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	

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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 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. Positive blood cultures in BACTEC FX, bacteria seen 2. Positive blood cultures in BACTEC FX, bacteria not seen 3. Gram stain results for blood culture bottles received >24 hours after collection 4. Positive blood cultures received from Inuvik laboratory

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:

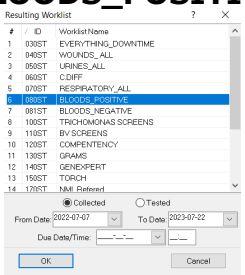
- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure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

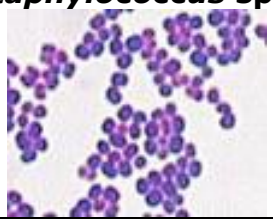
- Quality control is performed daily
- A TQC order is automatically generated daily to record the QC results
- Refer to MIC60060-Microbiology Stain Quality Control

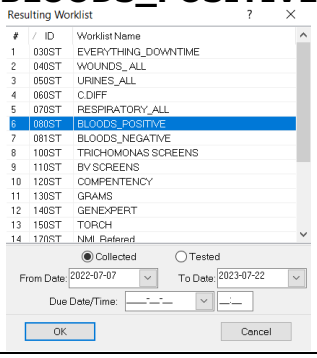
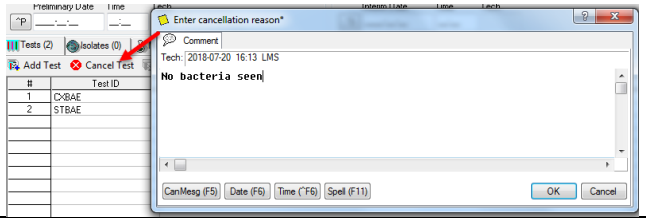
PROCEDURE INSTRUCTIONS:

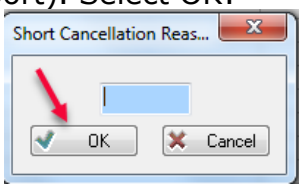
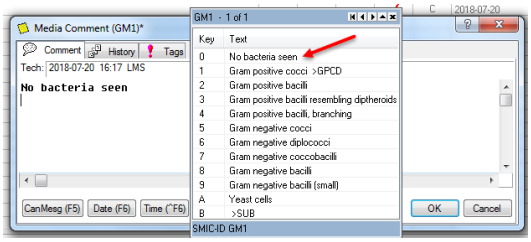
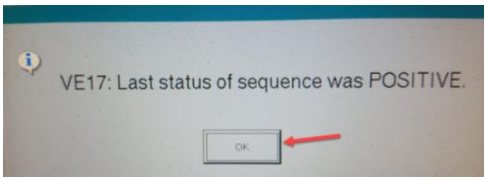
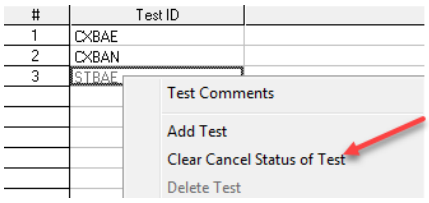
Step	Action
1. Reporting positive blood cultures in LIS, bacteria seen	
1	• Pending positive blood culture orders are found in the LIS Resulting Worklist: **Resulting Worklist → BLOODS_POSITIVE** 

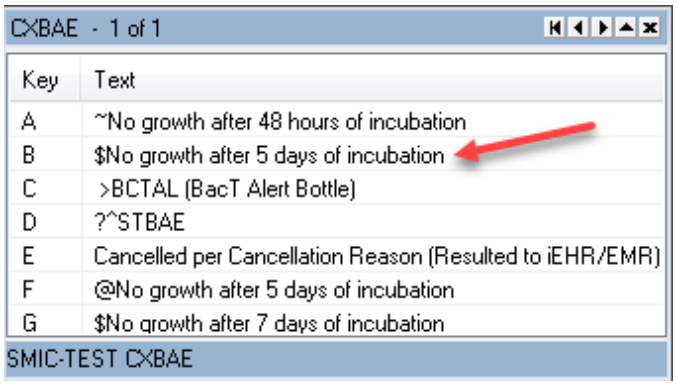
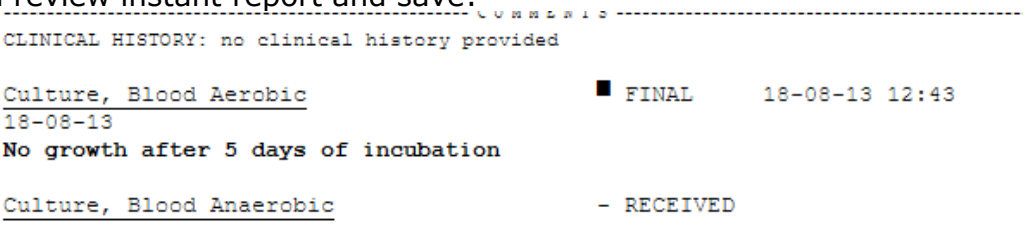
2	• Enter the accession number on the slide and select enter to mark the order • Select enter again to open Result Entry or double click on accession number to open
3	The **ST** order for the bottle that went positive is ordered in the LIS: **STBAE (aerobic)** **STBAN (anaerobic)** **STBPE (pediatric)** | # | Test ID | | # | Test ID | | # | Test ID | | 1 | CXBAE | | 1 | CXBAN | | 1 | CXBPE | | 2 | STBAE | | 2 | STBAN | | 2 | STBPE |
4	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. Use the **STBAE** and/or **STBAN** or **STBPE** keypad to report results.
5	Make sure the ST order matches the bottle that went positive. If the specimen collection label was placed on the wrong bottle, the LIS will place the wrong ST order.

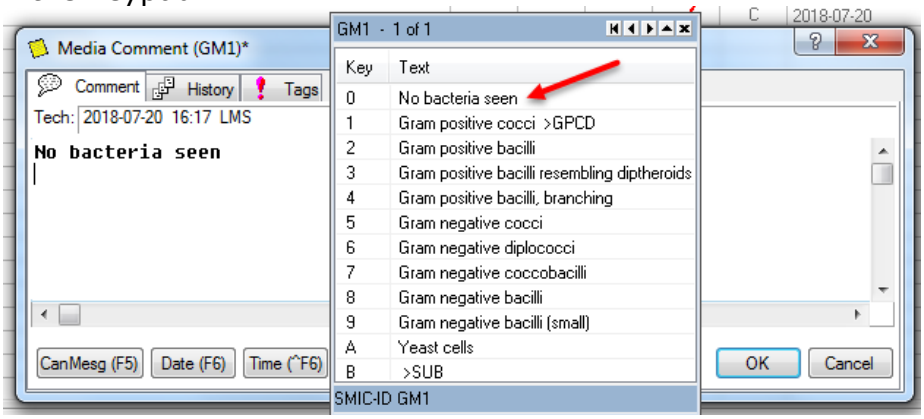
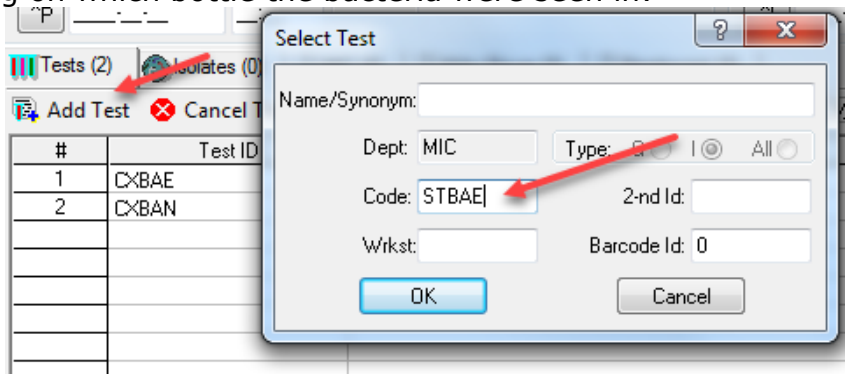
REPORTING INSTRUCTIONS FOR POSITIVE BC-BACTERIA SEEN:

IF	REPORT
Bacteria seen on gram stain	• Report using the **ST keypad** • Finalize **ST** order. Preview instant report and save • Bacteria seen in the gram stain of blood cultures is considered a critical result. Phone ordering location to give result • Document call in the **"Call"** box • If unable to reach ordering location, consult the hospital wide policy 15-10-V1-Laboratory Critical Results Procedure • Gently blot excess oil from slide using paper towel or gauze and save slides for further evaluation on the slide tray designated for day slide being read
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"** ***If sample location is Stanton Territorial Hospital or Inuvik Regional Hospital, copy appropriate infection control (SIPAC or IIPAC)*** **NOTE: Use caution if in doubt. If doubt exists, report as Gram positive cocci**

Step	Action
2. Reporting positive blood cultures in LIS, bacteria NOT seen	
1	• Pending positive blood culture orders are found in the LIS Resulting Worklist: **Resulting Worklist → BLOODS_POSITIVE** 
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	The **ST** order for the bottle that went positive is ordered in the LIS: **STBAE (aerobic)** **STBAN (anaerobic)** **STBPE (pediatric)** | # | Test ID | | # | Test ID | | # | Test ID | | 1 | CXBAE | | 1 | CXBAN | | 1 | CXBPE | | 2 | STBAE | | 2 | STBAN | | 2 | STBPE |
4	If no bacteria are seen: • Consider repeating smear • Consider performing acridine orange stain • Refer to MIC20100-Acridine Orange Stain Procedure
5	Check to see if CBC was performed on 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 present in the gram stain, perform the following in the LIS: 1. Remove the ✓ in the CX order + column by double clicking it:  2. Do NOT enter any results into the ST order 3. With the ST order selected, select "Cancel Test." Enter "No bacteria seen" in cancellation box: 

	4. In the "Short Cancellation Reason" box do **NOT** select any of the options (do not select Report). Select OK:  5. In the media resulting plate log, select Add Media to add the media **"GM1"** 6. Using the **GM1** keypad, select "No bacteria seen" to document that the gram stain was read:  7. Do NOT release a preliminary report, only select save
7	If the 5-hour window for bottle replacement into the BACTEC has NOT expired, it can be loaded back into the instrument: 1. Open the BACTEC door and scan the bottle. The following message will appear:  2. Select OK and load the bottle into the instrument. The bottle can be placed in any available station
8	If the bottle goes positive a second time and bacteria ARE seen: 1. Un-cancel the ST order that was cancelled 2. To un-cancel the ST order, right click the ST order, and select **"Clear Cancel Status of Test"**  3. Report the gram stain as above-Resulting positive blood cultures in LIS, bacteria seen 4. Place the positive Blood Culture bottle in the storage box in the O_2 incubator

9	If the bottle goes positive a second time and bacteria are still 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 FX has expired.
10	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 performed on the bottle daily for 5 days and fully sub-cultured on Day 5: 1. In the media resulting plate log, add the media **"5DAY"** 2. Ensure the ✓ is in the + column so that the order does not automatically finalize on day 5:  3. 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. 4. Place the bottle in the O_2 incubator on the top shelf.
11	If bacteria are seen on any of the daily gram stains or the day 5 subculture, un-cancel the ST test order and report as above.
12	If no bacteria are seen on any of the daily gram stains or the day 5 subculture, the order will need to be manually resulted: 1. In the test resulting log, under the test order that corresponds to the bottle that was sub-cultured (i.e., CXBAE for aerobic bottle CXBAN for anaerobic bottle or CXBPE for pediatric bottle), select **"Key B - $No growth after 5 days of incubation":**  2. Finalize the test 3. Preview instant report and save:  4. Discard the bottle in the sharp's container

Step	Action
3. Reporting of >24 hour blood culture bottles in 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 plate log, result the media **"GM1"**, using the **GM1** keypad.
5	• If bacteria are NOT seen in the gram stain, select "No bacteria seen" from the keypad  • Ensure the bottle has been loaded into the BACTEC FX
6	If bacteria ARE seen in the gram stain: 1. In the test resulting area, add test: **STBAE** or **STBAN** or **STBPE** depending on which bottle the bacteria were seen in:  2. Report the gram stain as above-Resulting positive blood cultures in LIS, bacteria seen 3. 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
4. Reporting of positive blood culture bottles received from Inuvik Laboratory	
1	Refer to MIC10900-Receiving Inuvik Positive Blood Culture Vial-Stanton Job Aid to receive the culture at Stanton.
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. Use the **STBAE** and/or **STBAN** or **STBPE** keypad to report results.
3	Make sure the ST order matches the bottle that went positive. If it does not, cancel the ST order and add the correct one.
4	If bacteria are seen on the slide, refer to above, "Reporting positive blood cultures in LIS, bacteria seen" instructions.
5	If bacteria are not seen, make another gram stain slide and re-examine. If bacteria are still not seen, select Key N from the ST Keypad to add the result "No bacteria seen" and final the ST order.
6	Monitor the culture media as per MIC34000-Blood Culture for growth. If growth is present, review original gram stain slide.

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. Carefully 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 Vial-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