UnityPoint Health Pekin
Department of Pathology
Pekin, IL 61554

Effective Date: 02/11/19
Date Reviewed/ Date Revised: 02/19/19

GRAM STAIN
Order and Result for Monthly Slide Competencies
VCGRAM

I. PRINCIPLE

For all techs to participate in monthly slide competencies for microbiology samples. Slides are prepared at Methodist Microbiology department and sent monthly. Some months we may need to stain the slides ourselves or they can come already stained for us. This gives us the opportunity to see where we may need to focus more attention and how each tech is performing since we do not perform gram stains on a daily basis.

II. SIGNIFICANCE

This allows us to report our monthly competency slide like our patient slides in SunQuest. Not only are we testing our gram stain skills we are taking this opportunity to test our skill in Sunquest. Along with following written instructions and meeting a deadline. Each month the techs will order, receive and result their gram stain competencies based off the type of slide Methodist has submitted to us.

III. SPECIMEN

A. Smears for gram stain will be sent from UPH Methodist microbiology dept. They will either send stained or unstained slides for monthly competencies.

IV. REAGENT

A. Crystal violet stain-purchased commercially
B. Safranin stain-purchased commercially
C. Gram Iodine-purchased commercially, prepared by adding the iodine concentrate to the gram iodine diluent and mix well.
D. Decolorizer-prepared by mixing one part 95% ethyl alcohol with one part acetone.
E. Storage
    1. Reagents are stored at room temperature.
    2. Gram iodine solution is stable for 3 months once prepared.

V. QUALITY CONTROL

A. All Gram stain reagents are quality controlled by lot # as they are received in the laboratory
B. Staff will perform a gram positive and gram negative (QC slide) control if the monthly slides need stained.
C. Gram stain QC results are logged on the appropriate daily/monthly QC chart at the workbench.

D. Monthly proficiency testing is performed by all staff responsible for testing to assure consistent reporting.

VI. PROCEDURE:

A. Order and receive gram stains from following instructions:

1. Log into SQ
2. Order entry
3. Patient ID: CAP-
4. Enter
5. Click Create
   Fill in:
   Last Name: Use your first name
   First Name: Slide # (Example: Slide 1, Slide 2, etc.....)
   DOB: Doesn't have to be your DOB. Just pick a date
   Time: Just enter through
   Sex: F or M
6. Click New Episode: (Middle of page Lt. Side)
7. Financial Class: SELF
8. Tab
9. Click SAVE
10. Fill in collect date and time: for time type "N" for now that way it will leave received date and time open to receive later.
11. Order: VCGRAM
12. TAB to Modifier box
    Use a modifier code like RT. Then -;which slide it is (Example: RT-;chest fluid)
13. Save
14. Pop up box for site will come up. Use codes from micro list (found in send out procedure book). Use proper code for specimen. (Example: FLUID-; chest fluid) This should be in the SDES box. SREQ type HIDE.
15. TAB out of box
16. Save
17. Go to General Lab and receive sample.
    NOTE: Do not order ahead of time and leave unresolved. This tends to add to the pending logs here and at Methodist. Order, result and finalize all in the same day.

B. Stepwise
1. Flood the fixed smear with crystal violet stain. Allow the stain to remain for 15 seconds to 1 minute.
2. Rinse the slide gently with tap water.
3. Flood the slide with working gram iodine solution and allow to stand for 15 seconds to 1 minutes.
4. Rinse the slide gently with gram decolorizer. Decolorization is complete when the solution runs clear from the slide. (Depends on thickness of the smear).
5. Rinse the slide gently with tap water.
6. Flood the slide with safranin stain and allow to stand for 15-30 seconds.
7. Rinse the slide gently with tap water.
8. Drain the slide and air dry it in an upright position or blot dry.
9. Examine the slide microscopically.
10. Examine the smear microscopically
11. Using the 10X objective, quantitate the number of inflammatory and epithelial cells.
12. Quantitate and identify bacterial morphologies and Gram stain reactions using the 100X objective (oil immersion).

C. Screening Sputum's for acceptability:
1. Gram stains of expectorated sputum specimens for routine culture are used to determine the acceptability of the specimen prior to culturing. Sputum gram stains are screened using low power. Specimens with greater than 10 squamous epithelial cells/low power field (10X objective) are rejected.
2. Specimen is acceptable if columnar epithelial cells or macrophages are present.
3. When specimen is not acceptable use the Direct Exam page note unacceptability. Type "SALV" or use key "U" = specimen microscopically resembles saliva, not cultured.
4. Then finalize

D. Resulting Sputum's for monthly competencies:
1. Micro result entry
2. Look up by accession number
3. The screen that comes up is the Direct exam page where you will enter your results or your rejection code "SALV" or to note any findings like bacteria, WBC's and EPI's.
4. Use F8 (See last page in procedure for keyboard interpretation) on your keyboard to display the Micro keyboard for resulting options.
5. Use one line for each organism or cellular element entry.
6. Once everything has been entered finalize report either using F8 to display the micro keyboard (Key-?/ FNL) or "?/" key on your keyboard.

Commented [U1]: What if a sputum has no bacteria or WBC's present? Reject?

7. Answer popup box
8. Save
9. Billing tab should appear. Do not enter anything here just click save.
10. Go to Lab inquiry to review results and make sure your gram stain is finalized. If so print out your sheet to hand in. If not go back and finalize.

NOTE: If you can't get your gram stain to finalize check to make sure your Site has been put in under the SDES box and HIDE is in SREQ.

VII. RESULTS FOR OTHER THAN SPUTUM SAMPLES

A. To pull up the micro keyboard press "F8".
B. Quantify epithelial cells and white blood cells using 10x objective as follows:
   1. Few = 0-10/lpf
   2. Moderate = 10-25/lpf
   3. Many = >25/lpf
C. Quantitate and identify bacterial morphologies as follows:
   1. Few = 1-5 in every field
   2. Moderate = 6-30 organisms/oil field
   3. Many = >30 organisms/oil field
D. Observe gram reaction as either gram positive (deep violet) or gram negative (pink or red). Gram variable organisms may also be observed.
E. Staining characteristics: even, bipolar, beaded or irregular staining may occur.
F. Shape of organisms: may appear as:
   1. Coccus
   2. Coccobacillary
   3. Rod
   4. Filament or yeast-like or pleomorphic (variation in shape).
G. Characteristic arrangements may be noted:
   1. Singles
   2. Pairs
   3. Chains
   4. Tetrads
   5. Clusters
   6. Palisades or Chinese letters.

H. Result slides using following instructions:
   1. Micro result entry
   2. Look up by accession number
   3. The screen that comes up is the Direct exam page.
   4. Use F8 (See last page in procedure for keyboard interpretation) on your keyboard to display the Micro keyboard for resulting options.

5. Use one line for each organism or cellular element entry.
6. Once everything has been entered finalize report either using F8 to display the micro keyboard (Key-?/ FNL) or "?/" key on your keyboard.
7. Answer popup box
8. Save
9. Billing tab should appear. Do not enter anything here just click save.
10. Go to Lab inquiry to review results and make sure your gram stain is finalized. If so print out your sheet to hand in. If not go back and finalize.

NOTE: If you can't get your gram stain to finalize check to make sure your Site has been put in under the SDES box and HIDE is in SREQ.

VIII. PROCEDURAL NOTES/PROBLEM SOLVING TIPS:

A. Excessive Decolorization

1. Excessive heat during fixation: Heat fixing the cells, when done to excess, alters the cell morphology and makes the cells more easily decolorized.
2. Low concentration of crystal violet: Concentrations of crystal violet up to 2% can be used successfully, however low concentrations result in stained cells that are easily decolorized. The standard 0.3% solution is good, if decolorization does not generally exceed 10 seconds.
3. Excessive washing between steps: The crystal violet stain is susceptible to wash-out with water (but not the crystal violet – iodine complex). Do not use more than a 5 second water rinse at any stage of the procedure.
4. Insufficient iodine exposure: The amount of the mordant available is important to the formation of the crystal violet – iodine complex. The lower the concentration, the easier to decolorize (0.33% - 1% commonly used). Also, QC of the reagent is important as exposure to air and elevated temperatures hasten the loss of Gram's iodine solution. A closed bottle (0.33% starting concentration) at room temperature will lose >50% of available iodine in 30 days, an open bottle >90%. Loss of 60% iodine results in erratic results.
5. Prolonged decolorization: 95% ethanol decolorizes more slowly, and may be recommended for inexperienced technicians while experienced workers can use the acetone-alcohol mix. Skill is needed to gauge when decolorization is complete.
6. Excessive counterstaining: As the counterstain is also a basic dye, it is possible to replace the crystal violet-iodine complex in gram positive cells with an over-exposure to the counterstain. The counterstain should not be left on the slide for more than 30 seconds.

B. Leukocytes

1. If properly decolorized, leukocytes will appear gram-negative (pink-to-red),

due to the retention of the safranin counterstain.

C. Troubleshooting
   1. If the smear is too thick, the cells can appear gram-positive in very thick area. You may see Gram-variability from the thick to the thin areas.
      Solution: Try to prepare a single cell layer of organism.
   2. If cells are prepared in hyper or hypotonic solutions, morphology may be disturbed. Solution: Smear the cells onto the slide dry with a sterile toothpick.
   3. Over warming the smear (this happens most often when smears are warmed prior to being completely air dried, or when flaming too much to fix the slides) will cause all cells to appear Gram-negative.
      Solution: Dry slide thoroughly prior to "heat fixing", be extremely careful when using flames.
   4. Gram-variability, this can be due to the organism itself, and not to the staining method.
      Solution: The vast majority of Gram-variable organisms are Gram-positive. Characteristically Gram-variable organisms (e.g. Corynebacterium variabilis) or those whose membrane alter with age and appear Gram-variable (e.g. Arthrobacterium spp.) are grouped with the Gram-positive organisms. Therefore, they are treated as Gram-positive organisms.

IX. REFERENCES

A. Clinical Microbiology Procedures Handbook, section 1.5 ASM, 1994.
B. Bailey and Scott's Diagnostic Microbiology 12th edition, 2007. Page 80-83.
C. The Microbiology Network, "The Gram Stain", Feb 2006. www.microiol.org. 04/2017.
D. CAP, 325 Waukegan Road, Northfield Illinois 60093-2750. Survey - Gram Stain D5-C 2016, slide D5-13 discussion.
E. Arrow Scientific. "Differentiating Gram-negative and Gram-positive Bacteria". 2013 www.arrowscientific.com. 04/2017

POLICY CREATION :	***Date***
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Medical Director: ***Lori Racsa, DO***	***02/11/19***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
SECTION MEDICAL DIRECTOR		

REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date

Reviewed by

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date

YELLOW HIGHLIGHTED = MOST COMMON

`/`= OCC = OCCASIONAL

!/1 = RARE= RARE

@/2 = FEW = FEW

#/3 = MOD = MODERATE

$/4 = MANY = MANY

%/5 = CELREM = CELLULAR REMNANTS

^/6 = CPUTD = CELLS PRESENT, UNABLE TO DIFFERENTIATE

&/7 = CHS = IN CHAINS

*/8 = CTRB = CALLED TO READ BACK BY

(/9 = CLS = IN CLUSTERS

)/0 = NURFGS = BACTERIAL MORPHOTYPES CONSISTENT WITH NORMAL UPPER RESPIRATORY FLORA

Q= WBCS = WBC's SEEN

W= EPIT = EPITHELIAL CELLS

E= CECP = COLUMNAR EPITHELAL CELLS PRESENT

R = RBSN= RBC's SEEN

T = MONCEL = MONONUCLEAR CELLS

Y= FUNGEP= FUNGAL ELEMENTS PRESENT

U= SALV = SPECIMEN MICROSCOPICALLY RESEMBLES SALIVA, NOT CULTURED

I = PREL = PRELIMINARY REPORT- SMEAR TO BE REVIEWED IN AM

O = FSR = FINAL SMEAR REPORT

P = YEAST = YEAST

{/[= YPSU = YEAST WITH PSEUDOHYPHAE

}/] = NOYF = NO YEASR OR FUNGAL ELEMENTS OBSERVED

A= GPC = GRAM POSITIVE COCCI

S =GPR= GRAM POSITIVE RODS

D = NBS= NO BACTERIA SEEN

F= NWMO = NO WBCs OR ORGANISMS SEEN

G= NOWBC = NO WBCs SEEN

H= GPDC = GRAM POSITIVE DIPLOCCI

J= GPCB= GRAM POSITIVE COCCOBACILLUS

K= POSI = POSITIVE

L = NEGA= NEGATIVE

;/: = othr = OPENS THE BOX TO SEARCH

Z = GNC = GRAM NEGATIVE COCCI

X = GRAM NEGATIVE RODS

C = GNCB = GRAM NEGATIVE COCCOBACILLI

V = GVR = GRAM VARIABLE RODS

? / / = fnl = FINAL REPORT