PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20115 – Gram Stain	Policy Number: 15-157-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 12/04/2024	Next Review Date: 12/04/2026
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 12/04/2024
Accreditation Canada Applicable Standard: NA	

GUIDING PRINCIPLE:
The Gram stain is a differential staining method for staining bacteria from cultures or patient specimens. It classifies bacteria on the basis of their cell wall structure and allows observations of their size and cellular morphology. Bacteria can stain as Gram-positive, Gram-negative or Gram-variable.

PURPOSE/RATIONALE:
This standard operating procedure describes how to perform the gram stain.

SCOPE/APPLICABILITY:
This standard operating procedure applies to Medical Laboratory Technologists (MLTs) performing the gram stain.

SAMPLE INFORMATION:

Type	• Patient specimens requiring Gram stain. Refer to MIC10100-Microbiology Specimen Processing • Culture organisms requiring Gram stain for identification

REAGENTS and/or MEDIA:
- Methanol
- Gram Crystal Violet
- Gram Iodine (Stabilized)
- Gram Decolorizer
- Gram Safranin

SUPPLIES:
- Glass microscope slide
- QC slide
- Immersion oil
- Slide storage tray

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EQUIPMENT

- Hot plate
- Microscope

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Quality control is performed daily
- A TQC order is automatically generated daily to record the QC results
- Refer to MIC60060-Microbiology Stain Quality Control

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the gram stain	
1	Prepare a smear of the specimen to be stained. Refer to MIC10000-Specimen Handling for slide preparation instructions.
2	Place slide on the slide warmer in the BSC until dry.
3	Once dry, fix smears with methanol for 1 minute. After 1 minute, drain off remaining methanol without rinsing, and allow the slide to air dry again.
4	Flood the fixed smear with crystal violet and allow stain to remain for 1 minute. Decant crystal violet and rinse slide gently with running tap water. Excessive rinsing in this step can cause crystal violet to be washed from Gram-positive cells.
5	Flood the smear with Gram's iodine and allow stain to remain for 1 minute. Rinse slide gently with running tap water.
6	Decolourize by letting the decolourizing reagent flow over the smear while the slide is held at an angle. Stop when the runoff becomes clear. Adjust decolourization time to thickness of smear. Remove excess decolorizer with gentle flow of tap water.

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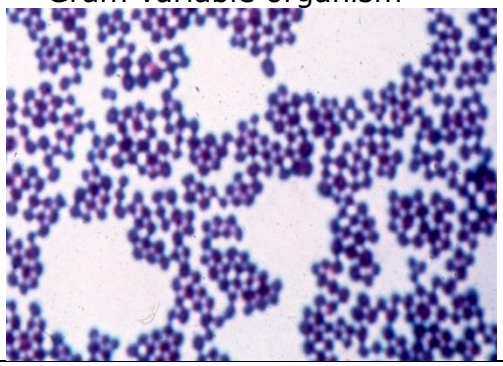
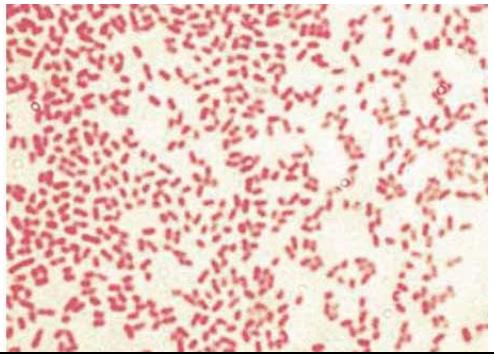
7	Flood smear with safranin and allow stain to remain for 1 minute. Remove excess counterstain with a gentle flow of tap water.
8	Drain slide and air dry in an upright position. Slides may be gently blotted with filter paper to remove excess water, but care should be taken to avoid wiping the stained material from the slide.

INTERPRETATION OF RESULTS:

Step	Action
1	• Deep violet = Gram-positive organism • Pink or red = Gram-negative organism • Both Gram-positive and Gram-negative cells with same morphology = Gram variable organism  
2	If unable to differentiate organisms from cellular debris or for positive blood cultures with no bacteria seen, perform acridine orange stain. Refer to MIC20100-Acridine Orange Stain.

REPORTING INSTRUCTIONS:

Refer to:

- MIC20200-Gram stain reporting in LIS-Routine Specimens
- MIC20300-Gram stain reporting in LIS-Respiratory Specimens
- MIC20400-Gram stain reporting in LIS-Sterile Fluids Specimens
- MIC20500-Gram stain reporting in LIS-Blood Culture Specimens
- MIC20600-Gram stain reporting in LIS-Bacterial Vaginosis Screens
- MIC20800-Gram stain reporting in LIS-Genital Specimens

LIMITATIONS:

1. Use an 18–24 hour culture as they have a greater affinity for the dyes than old cells.
2. Microorganisms that are physically disrupted by excess heat fixation will not react to the Gram staining as expected.
3. Gram stain results, including organism morphology, can be affected by the age of the isolate, autolytic enzymes, cultures transferred to media containing antibiotics, as well as specimens collected from patients on antibiotics.
4. Precipitate from crystal-violet stain can appear as irregular coccoid shapes or asters resembling fungal hyphae.

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CROSS-REFERENCES:

- MIC10000-Microbiology Specimen Handling
- MIC10100-Microbiology Specimen Processing
- MIC20100-Acridine Orange Stain
- MIC20200-Gram stain reporting in LIS-Routine Specimens
- MIC20300-Gram stain reporting in LIS-Respiratory Specimens
- MIC20400-Gram stain reporting in LIS-Sterile Fluids Specimens
- MIC20500-Gram stain reporting in LIS-Blood Culture Specimens
- MIC20600-Gram stain reporting in LIS-Bacterial Vaginosis Screens
- MIC20800-Gram stain reporting in LIS-Genital Specimens
- MIC60060-Microbiology Stain Quality Control
- LQM70620-Laboratory Critical Results List-Microbiology

REFERENCES:

1. Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
2. BD Gram Stain Kits and Reagents package insert, 2024-03

APPROVAL:

April 12, 2024
Date

Director, Laboratory and Diagnostic Imaging Services

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	07 Feb 19	Initial Release	L. Steven
2.0	31 Mar 22	Procedure reviewed and added to NTHSSA policy template	L. Steven
3.0	19 Feb 24	Procedure reviewed	L. Steven

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