PURPOSE: To report the Gram stain results of blood cultures in a consistent manner.

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 vials received > 24 hours after collection. 4. Positive blood cultures received from Inuvik. • Refer to MIC10230 – Microbiology Specimen Processing. • Refer to MIC70300 – BACTEC FX Instrument Procedures.

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 vial

SUPPLIES:

- Frosted end glass microscope slide
- Sub-culturing/aerobic venting unit
- 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.

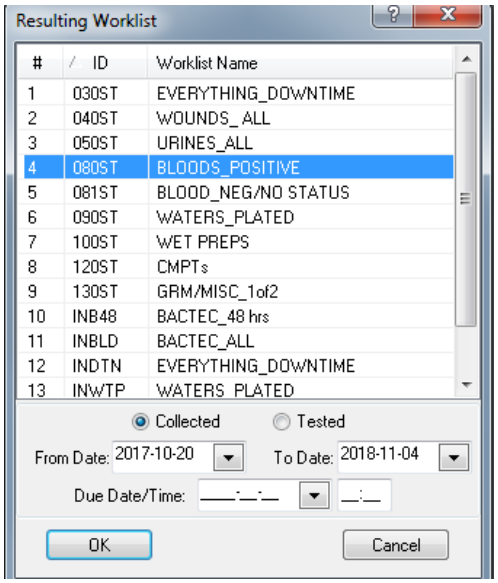
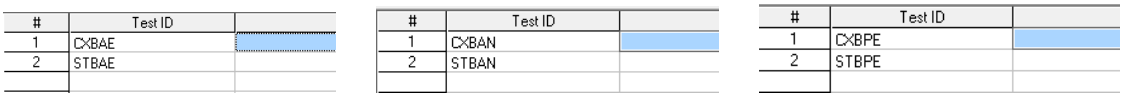
- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols,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

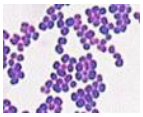
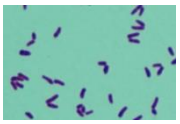
- Refer to MIC60060 – Microbiology Stain Quality Control.

PROCEDURE INSTRUCTIONS:

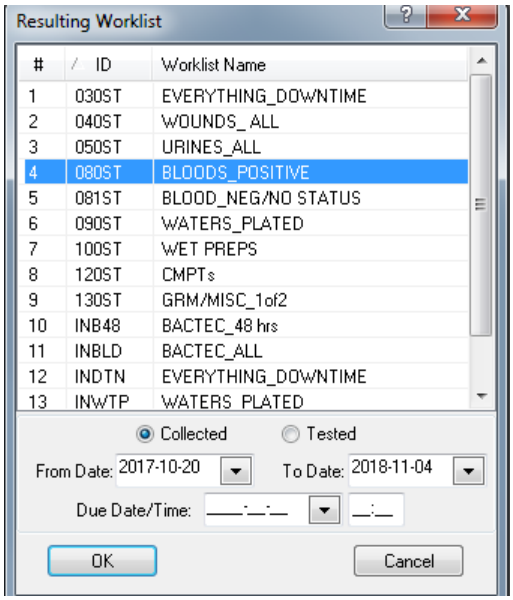
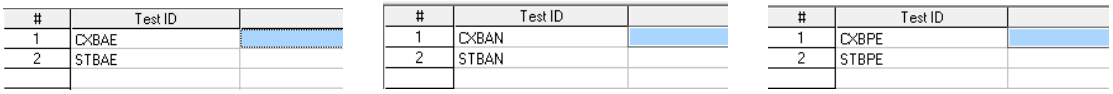
Step	Action
1. Resulting positive blood cultures in LIS, bacteria seen	
1	Pending positive blood culture orders are found in the LIS Resulting Worklist: **Resulting Worklist → BLOODS_POSITIVE** 
2	Enter the accession number on the slide and select enter to mark the order. Select enter again to open Result Entry or double click on accession number to open.
3	The **ST** order for the vial that went positive is automatically ordered in the LIS: **CXBAE (aerobic vial)** **CXBAN (anaerobic vial)** **CXBPE (pediatric vial)** 
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	Finalize **STBAE** and/or **STBAN** or **STBPE**. Preview instant report and save.
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.

NOTE: Please make sure the ST order matches the bottle that went positive. If the specimen collection label was placed on the wrong bottle, the wrong ST order will be placed by the LIS.

REPORTING OF RESULTS FOR POSITIVE BLOOD CULTURES (bacteria seen):

IF	REPORT
Bacteria seen on Gram stain	• Report using the **STBAE** and/or **STBAN** or **STBPE** keypad. • 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. Refer to LIS?????. • If unable to reach ordering location, consult the hospital wide policy "Laboratory: Critical Values – Responsible Party".
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.**

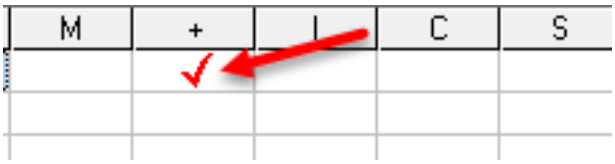
PROCEDURE INSTRUCTIONS:

Step	Action
2. Resulting positive blood cultures in LIS, bacteria NOT seen	
1	Pending Positive Blood culture orders are found in the LIS under: **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 vial that went positive is automatically ordered in the LIS: **CXBAE (aerobic vial)** **CXBAN (anaerobic vial)** **CXBPE (pediatric vial)** 
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.
5	If no bacteria seen: ➢ Consider repeating smear. ➢ Consider performing acridine orange stain. Refer to MIC20100 – Acridine Orange Stain Procedure.
6	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.

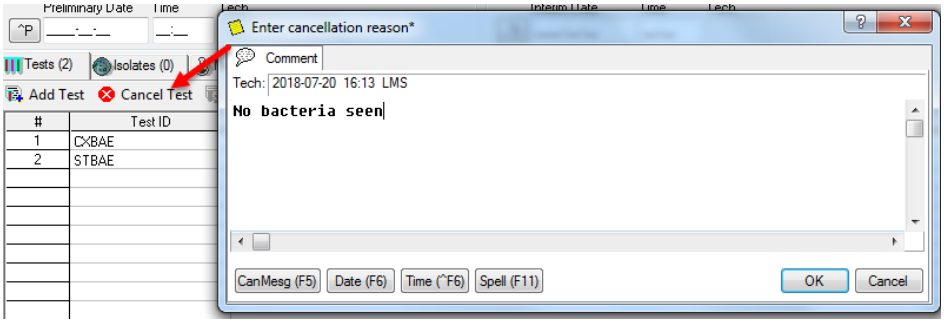
7

If certain that no bacteria are present in the Gram stain, perform the following in the LIS:

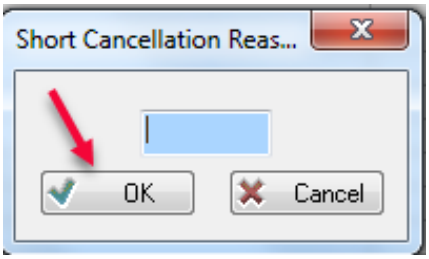
1. Remove the ✓ in the + column by double clicking it:



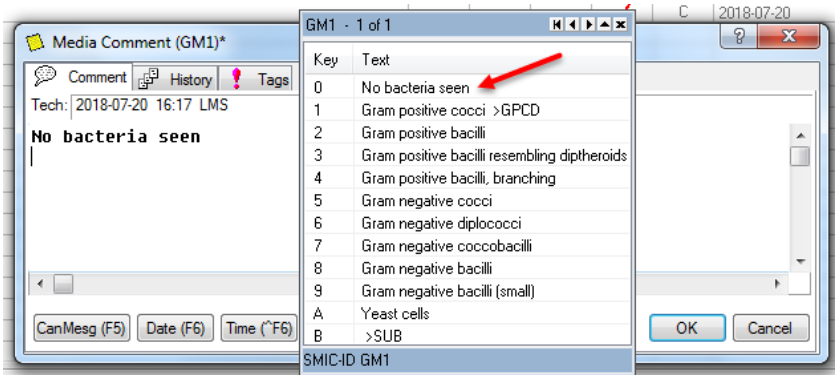
2. Do NOT enter any results into the ST order. With the ST order selected, select “Cancel Test”. Enter “No bacteria seen” in cancellation box:



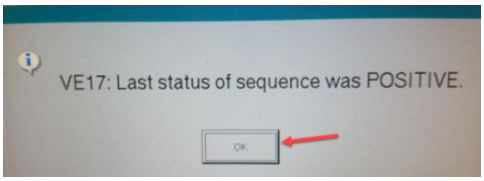
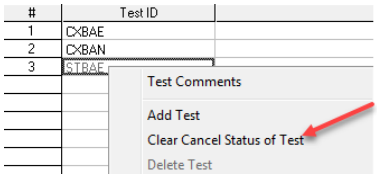
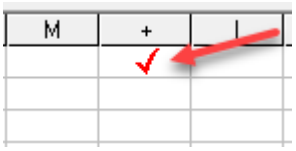
3. In the “Short Cancellation Reason” box do **NOT** select any of the options (do not select Report). Select OK:

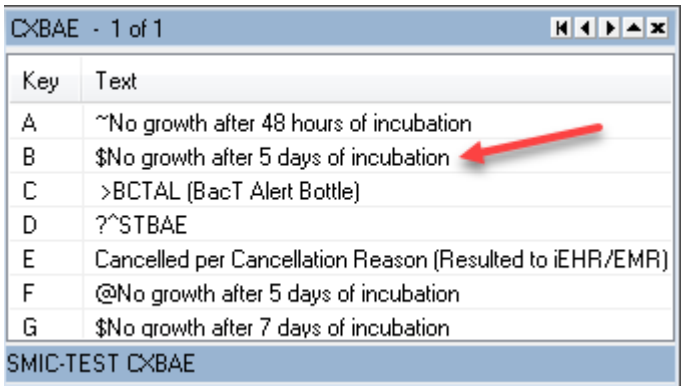
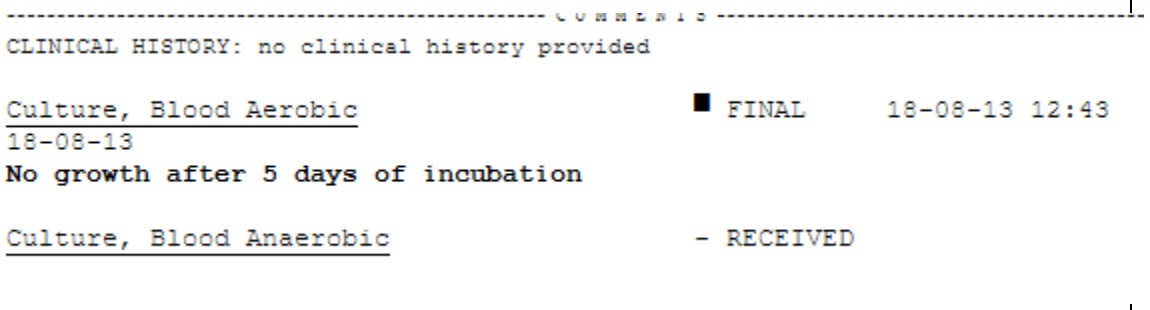


4. In the media resulting plate log, add the media **“GM1”**. Using the **GM1** keypad, select “No bacteria seen” to document that the Gram-stain was read:

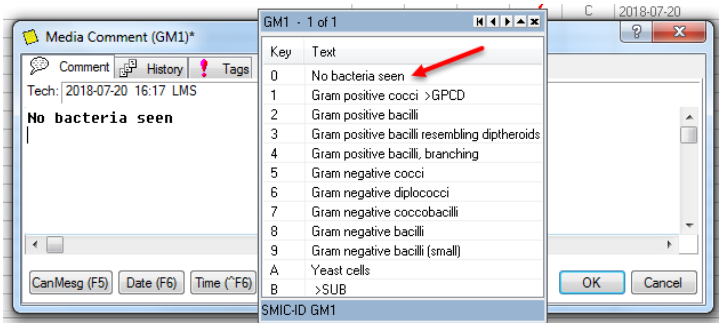
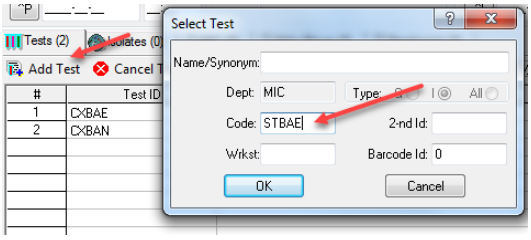


5. Do NOT release a preliminary report, only select save.

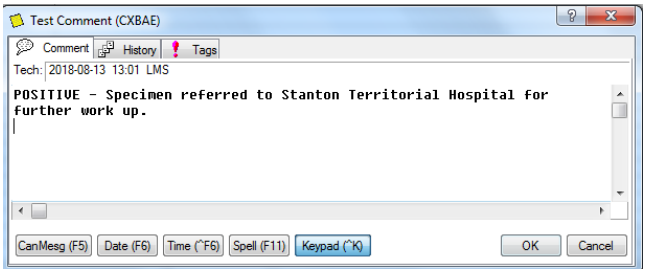
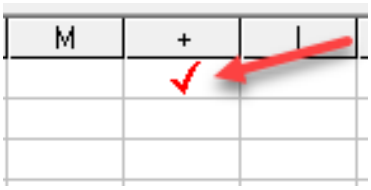
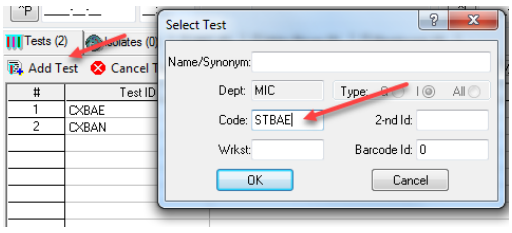
8	If the 5-hour window for vial replacement into the BACTEC has NOT expired it can be loaded back into the instrument: 1. Open the BACTEC door and scan the vial. The following message will appear:  2. Select OK and load the vial into the instrument. The vial can be placed in any available station.
9	If the vial goes positive a second time and bacteria ARE seen: 1. Un-cancel the ST order that was cancelled. To un-cancel the ST order, right click the ST order and select **"Clear Cancel Status of Test".**  2. Report the Gram stain as above - Resulting positive blood cultures in LIS, bacteria seen. 3. Place the positive Blood Culture vial in the storage box in the O_2 incubator.
10	If the vial goes positive a second time and bacteria are still not seen, do not re-load the vial a third time. Refer to instructions below, where 5-hour window for vial replacement into the BACTEC FX has expired.
11	If the 5-hour window for vial replacement into the BACTEC has expired, it cannot be loaded back into the instrument. Gram stain needs to be performed on the vial daily for 5 days and fully sub-cultured on Day 5. 1. In the media resulting plate log, add the media **"5DAY"**.Ensure the ✓ is in the + column so that the order does not automatically finalize on day 5.  2. Tape a note to the vial indicating the dates the Gram stains need to be performed and the date of the 5 day sub-culture. 3. Place the vial in the O_2 incubator on the white tray labelled **"5 DAY BC".**

12	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 listed above.
13	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 vial that was sub-cultured (i.e. CXBAE for aerobic vial, CXBAN for anaerobic vial or CXBPE for pediatric vial), select **"Key B - $No growth after 5 days of incubation"**.  2. Finalize the test. 3. Preview instant report and save.  4. Discard the vial in the sharps container.

PROCEDURE INSTRUCTIONS:

Step	Action
3. Reporting of blood culture vials received >24 hours after collection 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 MIC10230 – Microbiology Specimen Processing, Ordering >24 hour vials 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 vial 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 vial the bacteria were seen in:  2. Report the Gram stain as above - Resulting positive blood cultures in LIS, bacteria seen. 3. If the vial has already been loaded into the BACTEC instrument, remove and place in the positive blood culture storage box in the O_2 incubator.

PROCEDURE INSTRUCTIONS:

Step	Action
4. Reporting of positive blood culture vials received from Inuvik Laboratory	
1	Refer to LIS????? – Receiving positive blood cultures from Inuvik, to receive the culture to Stanton. Subculture vial as per MIC10230 – Microbiology Specimen Processing.
2	In Result Entry, enter the accession number on the slide and select enter.
3	When the blood culture goes positive in the BACTEC in Inuvik, the technologist releases a preliminary report that states: "POSITIVE - Specimen referred to Stanton Territorial Hospital for further work up." 
4	Ensure the ✓ is in the + column.  If the + column does not contain the ✓, double click the + column and the ✓ will be added.
5	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.
6	If bacteria ARE seen in the Gram stain, the ST test will need to be ordered:
7	In the test resulting area, add test: **STBAE** or **STBAN** or **STBPE** depending on which vial the bacteria were seen in: 

8	Report the Gram stain as above - Resulting positive blood cultures in LIS, bacteria seen.
9	Ensure the vial has not been loaded onto the BACTEC FX.
10	Place the positive Blood Culture vial in the storage box in the O_2 incubator.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016.
- Stanton Territorial Hospital, LIS Manual, 2018.

REVISION HISTORY:

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