PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20400 – Gram stain reporting in LIS-Sterile Fluid Specimens	Policy Number: 15-151-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 18/03/2024	Next Review Date: 18/03/2026
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 18/03/2024
Accreditation Canada Applicable Standard: NA	

GUIDING PRINCIPLE:
Critical fluid specimens, including CSF, need to be read extensively as low numbers of organisms may be seen and the presence of microorganisms from a normally sterile site is likely to indicate infection with that organism. Due to the nature of these specimens, fluid samples for microbiology culture are considered STAT and the gram stain needs to be read within 1 hour of receipt in the laboratory during regular microbiology hours.

PURPOSE/RATIONALE:
This standard operating procedure describes how to report the gram stain results of sterile fluids in the LIS in a consistent manner.

SCOPE/APPLICABILITY:
This standard operating procedure applies to Medical Laboratory Technologists (MLTs) reporting the gram stain of sterile fluid specimens in the LIS.

SAMPLE INFORMATION:

Type	1. Reporting sterile fluid specimens received in sterile containers, including CSF specimens 2. Reporting positive fluid cultures in blood culture bottles in LIS, bacteria seen 3. Reporting positive fluid cultures in blood culture bottles in LIS, bacteria NOT seen

REAGENTS and/or MEDIA:

- Methanol
- Gram Crystal Violet
- Gram Iodine (Stabilized)
- Gram Decolorizer
- Gram Safranin

SUPPLIES:

- Ringed cytology slide
- Sub-culturing/aerobic venting unit
- 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:

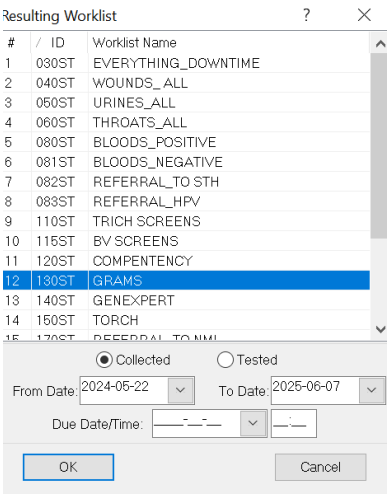
- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure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

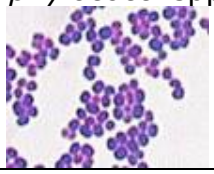
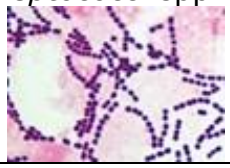
- Quality control is performed daily
- A TQC order is automatically generated daily to record the QC results
- Refer to MIC60060-Microbiology Stain Quality Control

PROCEDURE INSTRUCTIONS:

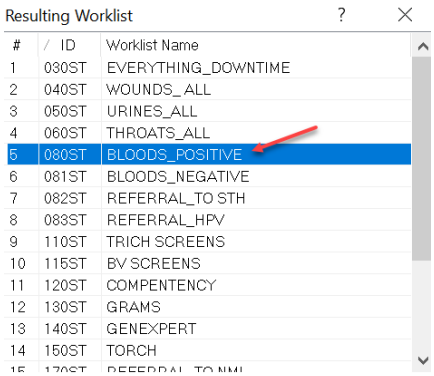
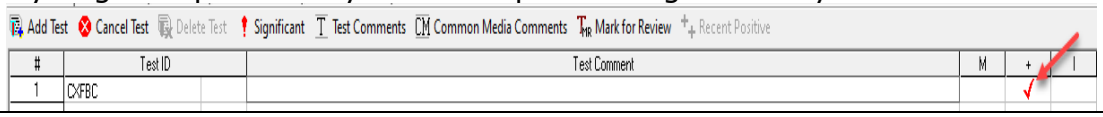
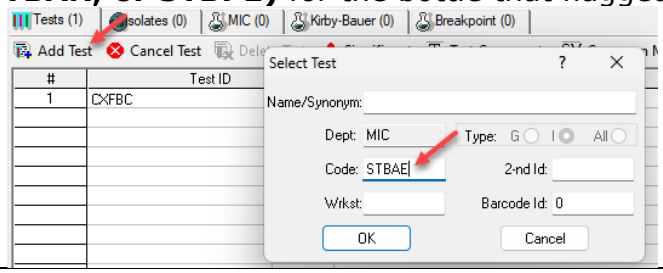
Step	Action
1. Reporting sterile fluid specimen received in sterile container in the LIS	
1	• Pending gram stain orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRAMS**  • Press enter or double click to open worklist
2	• Enter the accession number on the slide and select enter to mark the order • Select enter again to open Result Entry or double click on the 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
4	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.

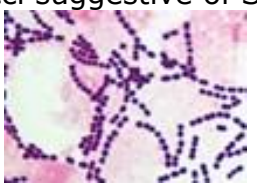
<table>
<tr><td>5</td><td>Under oil immersion (X100, OIF): quantitate, white blood cells, red blood cells and bacteria as follows:
<table>
<tr><th>None seen</th><th>No cells seen</th></tr>
<tr><td>1+</td><td>< 1 cell seen</td></tr>
<tr><td>2+</td><td>1 - 9 cells seen</td></tr>
<tr><td>3+</td><td>10 - 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 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 white blood cells, red blood cells and bacteria seen. Report cells in this order to maintain consistency with reporting.</td></tr>
</table>

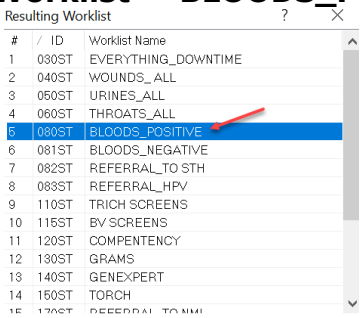
REPORTING INSTRUCTIONS:

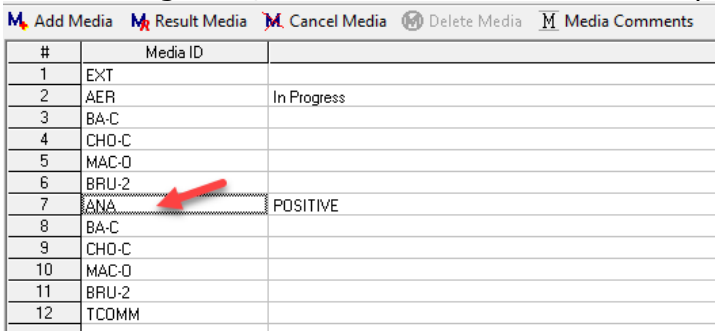
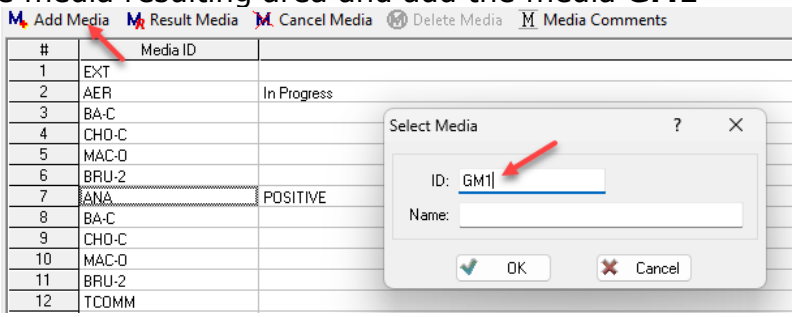
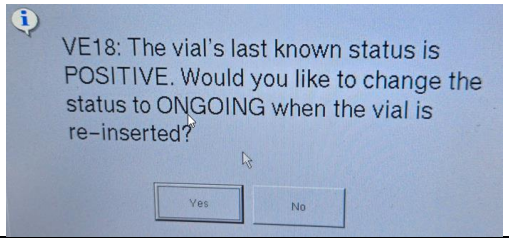
IF	REPORT
No white blood cells seen on gram stain	Report: **“No white blood cells seen”**
No bacteria seen on gram stain	Report: **“No bacteria seen”**
White blood cells or red blood cells seen on gram stain	• Quantitate and report using the **STGM1** keypad
Bacteria seen on gram stain	• Quantitate and report using the **STGM1** keypad • Bacteria seen in the gram stain of sterile fluids are considered a critical result ➢ Phone the ordering location to give result ➢ Document the call in the **“Call”** box ➢ If unable to reach ordering location, consult the hospital wide policy 15-10-V1-Laboratory Critical Results Procedure • Finalize the ST order, preview instant report and save
If the bacteria seen resembles *Staphylococci* spp.: 	• Report: Gram positive cocci suggestive of Staphylococci **NOTE:** Use caution. Report as Gram positive cocci if doubt exists
If the bacteria seen resembles *Streptococci* spp.: 	• Report: Gram positive cocci suggestive of Streptococci • If the ordering location of the positive fluid culture is Stanton Territorial Hospital or Inuvik Regional Hospital, copy appropriate infection control (SIPAC or IIPAC) **NOTE:** Use caution. Report as Gram positive cocci if doubt exists

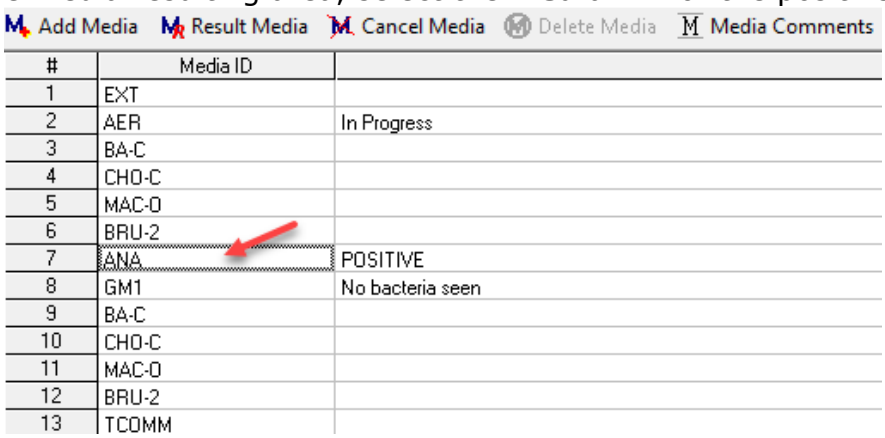
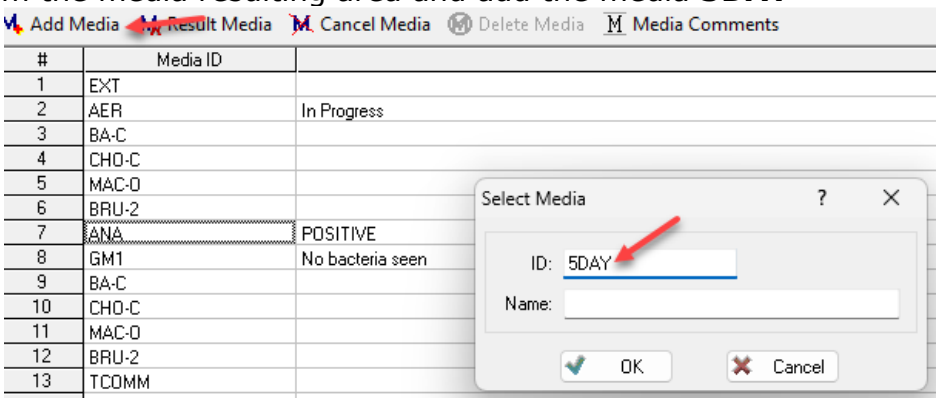
Step	Action
Complete reading of sterile fluid slides	
1	• Finalize **STGM1** • Preview instant report and save
2	Gently blot excess oil from slide using paper towel or gauze and save slides for further evaluation on the slide tray designated for day slides being read.

Step	Action
2. Reporting positive fluid cultures in blood culture bottles in LIS, bacteria seen	
1	• Pending positive fluid cultures received in blood culture bottles are found in the LIS Resulting Worklist: **Resulting Worklist → BLOODS_POSITIVE**  • 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 the accession number to open
3	Add one drop of immersion oil to the slide. Using the oil immersion lens (100X); examine 20 to 40 fields to observe gram reaction.
4	The CX order will be immediately flagged as positive ✓. This will prevent any negative preliminary or final reports being issued by SoftMic 
5	In the test resulting area, select **Add Test** and order the appropriate stain test **(STBAE, STBAN, or STBPE)** for the bottle that flagged positive 

6	Under the test code **STBAE**, **STBAN** or **STBPE** use corresponding ST keypad to report the bacteria that were seen.
7	<u>If the bacteria seen resembles ***Staphylococci* spp.**</u>: • Report: Gram positive cocci suggestive of Staphylococci  **NOTE:** Use caution. Report as Gram positive cocci if doubt exists <u>If the bacteria seen resembles ***Streptococci* spp.**</u>: • Report: Gram positive cocci suggestive of Streptococci  • If the ordering location of the positive fluid culture is Stanton Territorial Hospital or Inuvik Regional Hospital, copy appropriate infection control **(SIPAC or IIPAC)** **NOTE:** Use caution. Report as Gram positive cocci if doubt exists
8	Bacteria seen in the gram stain of fluid cultures is considered a critical result: • Phone the ordering location to give result • Document the call in the **Call** box • If unable to reach ordering location, consult the hospital wide policy 15-10-V1-Laboratory Critical Results Procedure
9	Finalize the ST order, preview instant report and save.
10	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 positive fluid cultures in blood culture bottles in LIS, bacteria NOT seen	
1	• Pending positive blood culture orders are found in the LIS Resulting Worklist: **Resulting Worklist → BLOODS_POSITIVE**  Resulting Worklist \# / ID Worklist Name 1 030ST EVERYTHING_DOWNTIME 2 040ST WOUNDS_ALL 3 050ST URINES_ALL 4 060ST THROATS_ALL 5 080ST BLOODS_POSITIVE 6 081ST BLOODS_NEGATIVE 7 082ST REFERRAL_TO STH 8 083ST REFERRAL_HPV 9 110ST TRICH SCREENS 10 115ST BV SCREENS 11 120ST COMPENTENCY 12 130ST GRAMS 13 140ST GENEXPERT 14 150ST TORCH • 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 the accession number to open
3	Add one drop of immersion oil to the slide. Using the oil immersion lens (100X); examine 20 to 40 fields to observe gram reaction.
4	If no bacteria are seen: • Consider repeating smear • Consider performing acridine orange stain
5	If certain that no bacteria are in the gram stain: • In the media resulting area, select the **Media ID** for the positive bottle  • With the Media ID for the positive bottle selected, select **Add Media** from the media resulting area and add the media **GM1**  • Using the GM1 keypad, select **Key 0-No bacteria seen** to document that the gram stain was read and that bacteria were not seen
6	If the 5-hour window for bottle replacement into the BACTEC has **NOT** expired, it can be loaded back into the instrument: • In the LIS, double click the positive flag ✓ to remove it. This will ensure that any preliminary or final reports will be automatically released by SoftMic and will move the bottle from the BLOODS_POSITIVE resulting worklist to the BLOODS_NEGATIVE resulting worklist • Open the BACTEC door and scan the bottle. A message will appear 

	• Select **Yes** and load the bottle into the instrument. The bottle can be placed in any available station
7	If the bottle goes positive a second time and bacteria ARE seen: • Order and report the gram stain as above-2. Reporting positive fluid cultures in blood culture bottles in LIS, bacteria seen
8	If the bottle goes positive a second time and bacteria are NOT seen: • Do NOT re-load the bottle a third time • Refer to instructions below, where 5-hour window for bottle replacement into the BACTEC has expired
9	If the 5-hour window for bottle replacement into the BACTEC has expired, it cannot be loaded back into the instrument: • Gram stain needs to be read from the bottle daily for 5 days and then fully sub-cultured on Day 5 • In the media resulting area, select the **Media ID** for the positive bottle  • With the Media ID for the positive bottle selected, select **Add Media** from the media resulting area and add the media **5DAY**  • Ensure the positive flag ✓ is in the + column so that no preliminary or final negative reports are released by SoftMic  • Tape a note to the bottle indicating the dates the gram stains need to be performed and the date of the 5-day sub-culture • Place the bottle in the O_2 incubator on the top shelf

<table>
<tr><td>10</td><td>Processing of 5-Day Media
<table>
<tr><td>Day One</td><td>• Bottle goes positive in BACTEC
• Positive bottle gram (Day 1 gram)
• Positive bottle media set up</td></tr>
<tr><td>Day Two</td><td>• Make gram from bottle (Day 2 gram)
• Read aerobic media</td></tr>
<tr><td>Day Three</td><td>• Make gram from bottle (Day 3 gram)
• Read aerobic media and discard
• Read anaerobic media and discard
• Issue the no growth after 48 hours preliminary report
➢ In the test resulting area, under the test order that corresponds to the bottle that was sub-cultured select Key 1-~No growth after 48 hours of incubation</td></tr>
<tr><td>Day Four</td><td>• Make gram from bottle (Day 4 gram)</td></tr>
<tr><td>Day Five</td><td>• Perform 5 day bottle subculture
• Read 5 day bottle subculture gram (Day 5 gram)</td></tr>
<tr><td>Day Six</td><td>• Read aerobic media and discard
• Read anaerobic media and discard
• Issue the no growth after 5 days final report
➢ In the test resulting area, under the test order that corresponds to the bottle that was sub-cultured select Key 2-No growth after 5 days of incubation</td></tr>
</table></td></tr>
<tr><td>11</td><td>If bacteria are seen on any of the daily gram stains or the day 5 subculture, report as above-2. Reporting positive fluid cultures in blood culture bottles in LIS, bacteria seen.</td></tr>
</table>

LIMITATIONS:

1. If rare or no organisms are seen from a normally sterile site, but the specimen appears purulent, or the specimen looks suspicious, perform more extensive review of the slide.
2. 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.
3. Careful adherence to procedure and interpretive criteria is required for accurate results. Accuracy is highly dependent on the training and skill of microscopists.
4. Be wary of interpretations made from observing very few organisms (especially in the absence of inflammation or if the organisms are unevenly distributed), as collection tubes, slides and media may harbor nonviable bacteria. For sterile fluids, where the results will define an infectious process, prepare a second smear to confirm rare findings of microorganisms.
5. 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.).

6. False gram stain results may be related to inadequately collected specimens or delays in transit.
7. Prior treatment with antimicrobial drugs may cause gram positive organisms to appear gram negative.

CROSS-REFERENCES:

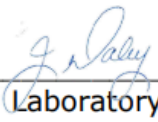
- MIC10100-Microbiology Specimen Processing
- MIC60060-Microbiology Stain Quality Control
- LQM70620-Laboratory Critical Results List-Microbiology

REFERENCES:

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

APPROVAL:

March 18, 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