PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20200 – Gram stain reporting in LIS-Routine Specimens	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director, Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
The gram stain has many uses: principally, it classifies bacteria on the basis of their cell wall structure and allows observation of their size and cellular morphology. Bacteria stain either gram positive or gram negative based on differences in cell wall composition.

PURPOSE/RATIONALE:
This standard operating procedure describes how to report the Gram stain results of routine specimens in the LIS in a consistent manner.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) reporting routine specimens in the LIS.

SAMPLE INFORMATION:

Type	• Wound, ear, eye, lower genital tract (excluding BV) and male urethra gonorrhoeae specimens • Refer to MIC10100-Microbiology Specimen Processing

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

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SUPPLIES:

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

EQUIPMENT

- Hot plate
- Microscope

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential 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
Reporting routine specimens in the LIS	
1	• Pending Gram stain orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRM/MISC_1of2**  • Press enter or double click to open worklist

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<table>
<tr><td>2</td><td>

Enter the accession number on the slide and select enter to mark the order
Select enter again to open Result Entry or double click on accession number to open

</td></tr>
<tr><td>3</td><td>
<u>Under low power (X10, LPF):</u> screen slide to locate good specimen areas to obtain an overall impression of cell types present.

Observe slide for stain crystals:

If an excess of precipitated stain is observed, prepare another smear
If precipitate continues, use freshly filtered crystal violet

Determine if slide has been properly decolorized:

Depending on the source of the specimen, the background should be generally clear or Gram negative
If white blood cells are present, they should appear completely Gram negative
If slide is over decolorized, prepare another smear

Determine if thickness of smear is appropriate:

For proper interpretation, areas must be no more than one cell thick, with no overlapping of cells. Prepare a new slide if unreadable

Examine for evidence of inflammation:

Determine areas representative of inflammation and areas of contamination with squamous epithelial cells

</td></tr>
<tr><td>4</td><td>Add one drop of immersion oil to the slide. In a representative area with predominance of inflammation or purulence using the oil immersion lens (100X), examine 20 to 40 fields to observe cell morphology and Gram reaction.</td></tr>
<tr><td>5</td><td>
<u>Under oil immersion (X100, OIF)</u>: quantitate epithelial cells, white blood cells, red blood cells and bacteria as follows:
<table>
<tr><th>None seen</th><th>No cells seen</th></tr>
<tr><td>1+</td><td>< 1 cell seen</td></tr>
<tr><td>2+</td><td>1 - 9 cells seen</td></tr>
<tr><td>3+</td><td>10 - 25 cells seen</td></tr>
<tr><td>4+</td><td>> 25 cells seen</td></tr>
</table>
NOTE: Only report “None seen” for white blood cells and bacteria. If no epithelial cells or red blood cells are seen, do not report this
</td></tr>
</table>

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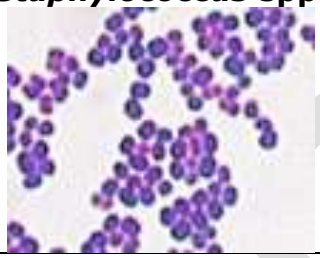
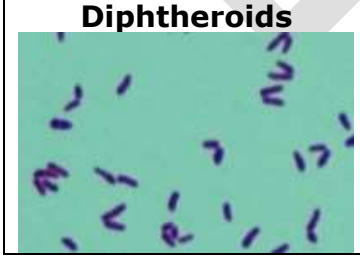
6	If 3-4+ Gram-negative bacilli are seen in the smear, add "**CNA-C**" plate in the media resulting plate log and subculture original specimen to CNA plate and incubate in the CO_2 incubator.
7	Under the test code: **STGM1,** use the **STGM1** keypad to report the quantity of epithelial cells, white blood cells, red blood cells and bacteria seen. Report cells in this order to maintain consistency with reporting.

REPORTING INSTRUCTIONS:

IF	REPORT
No white blood cells seen on Gram stain	Report: **"No white blood cells seen"**
No bacteria seen on Gram stain	Report: **"No bacteria seen"**
Epithelial cells, white blood cells and red blood cells seen on Gram stain	• Quantitate and report using the **STGM1** keypad
Bacteria seen on Gram stain	• Quantitate and report using the **STGM1** keypad
Bacteria resembles: ***Staphylococcus* spp.** 	Report: **"Gram positive cocci suggestive of Staphylococci"** **NOTE: Use caution. If doubt exists, report as Gram positive cocci.**
Bacteria resembles: ***Streptococcus* spp.** 	Report: **"Gram positive cocci suggestive of Streptococci"** **NOTE: Use caution. If doubt exists, report as Gram positive cocci.**
Bacteria resembles: **Diphtheroids** 	Report: **"Gram positive bacilli resembling diphtheroids"** **NOTE: Use caution. If doubt exists, report as Gram positive bacilli.**

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Step	Action
Complete reading of routine slides	
1	• If the specimen is routine, save the gram stain and do not finalize **STGM1** • If the specimen is STAT, finalize **STGM1** • Preview instant report and save • If finished reading slides, ensure Gram stains remaining on worklist have been prepared to be read at a later time
2	Gently blot excess oil from slide using paper towel or gauze and save slides for further evaluation on the slide tray designated for day slides being read.

LIMITATIONS:

1. Use results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., inclusion of selective media, etc.) to confirm findings suggested by Gram-stained smears.
2. Careful adherence to procedure and interpretive criteria is required for accurate results. Accuracy is highly dependent on the training and skill of microscopists.
3. Gram stain-positive, culture negative specimens may be the result of contamination of reagents and other supplies, presence of antimicrobial agents, or failure of organisms to grow under usual culture conditions (medium, atmosphere, etc.).
4. False Gram stain results may be related to inadequately collected specimens or delays in transit.
5. Prior treatment with antimicrobial drugs may cause Gram-positive organisms to appear Gram-negative.

CROSS-REFERENCES:

- MIC10100-Microbiology Specimen Processing
- MIC60060-Microbiology Stain Quality Control

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4th ed.) Washington, D.C.: ASM Press

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APPROVAL:

_____
Date

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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

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