Regional Medical Center®

Standard Operating Procedure

SUBJECT: Gram Stain			
ORIGINATION DATE:	March 2025	**PREPARED BY:**	**APPROVED BY (If Applicable):**
LAST REVIEWED:	NA	Lab	

PROCEDURE:

1. Verify Gram Stain order is for in-house and the specimen type & body site completed
2. Log into REGM Micro
    a. Free text pertinent information Free Text Source box
    b. Uncheck Print Label–OK
3. Prepare a thin, even smear of material on a labeled clean glass slide. Specimens from broth or liquid may be placed directly on the slide. Bacterial colonies may be emulsified in small amounts of saline and then placed on the slide.
4. Prepare a QC slide using Staphylococcus aureus and E.coli organisms from plated cultures. Label accordingly.
5. All reagents are stored at room temperature in the lower cupboard by the Micro sink.
    a. Reagents are stable until the expiration date on the bottle.
    b. The methanol bottle in use is stored in the lower cupboard by the Micro sink to protect from light.
    c. Stains in use are kept next to the Micro sink. Protect from light by placing the lid on the stain kit.
6. Allow the smear to air dry without heat.
7. Methanol fix the slide by flooding with absolute methanol (95%) for 1-2 minutes, then tilt the slide to drain off methanol and allow it to air dry.
8. Place the slide on a staining rack, cover with crystal violet solution, and let stand for 1 minute.
9. Pour off stain and rinse briefly with cold tap water.
10. Shake off excess water. Cover slide with iodine solution and let stand for 1 minute.
11. Pour off iodine and rinse briefly with cold tap water.
12. Rinse with decolorizer just until there is no more color coming off the slide. Quickly rinse off remaining decolorizer with cold tap water.
13. Shake off excess water. Cover with safranin solution for 1 minute.
14. Pour off safranin and rinse with cold tap water, air dry, and examine microscopically.

15. Interpret gram positive/negative and morphology
    a. If bacteria appear blue to purple, they are considered gram positive.
    b. If bacteria appear pink to red, they are considered gram negative.
    c. Morphology ie: cocci, bacilli, cocci in cluster, etc
16. Clinical specimens should also note presence or absence of any cellular elements or mucous (ie: yeast, WBC, etc)
17. From the Micro Pending Log select specimen and bridge to ARE(Results)
    a. Move cursor to Entry Box, hit F2 and choose Gram Stain Report, ADD
    b. Click in the empty white box in the Response section. Here you will enter your results:
        i. First enter the quantity (F=few, mod=moderate, M=many)
        ii. In the next box enter the bacteria seen (GPC=gram positive cocci)
        iii. Go down to the next line for each additional thing seen.
        iv. For a list of the comments available, press F2 on your keyboard.
        v. Document all called results.
18. All gram stains need to be prepared, stained and read by **two** techs.
    a. The 1st tech should choose **PERFORM** the Gram Stain Results in Cerner (Not Verify)
    b. The 2nd tech should do the gram stain and if the results agree then **VERIFY** the Gram Stain Report
    c. All discrepancies must be resolved prior to verifying. (Troubleshoot by repeating the gram stain or 3rd tech review, etc)

References:

Hardy Diagnostic Gram Stain Instructions for Use, IFU-1045(E)