| 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** | • Sputum, endotracheal aspirates (ETT) and auger suction specimens are Q-scored for quality<br>• 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 |

**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:

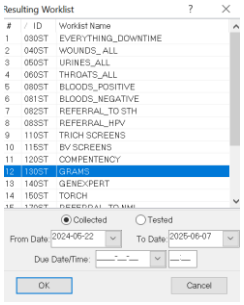
- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure 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 |
|---|---|
| **Reporting respiratory specimens in the LIS** | |
| **1** | • Pending gram stain orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRAMS**<br>  <br>• 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**

Under low power (X10, LPF): 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

**4**

Under **low** power (X10, LPF): average the number of epithelial cells and white blood cells:

| None seen | No cells seen |
|---|---|
| 1+ | < 1 cell seen |
| 2+ | 1 - 9 cells seen |
| 3+ | 10 - 25 cells seen |
| 4+ | > 25 cells seen |

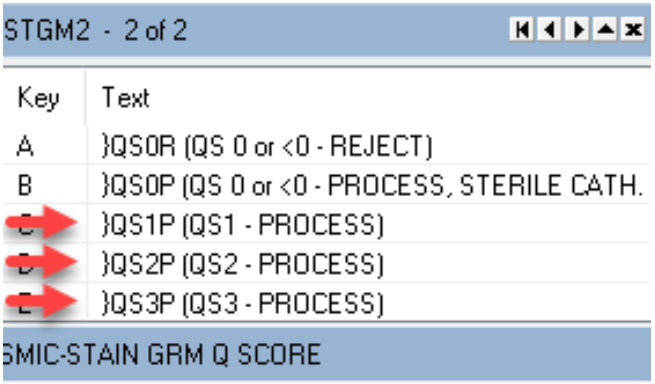
**5**

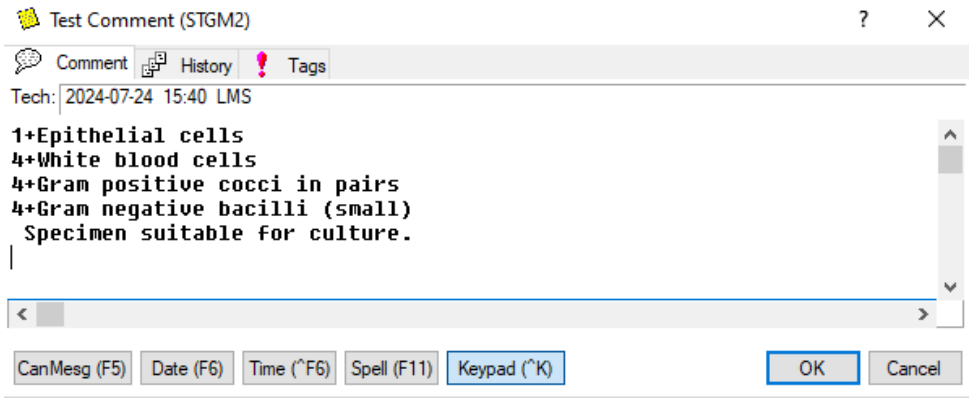
Calculate the Q-score of the specimen. The Q-score is calculated by assessing the quantity of epithelial cells and neutrophils. Examine 20 to 40 fields and interpret as follows:

| 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 |

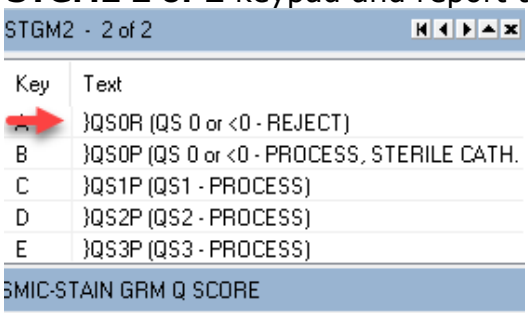
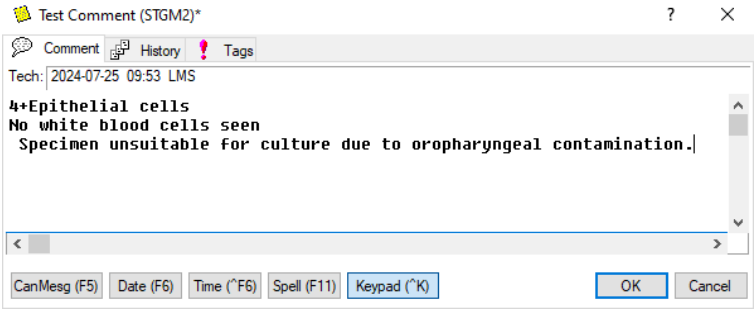
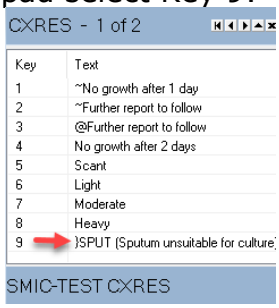
<table>
<tr><td>6</td><td>Do not perform the Q-score on bronchial aspirates (washings), bronchoalveolar lavage (BAL), specimens collected from sterile catheter down ETT and specimens from cystic fibrosis patients.</td></tr>
<tr><td>7</td><td>If the Q-score indicates the sample is of good quality (Q-score 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.</td></tr>
<tr><td>8</td><td>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.</td></tr>
<tr><td>9</td><td>Under oil immersion (X100, OIF): quantitate epithelial cells, white blood cells, red blood cells and bacteria as follows:<br><br>
<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>
<br>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<br>
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>10</td><td>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.</td></tr>
<tr><td>11</td><td>Reporting Mixed oropharyngeal flora in respiratory gram stain:<br>1. If smear has ≥2 morphotypes and neither are predominant or intracellular, mixed oropharyngeal flora can be reported<br>2. 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</td></tr>
</table>

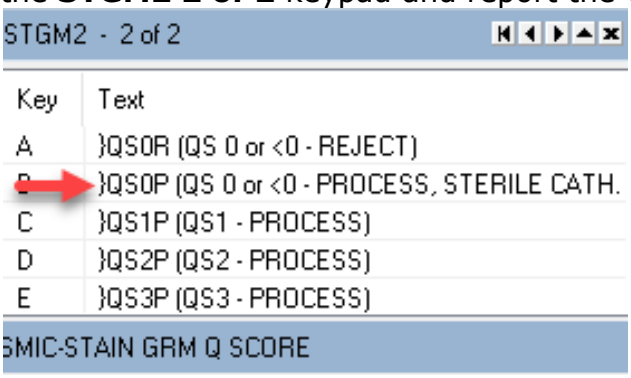
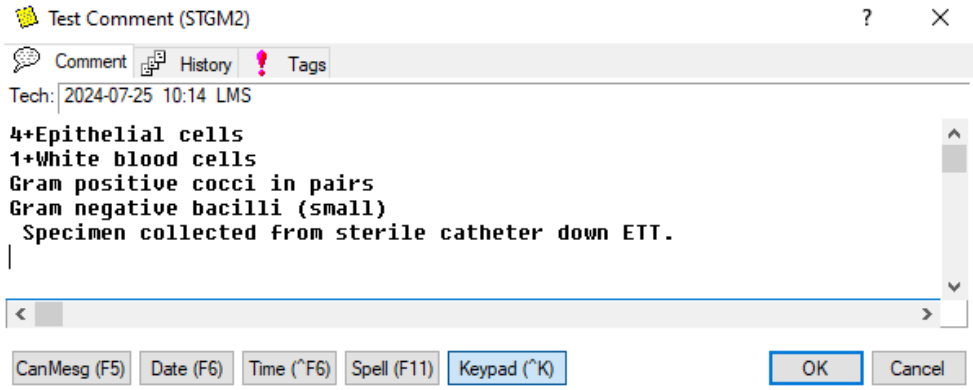
**REPORTING INSTRUCTIONS:**

<table>
<tr><th>IF</th><th>REPORT</th></tr>
<tr><td>Q-score is 1, 2 or 3<br><br>PROCESS</td><td>
1. Quantify and report epithelial cells, white blood cells and red blood cells on the STGM2 1 of 2 keypad<br>
2. Quantify and report bacteria on the STGM2 1 of 2 keypad<br>
3. Scroll to the STGM2 2 of 2 keypad and report the Q-score:<br>


<br>
4. Comment will appear as:<br>
“Specimen suitable for culture.”<br>


<br>
5. If the specimen is routine, save the gram stain and do not finalize STGM2<br>
6. If the specimen is STAT, finalize STGM2. Preview instant report and save. Refresh GRAMS worklist<br>
7. If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time<br>
8. 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
</td></tr>
</table>

| IF | REPORT |
|---|---|
| **Q-score is 0 or <0**<br><br>**DON'T PROCESS** | 1. Quantify and report epithelial cells, white blood cells and red blood cells on the **STGM2 1 of 2** keypad<br>2. Do NOT quantify and report bacteria on the **STGM2 1 of 2** keypad<br>3. Scroll to the **STGM2 2 of 2** keypad and report the Q-score:<br>  <br><br>1. Comment will appear as:<br>**"Specimen unsuitable for culture due to oropharyngeal contamination."**<br>  <br><br>2. Select **OK** and finalize **STGM2**<br>3. From the CXRES keypad select Key 9:<br>  <br><br>4. Comment will appear as:<br>**"Specimen unsuitable for culture due to oropharyngeal contamination"**<br>5. Finalize **CXRES**. Preview instant report and save<br>6. Refresh **GRAMS** worklist<br>7. If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time<br>8. 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 0 or <0**<br><br>**Specimen is from sterile catheter**<br><br>**PROCESS** | 1. Quantify and report epithelial cells, white blood cells and red blood cells on the **STGM2 1 of 2** keypad<br>2. Quantify and report bacteria on the **STGM2 1 of 2** keypad<br>3. Scroll to the **STGM2 2 of 2** keypad and report the Q-score:<br>  <br><br>4. Comment will appear as:<br>**"Specimen collected from sterile catheter down ETT"**<br>  <br><br>5. If the specimen is routine, save the gram stain and do not finalize **STGM2**<br>6. If the specimen is STAT, finalize **STGM2**. Preview instant report and save. Refresh **GRAMS** worklist<br>7. If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time<br>8. 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 if**<br><br>**Sample is bronchial aspirate (washings) OR bronchoalveolar lavage (BALs) OR Specimen is from a cystic fibrosis patient**<br><br>**PROCESS** | 1. Quantify and report epithelial cells, white blood cells and red blood cells on the **STGM2 1 of 2** keypad<br>2. Quantify and report bacteria on the **STGM2 1 of 2** keypad<br>3. If the specimen is routine, save the gram stain and do not finalize **STGM2**<br>4. If the specimen is STAT, finalize **STGM2**. Preview instant report and save. Refresh **GRAMS** worklist<br>5. If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time<br>6. 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

**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 |
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