PURPOSE: To report the Gram stain results of routine specimens in a consistent manner.

SAMPLE INFORMATION:

Type	• Wound, ear, eye, lower genital tract (excluding BV) and male urethra gonorrhoeae specimens. • 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 15º to 30º
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.

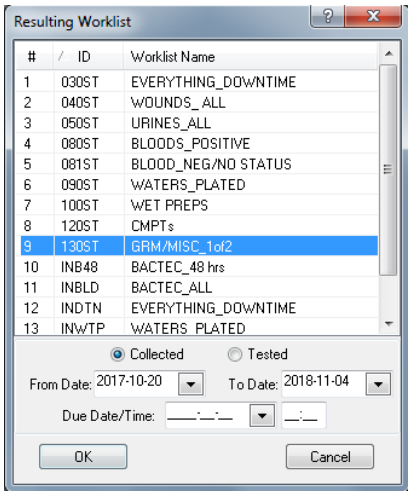
- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols,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

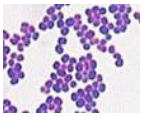
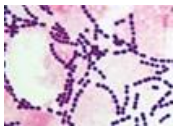
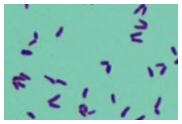
- Refer to MIC60060 – Microbiology Stain Quality Control.

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 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>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. Scan approximately 20 to 40 fields.</td></tr>
<tr><td>5</td><td>Epithelial cells, white blood cells and red blood cells 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>
NOTE: Only report “None seen” for white blood cells. If no epithelial cells or red blood cells are seen, do not report this.</td></tr>
<tr><td>6</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>7</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>
NOTE: If no bacteria are seen, report this result.</td></tr>
<tr><td>8</td><td>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.</td></tr>
<tr><td>9</td><td>Finalize STGM1. Preview instant report and save. Refresh GRM/MISC1of2 worklist. If finished reading slides, ensure Gram stains remaining on worklist have been prepared to be read at a later time.</td></tr>
<tr><td>10</td><td>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>

REPORTING OF RESULTS:

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 in doubt. If doubt exists, report as Gram positive cocci.**
Bacteria resembles: ***Streptococcus spp.*** 	Report: **"Gram positive cocci suggestive of Streptococci"** **NOTE: Use caution if in doubt. If doubt exists, report as Gram positive cocci.**
Bacteria resembles: **Diphtheroids** 	Report: **"Gram positive bacilli resembling diphtheroids"** **NOTE: Use caution if in doubt. If doubt exists, report as Gram positive bacilli.**

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. Carefully 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.

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