PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20800 – Gram stain reporting in LIS-Genital Specimens	Policy Number: 15-163-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 15/05/2024	Next Review Date: 15/05/2026
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 15/05/2024
Accreditation Canada Applicable Standard: NA	

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GUIDING PRINCIPLE:

Cultures from female genital sites are sent to the clinical microbiology laboratory for detection of microorganisms from prepubescent females (≤13 years of age) and adult females and postmenopausal women meeting select criteria. Male urethritis is usually caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*.

PURPOSE/RATIONALE:

This standard operating procedure describes how to report the gram stain results of genital specimens in the LIS in a consistent manner.

SCOPE/APPLICABILITY:

This standard operating procedure applies to Medical Laboratory Technologists (MLTs) reporting the gram stain of male urethra specimens and vaginal culture specimens in the LIS.

SAMPLE INFORMATION:

Type	1. Cervix swab 2. Endometrium swab 3. Labia swab 4. Penis swab 5. Placenta swab	6. Semen sample 7. Vaginal swab 8. Vulva swab 9. Male urethra swab

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

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:

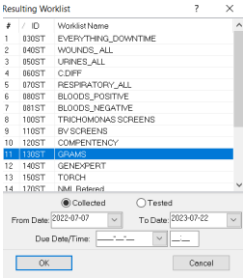
- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure 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

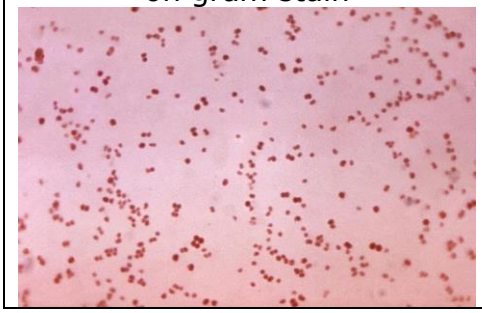
PROCEDURE INSTRUCTIONS:

Step	Action
1. Reporting male urethra gonorrhoeae specimen in the LIS	
1	• Pending male urethra gonorrhoeae specimen orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRAMS**  • Press enter or double click to open worklist

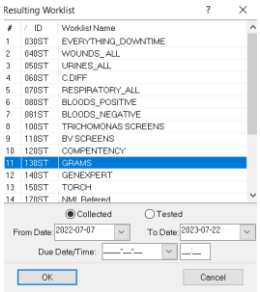
<table>
<tr><td>2</td><td>• Enter the accession number on the slide and select enter to mark the order
• Select enter again to open Result Entry or double click on accession number to open</td></tr>
<tr><td>3</td><td><u>Under low power (X10, LPF):</u> screen slide to locate good specimen areas to obtain 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 white blood cells and gram negative diplococci 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></td></tr>
<tr><td>6</td><td>Under the test code: STGM4, use the STGM4 keypad to report the quantity of white blood cells and gram negative diplococci seen. Report cells in this order to maintain consistency with reporting.</td></tr>
</table>

REPORTING INSTRUCTIONS:

IF	REPORT
No white blood cells seen on gram stain	Report: **"No white blood cells seen"**
No gram negative diplococci seen on gram stain	Report: **"No gram negative diplococci seen"**
White blood cells seen on gram stain	• Quantitate and report using the **STGM4** keypad
Gram negative diplococci seen on gram stain 	• Quantitate and report using the **STGM4** keypad

Step	Action
Complete reading of male urethra gonorrhoeae specimen slides	
1	• If the specimen is routine, save the gram stain and do not finalize **STGM4** • 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.

Step	Action
2. Reporting vaginal culture gram stains in the LIS	
1	• Pending vaginal culture specimen orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRAMS**  • Press enter or double click to open worklist

<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:

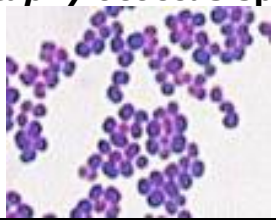
Determine areas representative of inflammation and areas of contamination with squamous epithelial cells

</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>
<tr><td>6</td><td>If patient is >13, Bacterial vaginosis screen needs to be performed. Refer to MIC20600-Gram stain reporting in LIS-Bacterial Vaginosis Screen.</td></tr>
<tr><td>7</td><td>
Under the test code STGM3:

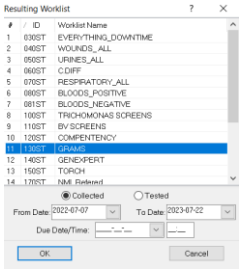
Use the STGM3 1 of 2 keypad to report the quantity of epithelial cells, white blood cells, red blood cells and bacteria seen. Report cells in this order to maintain consistency with reporting
Use the STGM3 2 of 2 keypad to report the BV results

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

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, red blood cells seen on gram stain	• Quantitate and report using the **STGM3** keypad
Bacteria seen on gram stain	• Quantitate and report using the **STGM3** 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**
N-Score is 0 - 3	Report: "**Smear NEGATIVE for Bacterial Vaginosis"**
N-Score is 4 - 6	Report: "**Gram stain shows altered vaginal flora. Results are indeterminate for Bacterial Vaginosis"**
N-Score is 7 - 10	Report: "**Smear consistent with Bacterial Vaginosis"**
Scant or no cells seen on smear	Report: **"Insufficient sample to assess for vaginitis. Please recollect if clinically indicated"**

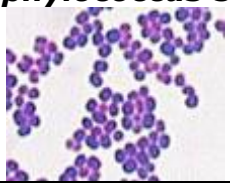
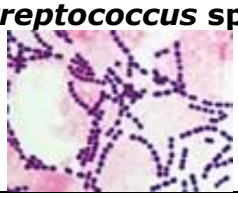
Step	Action
Complete reading of vaginal culture specimen slides	
1	• If the specimen is routine, save the gram stain and do not finalize **STGM3** • Preview instant report and save • If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time
2	Gently 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.

Step	Action
3. Reporting other genital specimen culture gram stains in the LIS	
1	• Pending gram stain orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRAMS**  • Press enter or double click to open worklist
2	• 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
3	<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

<table>
<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>
<tr><td>6</td><td>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.</td></tr>
</table>

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, 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.**

Step	Action
Complete reading of other genital specimen culture slides	
1	• If the specimen is routine, save the gram stain and do not finalize **STGM1** • If the specimen is STAT, save and 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.
6. The presence or absence of clue cells is not part of the Nugent score and not required for diagnosis.
7. For post-menopausal patients, laboratory diagnosis of bacterial vaginosis has not been validated and interpretation of gram stain results needs to be considered. Ensure comment is added.
8. For pre-pubescent girls (≤13 years), Bacterial Vaginosis should not be reported.
9. A negative genital specimen culture does not eliminate the possibility of a genital tract infection. Organisms such as viruses, *Mycoplasmas* and *Chlamydia* are not detected by routine culture. Inadequate specimen collection, improper specimen handling and low organism levels in the specimen may yield a false negative result.

CROSS-REFERENCES:

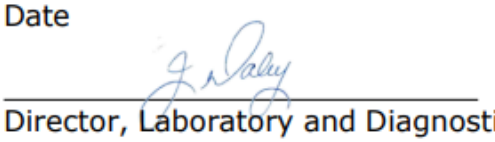
- MIC10100-Microbiology Specimen Processing
- MIC20600-Gram stain reporting in LIS-Bacterial Vaginosis Screen
- MIC60060-Microbiology Stain Quality Control

REFERENCES:

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

APPROVAL:

May 15, 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	20 Feb 24	Procedure reviewed	L. Steven