Clinical Laboratory Department
POLICY AND PROCEDURE

POLICY NUMBER: 1181
VERSION: 3

SUBJECT: Gram Stain

Principle

Prokaryotes will differentially retain crystal violet depending on cell wall characteristics. Bacteria can be grouped initially based on their Gram stain reactions. Testing may be performed on direct clinical specimens or culture isolates.

Materials and Reagents

Crystal Violet - Primary stain
Grams Iodine - Mordant
Decolorizer
Safranin - Counterstain
Microscope slides
bacteriological loop
microscope with oil immersion lens

Storage

Store all reagents at 15-30°C.

Precautions

Refer to SDS for hazards of these chemicals.

As with all techniques involving pathogenic and potentially pathogenic microorganisms, established aseptic practices should be consistently applied throughout this procedure.

Specimen

Apply the test specimen to a clean glass microscope slide in a thin, uniform smear preferably by rolling the swab onto the slide. Emulsify colonies from an 18-24 hr culture in saline to obtain the proper density. Allow the smear to air dry. Fix the smear to the slide by flooding with absolute methanol for 1-2 minutes and rinse with tap water before staining.

Procedure

1. Flood the fixed smear with primary stain, Crystal Violet, and stain for 1 minute.
2. Remove the primary stain by gently washing with cold water.
3. Flood the slide with Grams iodine working solution and stain for 1 minute.
4. Remove the mordant by gently washing with water.

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5. Briefly decolorize until solvent running from the slide is colorless, 30-60 seconds maximum.
6. Flood the slide with counterstain -Safranin for 30-60 seconds.
7. Wash the slide with cold water. Gently blot with paper towel and allow to air dry.
8. Examine the smear under an oil immersion lens.

Quality Control

Run controls each day of use using a known gram positive and gram negative microorganism, *i.e. Staphylococcus aureus* ATCC 25923 and *E. coli* ATCC 25922

Results

Gram positive organisms will appear microscopically as purple-black cells. Gram negative organisms will appear as pink to red cells.

Reporting

Scan the smear on low power and report according to the following scheme:

	Epithelial cells	WBC
4+	>100 cells/LPF	>150 cells/HPF
3+	50-100 cells/LPF	76-150 cells/HPF
2+	10-49 cells/LPF	25-75 cells/LPF
1+	<10 cells/LPF	<25 cells/LPF

Examine the smear on high power and report according to the following:

	Micro-organisms
4+	>100 org/HPF
3+	50-99 org/HPF
2+	20-49 org/HPF
1+	5-20 org/HPF
FEW	<5 org/HPF

Gram stains are to be read and reported using the Microbiology Coded Response Sheet as soon as possible, preferably within 24 hours of set up.

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Limitations of Procedure

The Gram stain provides preliminary identification information only and is not a substitute for cultural studies of the specimen. Gram reactivity is not an absolute characteristic and is influenced by several factors. The age of a culture affects its degree of Gram positivity; in general young actively growing cultures retain the crystal violet complex more avidly than cells from older cultures.

Prior treatment with antibacterial drugs may cause gram positive organisms from a specimen to appear gram negative.

References

Baron, Ellen Jo and Finegold, Sydney M., *Bailey and Scott=s Diagnostic Microbiology* 8th edition, St. Louis MO, 1990.

Sacher, Ronald A., McPherson, Richard A., *Widmann=s Clinical Interpretation of Laboratory Tests*, 10th edition. Philadelphia, PA, 1991.

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Date: 05/26/2017	**Original Date:** 01/15/1992
Reviewed: 05/26/2017	**Next Review Date:** 05/26/2018
Revised: 4/15/04db-revised language to HDHS, 7/23/04jh-changed time of reading/reporting, 5/19/10 jh-removed anaerobe references, 6/1/12 & 1/29/13 jh changes reflect purchase of reagents from different vendor, 12/25/14jh removed heat fixation references, 4/20/17jh changed approver to Dr.Yee	
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