

PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20300 – Gram stain reporting in LIS-Respiratory Specimens	Policy Number: 15-160-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 14/05/2024	Next Review Date: 14/05/2026
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 14/05/2024
Accreditation Canada Applicable Standard: NA	

GUIDING PRINCIPLE:

The culture of poorly collected respiratory specimens is a wasteful use of laboratory resources and can lead to erroneous reporting and treatment of patients. These specimens need to be scored for acceptability using the Q-score method.

PURPOSE/RATIONALE:

This standard operating procedure describes how to report the gram stain results of respiratory 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 respiratory specimens in the LIS.

SAMPLE INFORMATION:

Type	<ul style="list-style-type: none">• Sputum, endotracheal aspirates (ETT) and auger suction specimens are Q-scored for quality• Bronchial aspirates (washings), bronchoalveolar lavage (BAL) specimens, specimens collected from sterile catheter down ETT and specimens from cystic fibrosis patients are NOT Q-scored for quality
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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 potentially infectious materials or cultures:

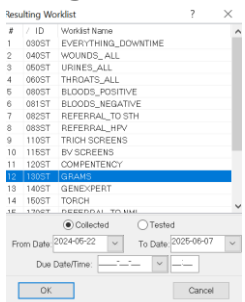
- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure 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
Reporting respiratory specimens in the LIS	
1	<ul style="list-style-type: none">• Pending gram stain orders are found in the LIS Resulting Worklist: Resulting Worklist → GRAMS  <ul style="list-style-type: none">• Press enter or double click to open worklist

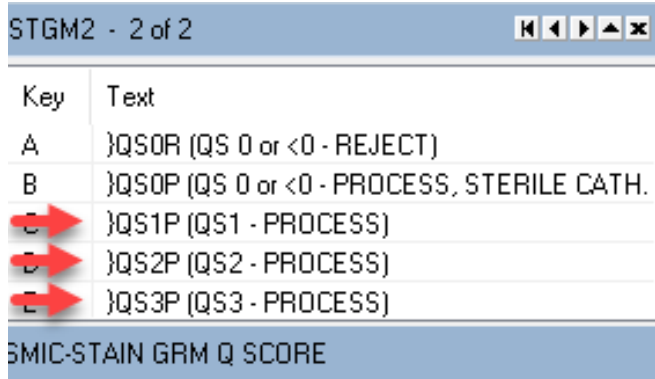
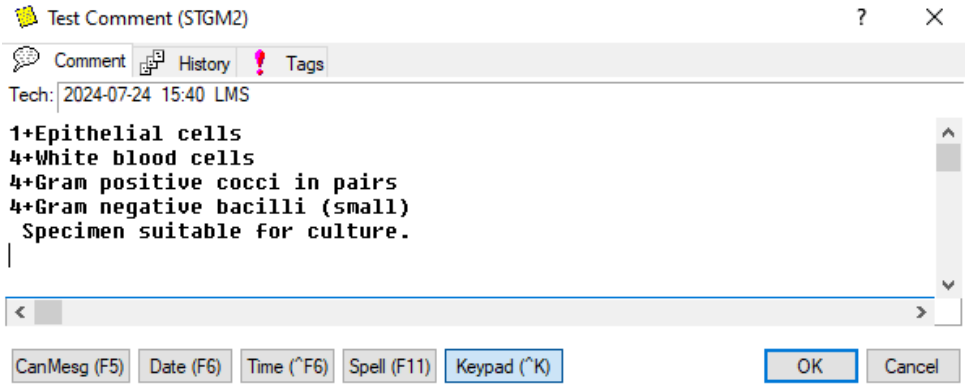
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2	<ul style="list-style-type: none">Enter the accession number on the slide and select enter to mark the orderSelect enter again to open Result Entry or double click on accession number to open																																		
3	<p><u>Under low power (X10, LPF):</u> screen slide to locate good specimen areas to obtain an overall impression of cell types present.</p> <ul style="list-style-type: none">Observe slide for stain crystals:<ul style="list-style-type: none">➤ If an excess of precipitated stain is observed, prepare another smear➤ If precipitate continues, use freshly filtered crystal violetDetermine if slide has been properly decolorized:<ul style="list-style-type: none">➤ 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 smearDetermine if thickness of smear is appropriate:<ul style="list-style-type: none">➤ For proper interpretation, areas must be no more than one cell thick, with no overlapping of cells. Prepare a new slide if unreadableExamine for evidence of inflammation:<ul style="list-style-type: none">➤ Determine areas representative of inflammation and areas of contamination with squamous epithelial cells																																		
4	<p><u>Under low power (X10, LPF):</u> average the number of epithelial cells and white blood cells:</p> <table><tr><td>None seen</td><td>No cells seen</td></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>	None seen	No cells seen	1+	< 1 cell seen	2+	1 - 9 cells seen	3+	10 - 25 cells seen	4+	> 25 cells seen																								
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5	<p>Calculate the Q-score of the specimen. The Q-score is calculated by assessing the quantity of epithelial cells and neutrophils. Examine 20 to 40 fields and interpret as follows:</p> <table><tr><th colspan="5">Q-score Table</th></tr><tr><th rowspan="2">Epi cells/LPF</th><th colspan="4">White blood cells /LPF</th></tr><tr><th>0</th><th>1-9</th><th>10-25</th><th>>25</th></tr><tr><td>0</td><td>Q 0</td><td>Q 1</td><td>Q 2</td><td>Q 3</td></tr><tr><td>1-9</td><td>Q-1</td><td>Q 0</td><td>Q 1</td><td>Q 2</td></tr><tr><td>10-25</td><td>Q-2</td><td>Q-1</td><td>Q 0</td><td>Q 1</td></tr><tr><td>>25</td><td>Q-3</td><td>Q-2</td><td>Q-1</td><td>Q 0</td></tr></table>	Q-score Table					Epi cells/LPF	White blood cells /LPF				0	1-9	10-25	>25	0	Q 0	Q 1	Q 2	Q 3	1-9	Q-1	Q 0	Q 1	Q 2	10-25	Q-2	Q-1	Q 0	Q 1	>25	Q-3	Q-2	Q-1	Q 0
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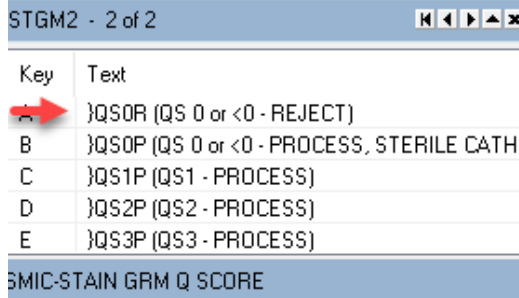
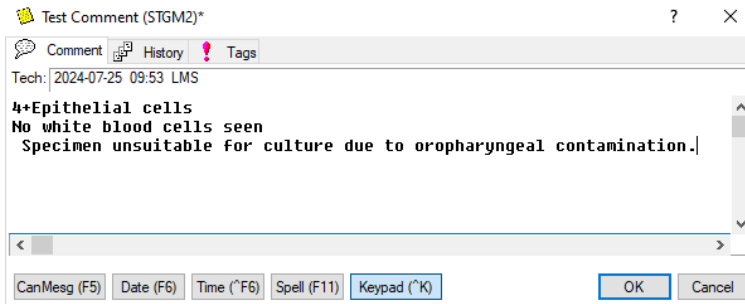
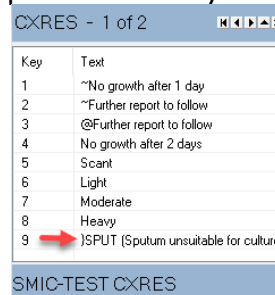
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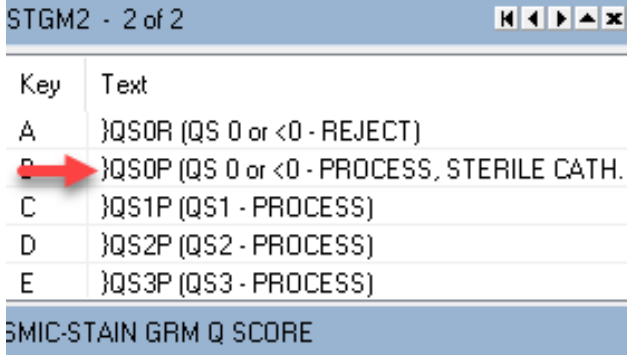
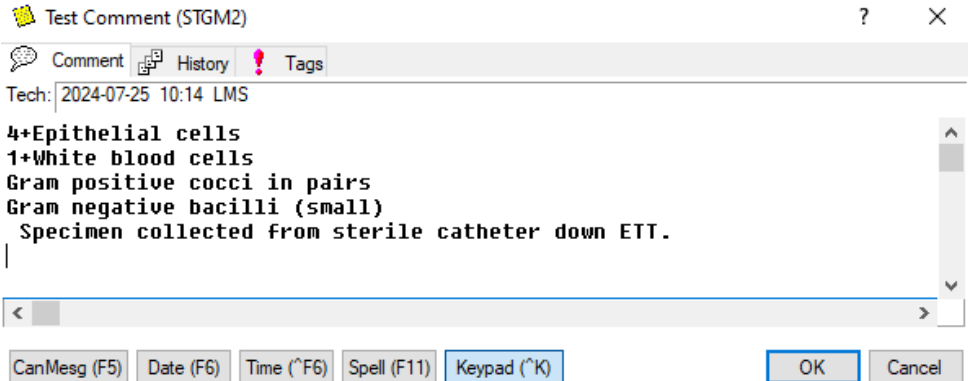
6	Do not perform the Q-score on bronchial aspirates (washings), bronchoalveolar lavage (BAL), specimens collected from sterile catheter down ETT and specimens from cystic fibrosis patients.										
7	If the Q-score indicates the sample is of good quality (Q-score 1-3) or if the specimen/sample type is bronchial aspirate (washings), bronchoalveolar lavage (BALs), from sterile catheter down ETT, or from a cystic fibrosis patient, 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.										
8	If the Q-score indicates the sample is not of good quality, and is not a bronchial aspirate (washings), a bronchoalveolar lavage (BALs), from sterile catheter down ETT, or from a cystic fibrosis patient, do not add immersion oil to the slide to observe bacteria.										
9	<p><u>Under oil immersion (X100, OIF):</u> quantitate epithelial cells, white blood cells, red blood cells and bacteria as follows:</p> <table border="1"> <thead> <tr> <th>None seen</th><th>No cells seen</th></tr> </thead> <tbody> <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> </tbody> </table> <p>NOTE: Bacteria are not reported if the Q-score indicates specimen is unsatisfactory for culture and the sample is not a bronchial aspirate (washings), a bronchoalveolar lavage (BALs), from sterile catheter down ETT, or from a cystic fibrosis patient</p> <p>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</p>	None seen	No cells seen	1+	< 1 cell seen	2+	1 - 9 cells seen	3+	10 - 25 cells seen	4+	> 25 cells seen
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10	Under the test code: STGM2 , use the STGM2 keypad to report the quantity of epithelial cells, white blood cells, red blood cells and bacteria if indicated by Q-score. Report cells in this order to maintain consistency with reporting.										
11	<p>Reporting Mixed oropharyngeal flora in respiratory gram stain:</p> <ol style="list-style-type: none"> If smear has ≥ 2 morphotypes and neither are predominant or intracellular, mixed oropharyngeal flora can be reported If smear has ≥ 2 morphotypes and one or more are predominant or intracellular, the predominant or intracellular morphotypes are reported individually and other morphotypes are reported as mixed oropharyngeal flora 										

REPORTING INSTRUCTIONS:

IF	REPORT
<p>Q-score is 1, 2 or 3</p> <p>PROCESS</p>	<ol style="list-style-type: none"> Quantify and report epithelial cells, white blood cells and red blood cells on the STGM2 1 of 2 keypad Quantify and report bacteria on the STGM2 1 of 2 keypad Scroll to the STGM2 2 of 2 keypad and report the Q-score: <div data-bbox="613 453 1263 829">  </div> Comment will appear as: "Specimen suitable for culture." <div data-bbox="462 945 1421 1333">  </div> If the specimen is routine, save the gram stain and do not finalize STGM2 If the specimen is STAT, finalize STGM2. Preview instant report and save. Refresh GRAMS worklist If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time 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

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IF	REPORT																																
<p>Q-score is 0 or <0</p> <p>DON'T PROCESS</p>	<ol style="list-style-type: none"> Quantify and report epithelial cells, white blood cells and red blood cells on the STGM2 1 of 2 keypad Quantify and report bacteria on the STGM2 1 of 2 keypad Scroll to the STGM2 2 of 2 keypad and report the Q-score: <div data-bbox="695 386 1211 682">  <p>STGM2 - 2 of 2</p> <table border="1"> <thead> <tr> <th>Key</th> <th>Text</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>}QS0R (QS 0 or <0 - REJECT)</td> </tr> <tr> <td>B</td> <td>}QS0P (QS 0 or <0 - PROCESS, STERILE CATH.</td> </tr> <tr> <td>C</td> <td>}QS1P (QS1 - PROCESS)</td> </tr> <tr> <td>D</td> <td>}QS2P (QS2 - PROCESS)</td> </tr> <tr> <td>E</td> <td>}QS3P (QS3 - PROCESS)</td> </tr> </tbody> </table> <p>SMIC-STAIN GRM Q SCORE</p> </div> Comment will appear as: "Specimen unsuitable for culture due to oropharyngeal contamination." <div data-bbox="583 793 1323 1094">  <p>Test Comment (STGM2)*</p> <p>Comment History Tags</p> <p>Tech: 2024-07-25 09:53 LMS</p> <p>4+Epithelial cells No white blood cells seen Specimen unsuitable for culture due to oropharyngeal contamination.</p> <p>CanMsg (F5) Date (F6) Time (^F6) Spell (F11) Keypad (^K) OK Cancel</p> </div> Select OK and finalize STGM2 From the CXRES keypad select Key 9: <div data-bbox="816 1167 1088 1459">  <p>CXRES - 1 of 2</p> <table border="1"> <thead> <tr> <th>Key</th> <th>Text</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>~No growth after 1 day</td> </tr> <tr> <td>2</td> <td>~Further report to follow</td> </tr> <tr> <td>3</td> <td>@Further report to follow</td> </tr> <tr> <td>4</td> <td>No growth after 2 days</td> </tr> <tr> <td>5</td> <td>Scant</td> </tr> <tr> <td>6</td> <td>Light</td> </tr> <tr> <td>7</td> <td>Moderate</td> </tr> <tr> <td>8</td> <td>Heavy</td> </tr> <tr> <td>9</td> <td>}SPUT (Sputum unsuitable for culture)</td> </tr> </tbody> </table> <p>SMIC-TEST CXRES</p> </div> Comment will appear as: "Specimen unsuitable for culture due to oropharyngeal contamination" Finalize CXRES. Preview instant report and save Refresh GRAMS worklist If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time 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 	Key	Text	A	}QS0R (QS 0 or <0 - REJECT)	B	}QS0P (QS 0 or <0 - PROCESS, STERILE CATH.	C	}QS1P (QS1 - PROCESS)	D	}QS2P (QS2 - PROCESS)	E	}QS3P (QS3 - PROCESS)	Key	Text	1	~No growth after 1 day	2	~Further report to follow	3	@Further report to follow	4	No growth after 2 days	5	Scant	6	Light	7	Moderate	8	Heavy	9	}SPUT (Sputum unsuitable for culture)
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IF	REPORT
<p>Q-score is 0 or <0</p> <p>Specimen is from sterile catheter</p> <p>PROCESS</p>	<ol style="list-style-type: none"> Quantify and report epithelial cells, white blood cells and red blood cells on the STGM2 1 of 2 keypad Quantify and report bacteria on the STGM2 1 of 2 keypad Scroll to the STGM2 2 of 2 keypad and report the Q-score: <div data-bbox="646 390 1269 743">  </div> Comment will appear as: "Specimen collected from sterile catheter down ETT" <div data-bbox="480 823 1442 1201">  </div> If the specimen is routine, save the gram stain and do not finalize STGM2 If the specimen is STAT, finalize STGM2. Preview instant report and save. Refresh GRAMS worklist If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time 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

IF	REPORT
Q-score is not performed Sample is bronchial aspirate (washings) OR bronchoalveolar lavage (BALs)	<ol style="list-style-type: none">1. Quantify and report epithelial cells, white blood cells and red blood cells on the STGM2 1 of 2 keypad2. Quantify and report bacteria on the STGM2 1 of 2 keypad3. If the specimen is routine, save the gram stain and do not finalize STGM24. If the specimen is STAT, finalize STGM2. Preview instant report and save. Refresh GRAMS worklist5. If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time6. 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:

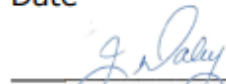
- MIC10100-Microbiology Specimen Processing
- MIC60060-Microbiology Stain Quality Control

REFERENCES:

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

APPROVAL:

May 14, 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
4.0	25 July 24	Procedure updated to reflect new Q score reporting and not cancelling specimens	L. Steven

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