PURPOSE: To report the Q-score and Gram stain results of respiratory specimens in a consistent manner.

SAMPLE INFORMATION:

Type	• Sputum, Endotracheal aspirates (ETT) and Auger Suction specimens are Q-scored for quality. • Bronchial aspirates (washings), Bronchoalveolar lavage (BAL) specimens and specimens from cystic fibrosis patients are **NOT** Q-scored for quality and the Q-score is **NOT** reported. • Refer to MIC10230 – Microbiology Specimen Processing.

REAGENTS INFORMATION:

Type	BD™ Gram Crystal Violet, 3.8 L, B4312526 BD™ Gram Iodine (Stabilized), 3.8 L, B4312543 BD™ Gram Decolorizer, 3.8 L, B4312528 BD™ Gram Safranin, 3.8 L, B4312531
Source	Fisher Scientific Canada
Storage	Store at 15° to 30°
Stability	As per expiry date listed on bottle

SUPPLIES:

- Frosted end glass microscope slide
- QC slide
- Methanol, absolute
- Immersion oil
- Microscope
- Slide storage tray

SPECIAL SAFETY PRECAUTIONS:

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

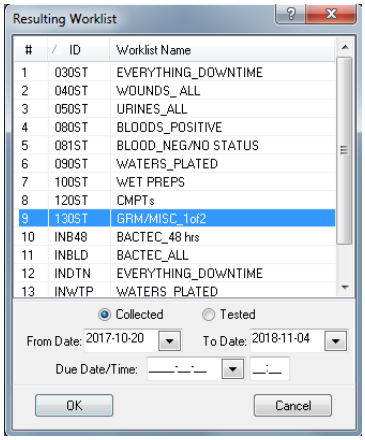
- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, 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. Universal precautions 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:

- Refer to MIC60060 – Microbiology Stain Quality Control.

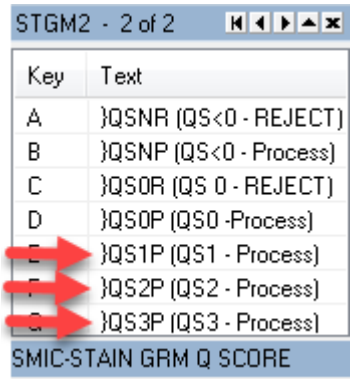
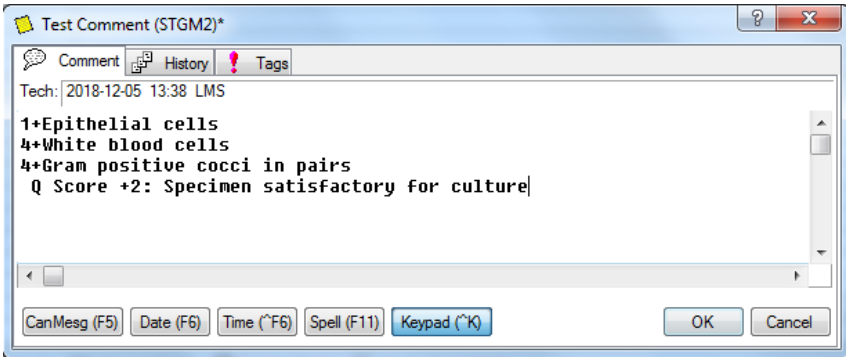
PROCEDURE INSTRUCTIONS:

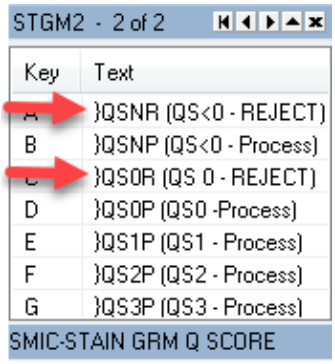
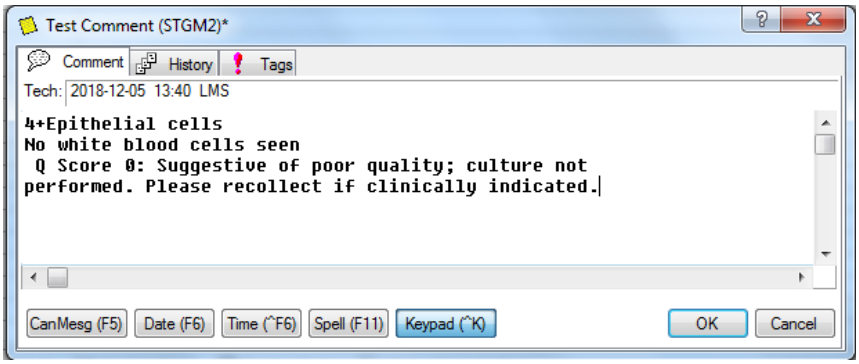
Step	Action
1	Pending Gram stain orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRM/MISC_1of2**  Press enter or double click to open worklist.
2	Enter the accession number on the slide and select enter to mark the order. Select enter again to open Result Entry or double click on accession number to open.
3	Under low power (10X/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 WBC 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.

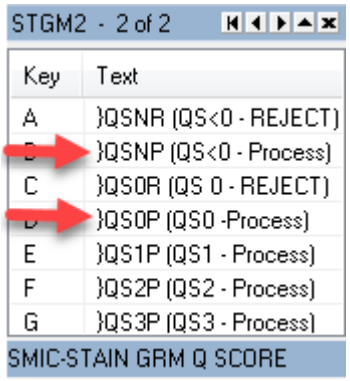
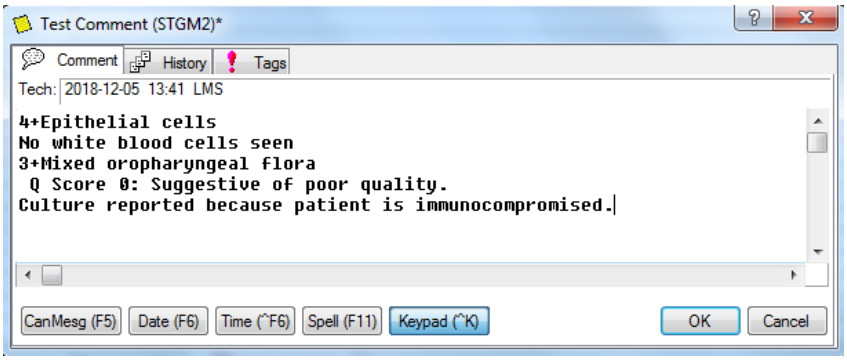
<table>
<tr><td>4</td><td>Under the test code: STGM2, use the STGM2 keypad to report the quantity of epithelial cells and neutrophils. Scan approximately 20 to 40 fields.</td></tr>
<tr><td>5</td><td>Epithelial cells and neutrophils are quantified as follows under LPF (10X):
<table>
<tr><td>None seen</td><td>No cells seen</td></tr>
<tr><td>1+</td><td>< 1 cells 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>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:
<table>
<tr><th colspan="5">Q-score Table</th></tr>
<tr><th rowspan="2">Epithelial 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>Q0</td><td>Q1</td><td>Q2</td><td>Q3</td></tr>
<tr><td>1-9</td><td>Q-1</td><td>Q0</td><td>Q1</td><td>Q2</td></tr>
<tr><td>10-25</td><td>Q-2</td><td>Q-1</td><td>Q0</td><td>Q1</td></tr>
<tr><td>>25</td><td>Q-3</td><td>Q-2</td><td>Q-1</td><td>Q0</td></tr>
</table></td></tr>
<tr><td>7</td><td>Do not perform or report the Q-score on Bronchial aspirates (washings), Bronchoalveolar lavage (BAL) and specimens from cystic fibrosis patients.</td></tr>
<tr><td>8</td><td>If the Q-score indicates the sample is of good quality (Q-score 1-3 or Q-score 0 or <0 if patient is immunocompromised), 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>9</td><td>Bacterial and yeast cells are quantified as follows under OIF (100x):
<table>
<tr><td>None seen</td><td>No cells seen</td></tr>
<tr><td>1+</td><td>< 1 cells 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>
</table>

10	**NOTE:** If no bacteria are seen, report this result **NOTE:** Bacteria are not reported if the Q-score indicates specimen is unsatisfactory for culture.
11	Reporting **Mixed oropharyngeal flora** in respiratory gram stain: 1. If smear has >= 2 morphotypes and neither are predominant or intracellular, mixed oral pharyngeal flora can be reported. 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.

REPORTING OF RESULTS:

IF	REPORT
Q-score is 1, 2 or 3	1. Quantify and report epithelial cells and white blood cells. 2. Quantify and report bacteria. 3. Scroll to the **STGM2 2 of 2** keypad and report the Q-score:  4. Comment will appear as: **"Q-score +1, +2 or +3: Specimen satisfactory for culture"**  5. Finalize **STGM2**. Preview instant report and save. Refresh **GRM/MISC1of 2** worklist. If finished reading slides, ensure Gram stains remaining on worklist have been prepared to be read at a later time. 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.

IF	REPORT
Q-score 0 or < 0 **Patient is NOT immunocompromised**	1. Quantify and report epithelial cells and white blood cells. 2. Do not report bacteria. 3. Scroll to the **STGM2 2 of 2** keypad and report the Q-score:  4. Comment will appear as: **"Q Score 0 or <0: Suggestive of poor quality; culture not performed. Please recollect if clinically indicated"**  5. Finalize **STGM2**. Standard deviation rule violation box will pop up indicating that the culture will be cancelled. Select **OK**. 6. Short cancellation reason box will pop up. Select **Key 0 – Report** and select **OK**. This will cancel the culture. 7. Finalize **STGM2**. Preview instant report and save. Refresh **GRM/MISC1of 2** worklist. If finished reading slides, ensure gram stains remaining on worklist have been prepared to be read at a later time. 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 0 or < 0 **Patient IS immunocompromised**	1. Quantify and report epithelial cells and white blood cells. 2. Quantify and report bacteria. 3. Scroll to the **STGM2 2 of 2** keypad and report the Q-score:  4. Comment will appear as: **"Q Score 0 or <0: Suggestive of poor quality. Culture reported as the patient is immunocompromised"**  5. Finalize **STGM2**. Preview instant report and save. Refresh **GRM/MISC1of 2** worklist. If finished reading slides, ensure Gram stains remaining on worklist have been prepared to be read at a later time. 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. Culture plates will be inoculated immediately upon receipt without waiting for the results of the smear. Plates from rejected specimens can be discarded.
2. The culture of a poorly collected respiratory specimen is a wasteful use of laboratory resources and can lead to erroneous reporting and treatment of patients.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven