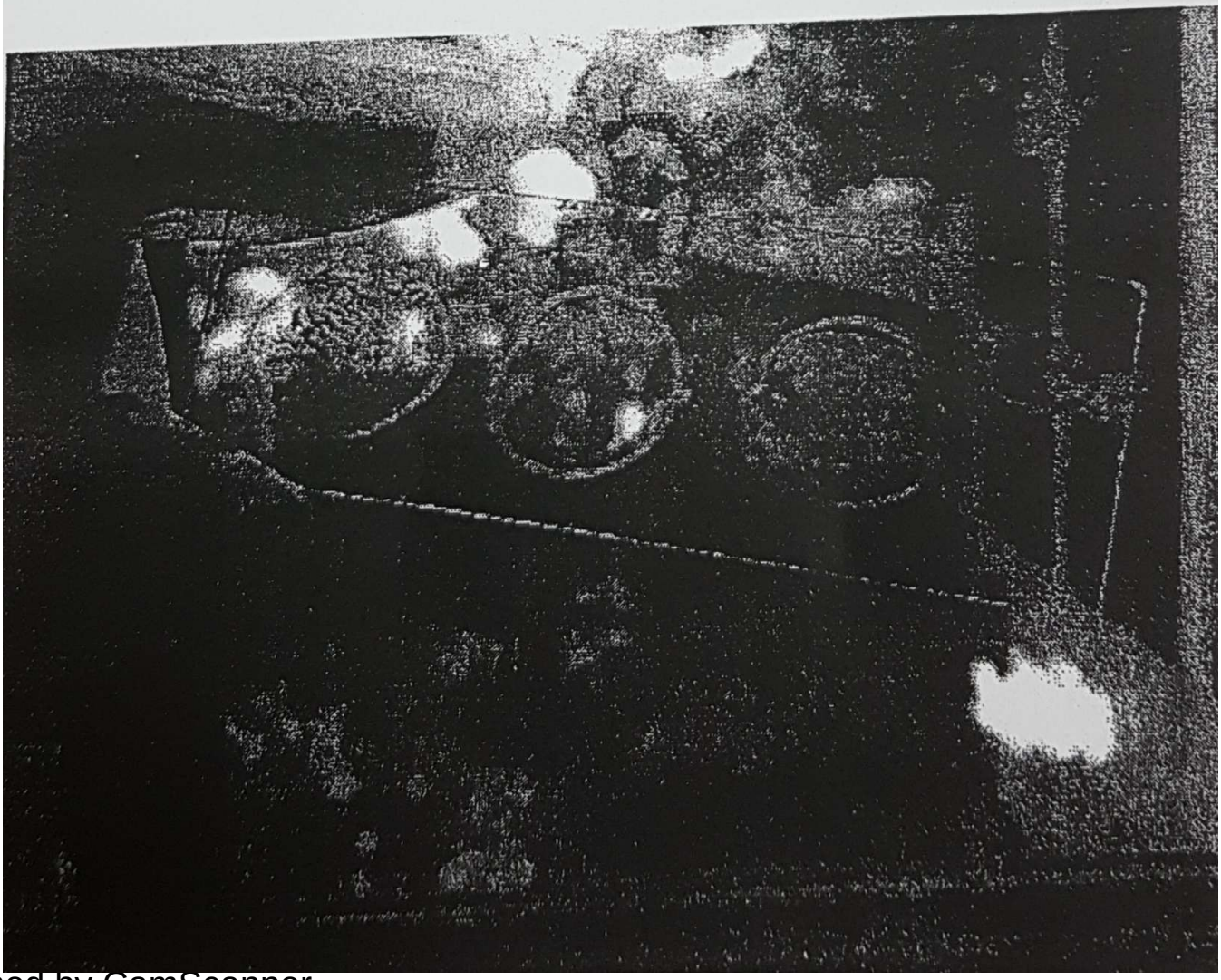


(b)

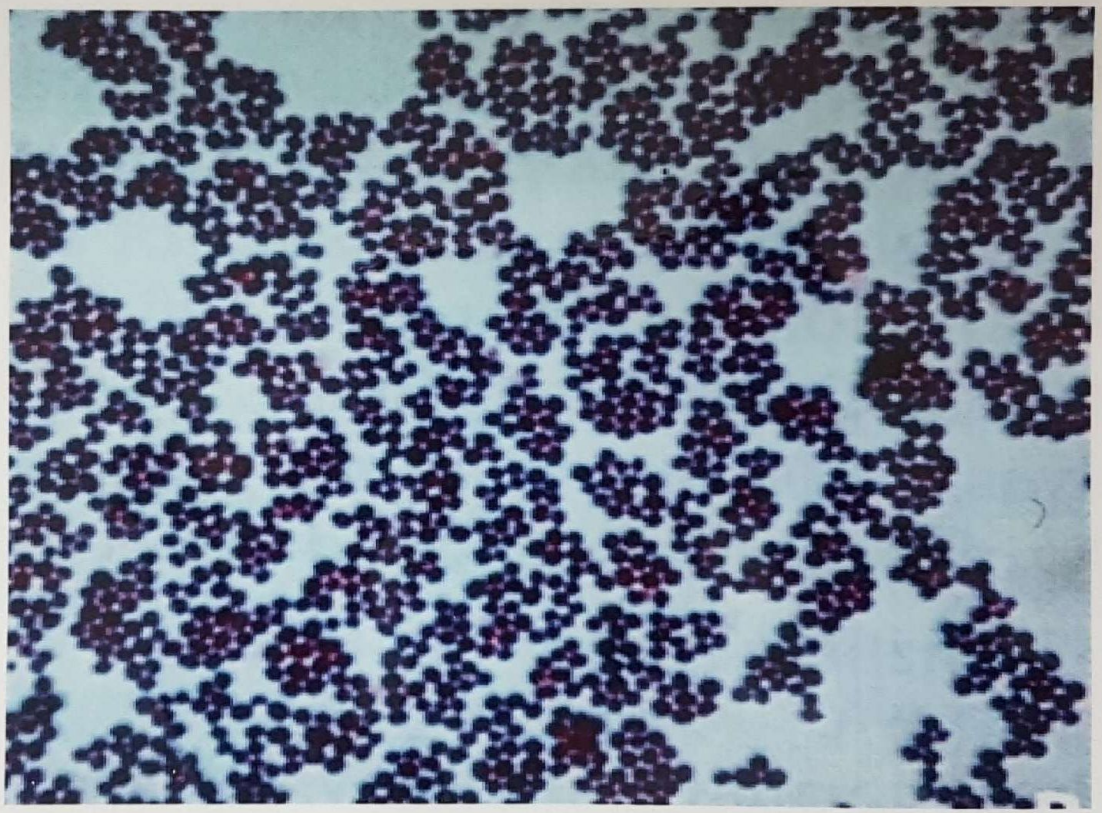


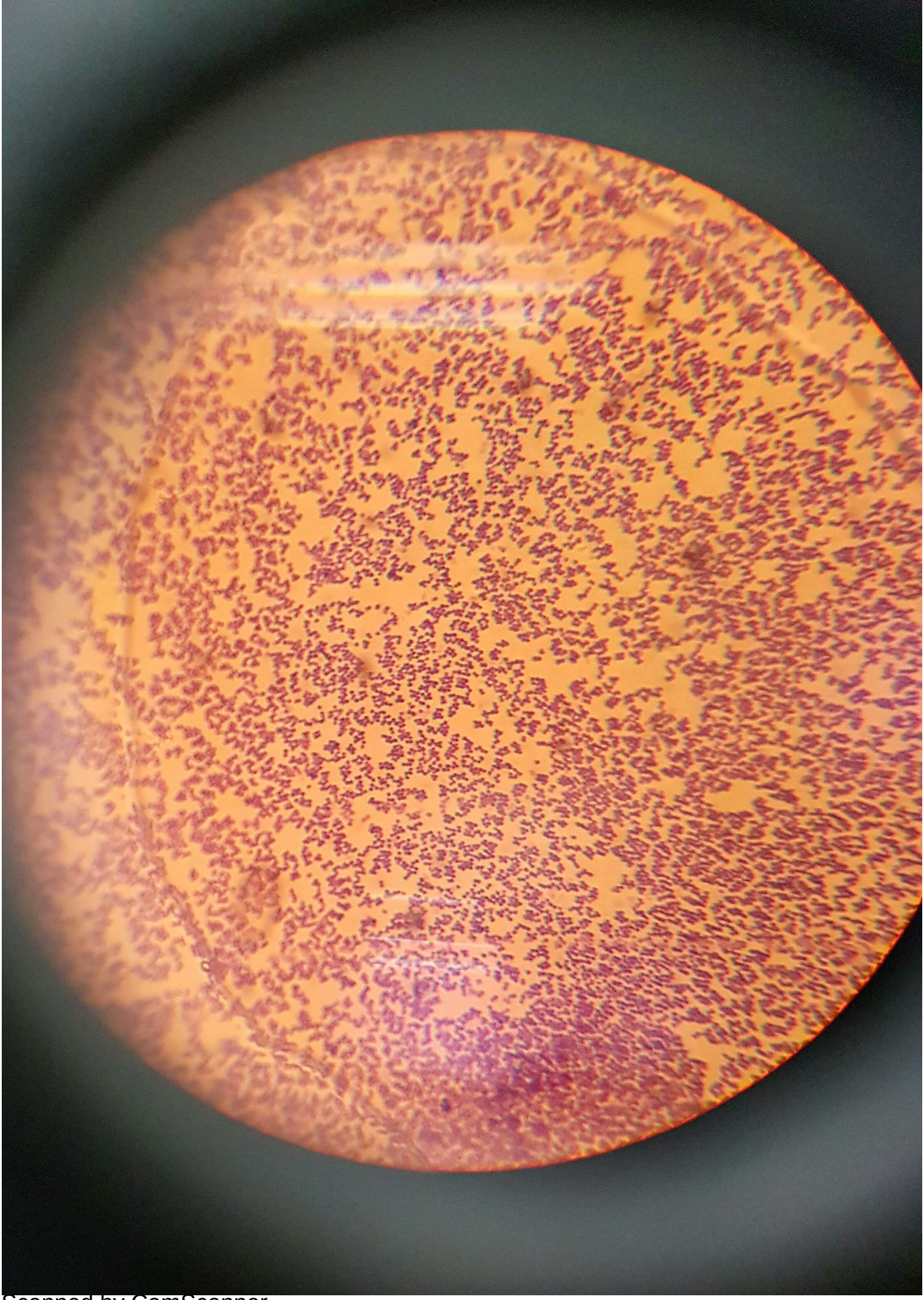






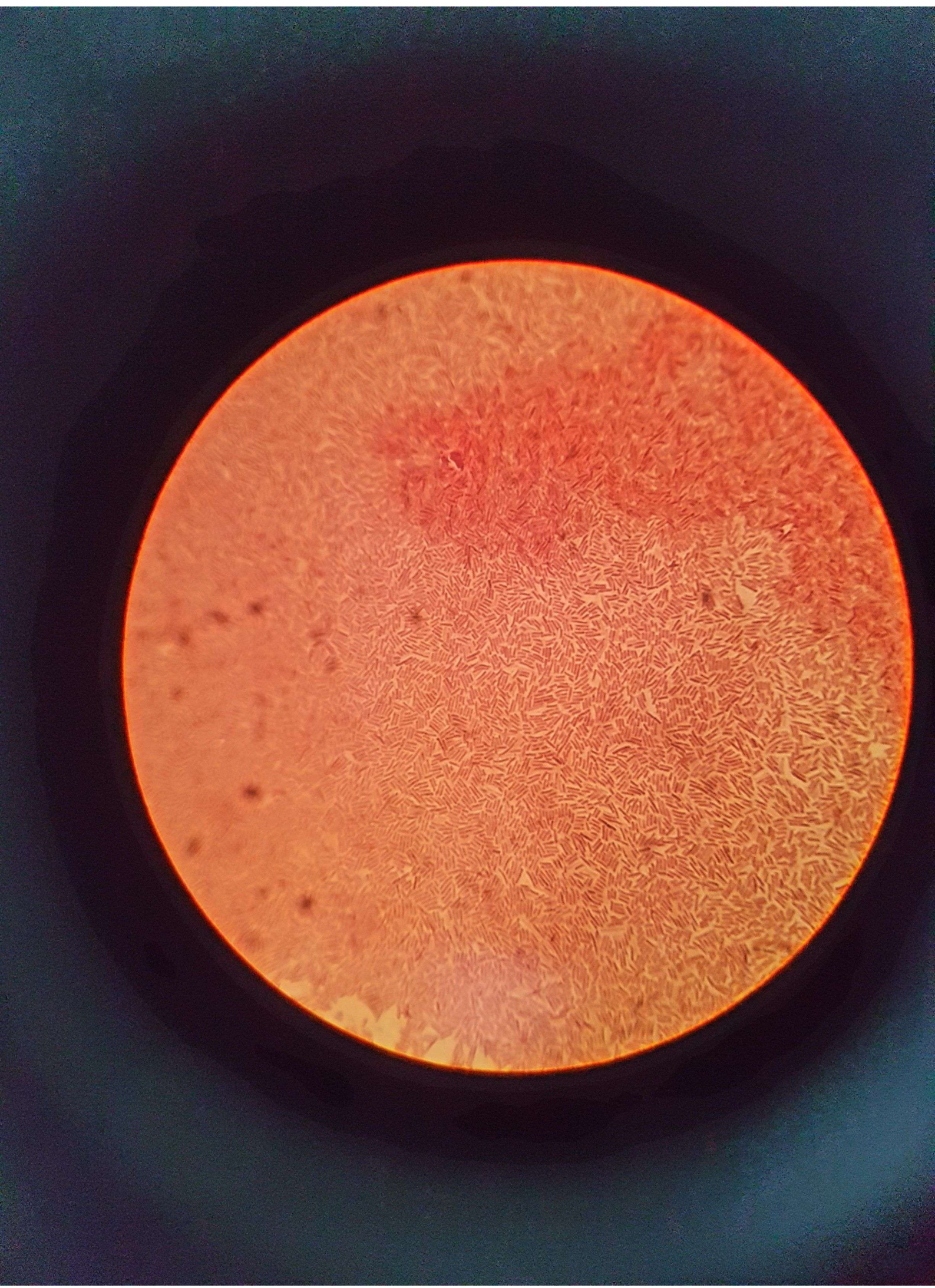
Staphylococcus





Gram negative Bacilli

E.Coli



A gram stained bacterial suspension containing a mixture of Gram negative bacilli, and Gram positive cocci arranged in bunches (Staphylococci spp)