PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20500 – Gram stain reporting in LIS-Blood Culture Specimens	Policy Number: 15-161-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:
Blood cultures are collected from patients with suspected sepsis or bacteremia. Due to the nature of these specimens, positive blood cultures are considered STAT, and the gram stain needs to be read within 1 hour of positive notification or within 1 hour of receipt of the positive bottle during regular microbiology laboratory hours.

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

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

SAMPLE INFORMATION:

Type	1. Reporting positive blood cultures in LIS, bacteria seen 2. Reporting positive blood cultures in LIS, bacteria NOT seen 3. Reporting positive blood cultures from Inuvik in LIS, bacteria seen 4. Reporting positive blood cultures from Inuvik in LIS, bacteria NOT seen 5. Reporting of >24 hour blood culture bottles in LIS 6. Reporting of >24 hour blood culture bottles from Inuvik in LIS

REAGENTS and/or MEDIA:

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

SUPPLIES:

- Glass microscope 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:

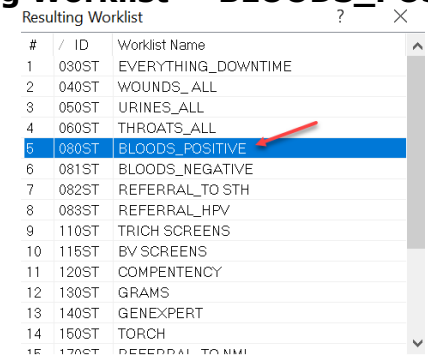
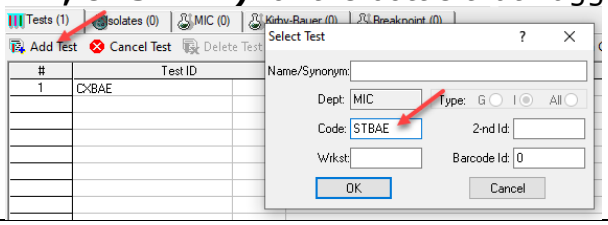
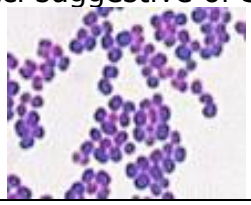
- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure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

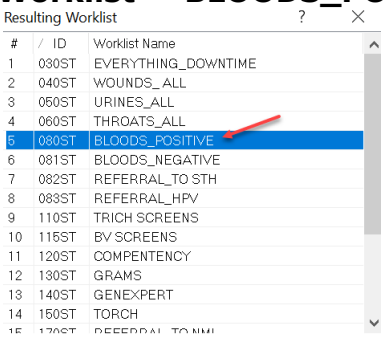
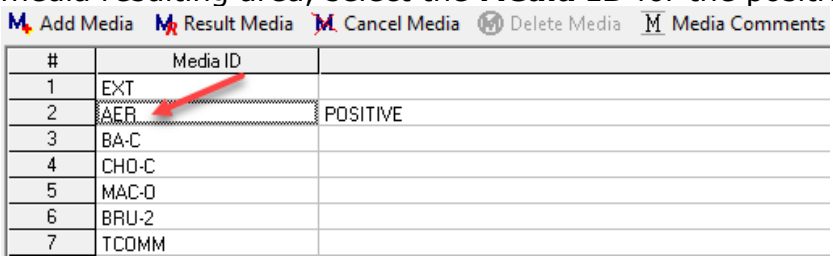
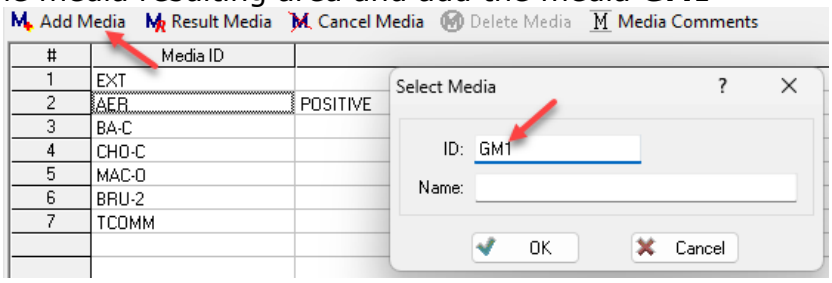
QUALITY CONTROL:

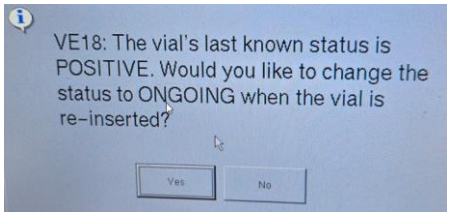
- Quality control is performed daily
- A TQC order is automatically generated daily to record the QC results
- Refer to MIC60060-Microbiology Stain Quality Control

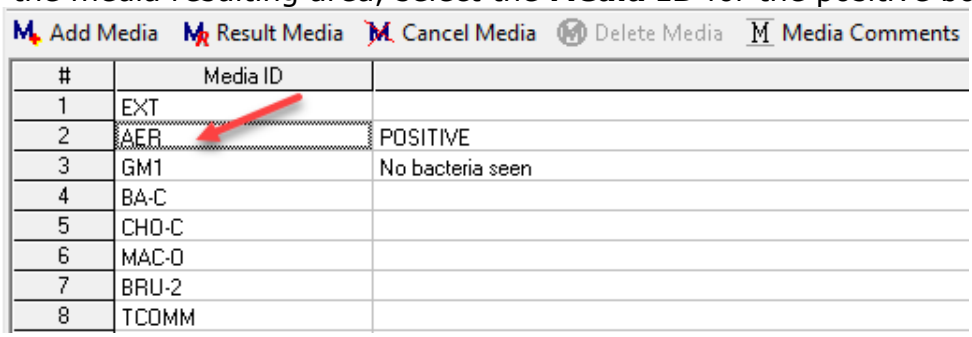
PROCEDURE INSTRUCTIONS:

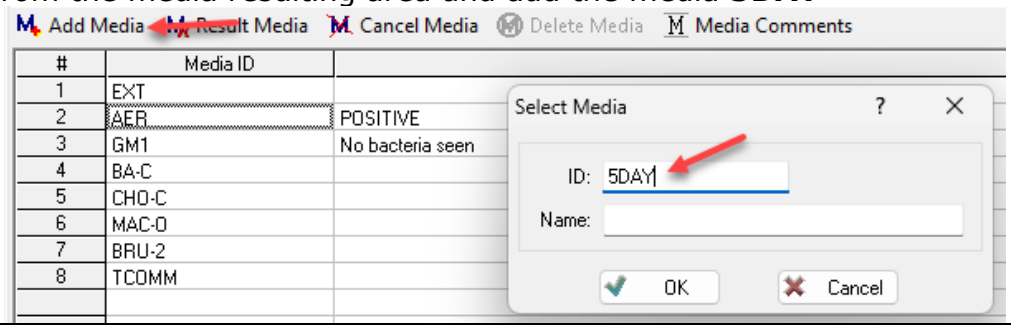
Step	Action
1. Reporting positive blood cultures in the LIS, bacteria seen	
1	• Pending positive blood culture orders are found in the LIS Resulting Worklist: **Resulting Worklist → BLOODS_POSITIVE**  • Press enter or double click to open worklist
2	• Enter the accession number on the slide and select 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 for the bottle that went positive 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 

	<u>If the bacteria seen resembles ***Streptococci* spp.**</u>: • Report: Gram positive cocci suggestive of Streptococci  • If the ordering location of the positive blood culture is Stanton Territorial Hospital, copy Stanton Infection Prevention and Control **(SIPAC)** **NOTE:** Use caution. Report as Gram positive cocci if doubt exists
8	Bacteria seen in the gram stain of blood 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
2. Reporting positive blood cultures in the 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	Check to see if a CBC was performed on the patient. Instrument false positives have been attributed to background CO_2 production that can be caused by increased white blood cell counts.
6	If certain that no bacteria are in the gram stain: • In the media resulting area, select the **Media ID** for the positive bottle  Add Media Result Media Cancel Media Delete Media Media Comments \# Media ID 1 EXT 2 AER POSITIVE 3 BA-C 4 CHO-C 5 MAC-O 6 BRU-2 7 TCOMM • With the Media ID for the positive bottle selected, select **Add Media** from the media resulting area and add the media **GM**1  Add Media Result Media Cancel Media Delete Media Media Comments \# Media ID 1 EXT 2 AER POSITIVE 3 BA-C 4 CHO-C 5 MAC-O 6 BRU-2 7 TCOMM Select Media ID: GM1 Name: OK Cancel • Using the GM1 keypad, select **Key 0-No bacteria seen** to document that the gram stain was read and that bacteria were not seen

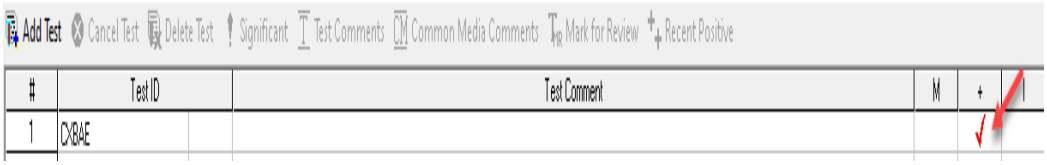
<table>
<tr><td>7</td><td>If the 5-hour window for bottle replacement into the BACTEC has NOT expired, it can be loaded back into the instrument:
In the LIS, double click the positive flag ✓ to remove it. This will ensure that any preliminary or final reports will be automatically released by SoftMic 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</td></tr>
<tr><td>8</td><td>If the bottle goes positive a second time and bacteria ARE seen:
Order and report the gram stain as above-1. Resulting positive blood cultures in LIS, bacteria seen</td></tr>
<tr><td>9</td><td>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 the 5-hour window for bottle replacement into the BACTEC has expired</td></tr>
<tr><td>10</td><td>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 
</td></tr>
</table>

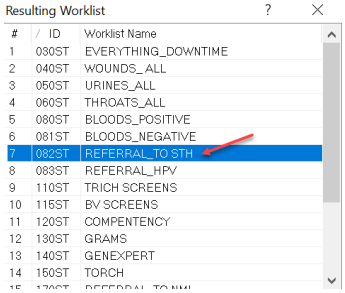
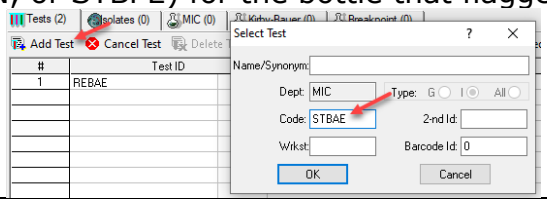
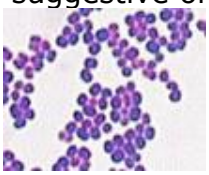
<table>
<tr><td></td><td>

Ensure the positive flag ✓ is in the + column so that no preliminary or final negative reports are released by SoftMic

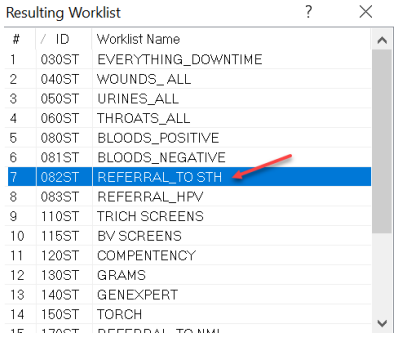
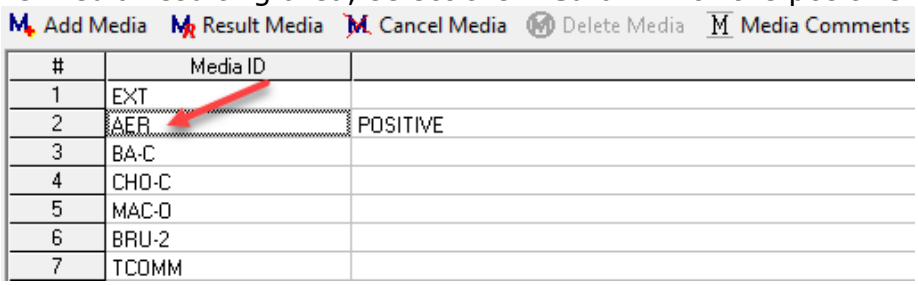
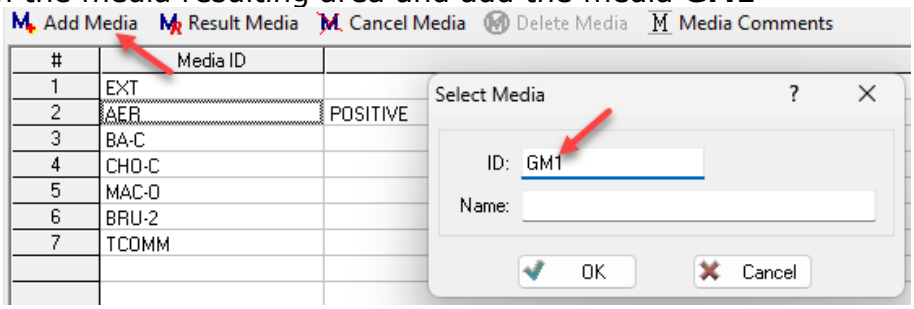


Tape a note to the bottle indicating the dates the gram stains need to be performed and the date of the 5-day sub-culture
Place the bottle in the O_2 incubator on the top shelf

</td></tr>
<tr><td>11</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>12</td><td>If bacteria are seen on any of the daily gram stains or the day 5 subculture, report as above-1. Reporting positive blood cultures in LIS, bacteria seen.</td></tr>
</table>

Step	Action
3. Reporting positive blood cultures from Inuvik in the LIS, bacteria seen	
1	Refer to MIC10900-Receiving Inuvik Positive Blood Culture Bottle-Stanton Job Aid to receive the bottle in the LIS at Stanton.
2	• Pending positive blood culture orders from Inuvik are found in the LIS Resulting Worklist: **Resulting Worklist → REFFERAL_TO STH**  • Press enter or double click to open worklist
3	• 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
4	Add one drop of immersion oil to the slide. Using the oil immersion lens (100X); examine 20 to 40 fields to observe gram reaction.
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  <u>If the bacteria resembles ***Streptococci* spp.**</u>: • Report: Gram positive cocci suggestive of Streptococci  • If the ordering location of the positive blood culture is Inuvik Regional Hospital, copy Inuvik Infection Prevention and Control **(IIPAC)** **NOTE:** Use caution. Report as Gram positive cocci if doubt exists

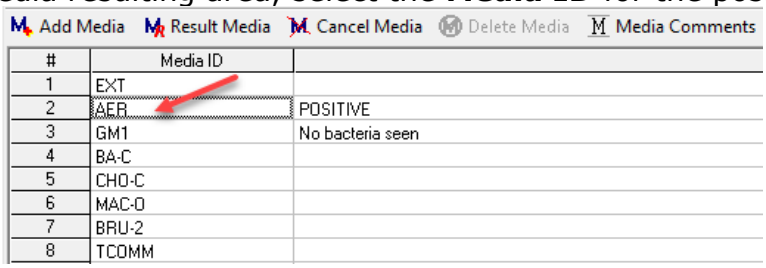
8	Bacteria seen in the gram stain of blood cultures is considered a critical result: • Phone the ordering location to give result • Document the call in the Call box • If unable to reach ordering location, consult the hospital wide policy 15-10-V1-Laboratory Critical Results Procedure
9	Finalize the 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
4. Reporting positive blood cultures from Inuvik in the LIS, bacteria NOT seen	
1	• Pending positive blood culture orders from Inuvik are found in the LIS Resulting Worklist: **Resulting Worklist → REFFERAL_TO STH**  • 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	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

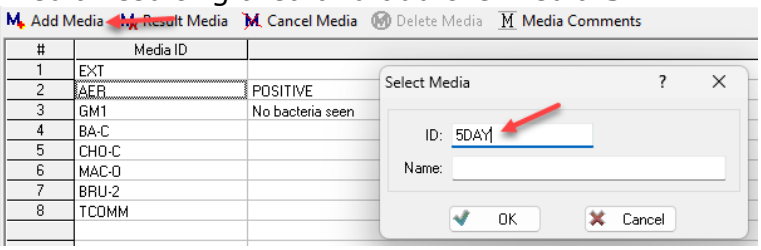
6

The bottle cannot be loaded onto the STH BACTEC:

- 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**



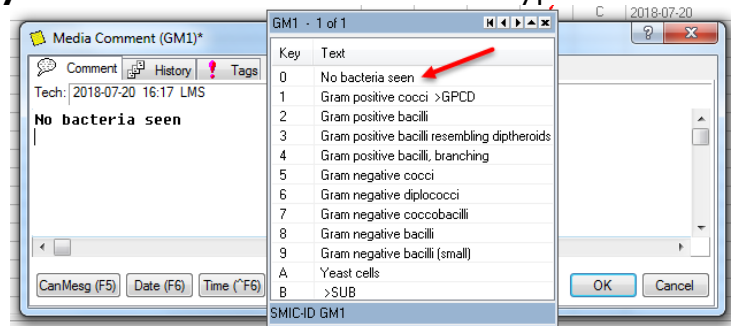
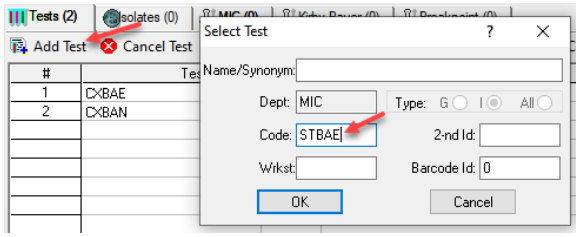
- Tape a note to the bottle indicating the dates the gram stains need to be performed and the date of the 5-day sub-culture
- Place the bottle in the O_2 incubator on the top shelf

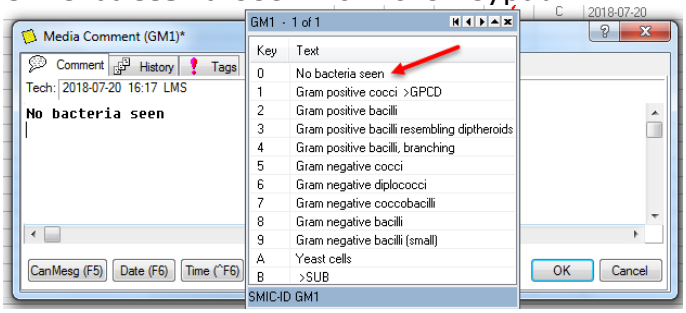
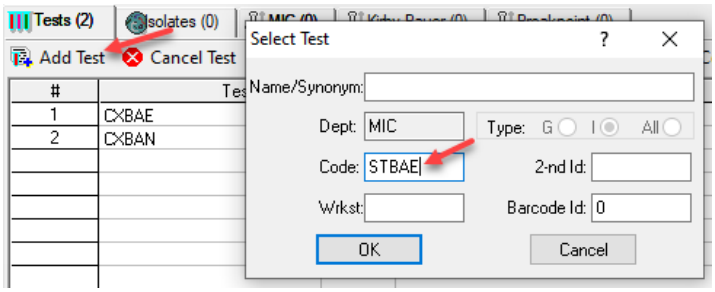
7

Processing of 5-Day Media

Day One	• Bottle goes positive in BACTEC • Positive bottle gram (Day 1 gram) • Positive bottle media set up
Day Two	• Make gram from bottle (Day 2 gram) • Read aerobic media
Day Three	• 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**
Day Four	• Make gram from bottle (Day 4 gram)
Day Five	• Perform 5 day bottle subculture • Read 5 day bottle subculture gram (Day 5 gram)
Day Six	• 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**

8	If bacteria are seen on any of the daily gram stains or the day 5 subculture, report as above-3. Reporting positive blood cultures from Inuvik in the LIS, bacteria seen

Step	Action
5. Reporting of >24 hour blood culture bottles in the LIS	
1	In Result Entry, enter the accession number on the slide and select enter.
2	Add one drop of immersion oil to the slide. Using the oil immersion lens (100X), examine 20 to 40 fields to observe cell morphology and gram reaction.
3	Confirm the media >24 hour has been ordered. If not, refer to MIC10100-Microbiology Specimen Processing Ordering >24-hour bottles to order this media.
4	In the media resulting area, result the media **GM1**, using the **GM1** keypad.
5	If bacteria are NOT seen in the gram stain: • Select **Key 0 No bacteria seen** from the keypad  • Ensure the bottle has been loaded into the BACTEC
6	If bacteria ARE seen in the gram stain: • Add the positive flag ✓ by double clicking in the + column  • In the test resulting area, add test STBAE, STBAN or STBPE, depending on which bottle the bacteria were seen in  • Report the gram stain as above-1. Reporting positive blood cultures in LIS, bacteria seen • If the bottle has already been loaded into the BACTEC instrument, remove and place in the positive blood culture storage box in the O_2 incubator

Step	Action
6. Reporting of >24 hour blood culture bottles from Inuvik in the LIS	
1	In Result Entry, enter the accession number on the slide and select enter.
2	Add one drop of immersion oil to the slide. Using the oil immersion lens (100X), examine 20 to 40 fields to observe cell morphology and gram reaction.
3	Confirm the media >24 hour has been ordered. If not, refer to MIC10100-Microbiology Specimen Processing Ordering >24-hour bottles to order this media.
4	In the media resulting area, result the media **GM1**, using the **GM1** keypad.
5	If bacteria are NOT seen in the gram stain: • Select **Key 0 No bacteria** seen from the keypad  • Ensure the bottle has been loaded into the BACTEC
6	If bacteria ARE seen in the gram stain: • Add the positive flag ✓ by double clicking in the + column  • In the test resulting area, add test STBAE, STBAN or STBPE depending on which bottle the bacteria were seen in:  • Report the gram stain as above-1. Reporting positive blood cultures in LIS, bacteria seen • If the bottle has already been loaded into the BACTEC instrument, remove and place in the positive blood culture storage box in the O_2 incubator

LIMITATIONS:

1. The presence of a microorganism from a normally sterile site is likely to indicate infection with that organism.
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. 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.).
5. False gram stain results may be related to inadequately collected specimens or delays in transit.
6. Prior treatment with antimicrobial drugs may cause gram positive organisms to appear gram negative.

CROSS-REFERENCES:

- MIC10100-Microbiology Specimen Processing
- MIC10900-Receiving Inuvik Positive Blood Culture Bottle-Stanton Job Aid
- MIC20100-Acridine Orange Stain
- MIC60060-Microbiology Stain Quality Control
- LQM70620-Laboratory Critical Results List-Microbiology LQM70620-Laboratory Critical Results List-Microbiology
- LQM70620-Laboratory Critical Results List-Microbiology LQM70620-Laboratory Critical Results List-Microbiology
- 15-10-V1-Laboratory Critical Results Procedure

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	20 Feb 24	Procedure reviewed	L. Steven