Title: MIC20400-Gram stain reporting in LIS-Sterile Fluid Specimens

Issuing Authority: Director, Health Services

Next Review Date:

Type: Laboratory Services Program SOP

Policy Number: Date Approved:

PROGRAM Standard Operating Procedure – Laboratory Services			
Title: MIC20400 – Gram stain reporting in LIS-Sterile Fluid Specimens	Policy Number:		
Program Name: Laboratory Services			
Applicable Domain: Lab, DI and Pharmacy Services			
Additional Domain(s):			
Effective Date:	Next Review Date:		
Issuing Authority:	Date Approved:		
Director, Health Services			
Accreditation Canada Applicable Standard: N/A			

## **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 procedure applies to Medical Laboratory Technologists (MLTs) reporting gram stain results for sterile fluids in the LIS.

### **SAMPLE INFORMATION:**

IVDA	•	Sterile fluids, including CSF
	•	Refer to MIC10100-Microbiology Specimen Processing

# **REAGENTS and/or MEDIA:**

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

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#### **SUPPLIES:**

Ringed cytology slide

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 potential infectious materials or cultures.

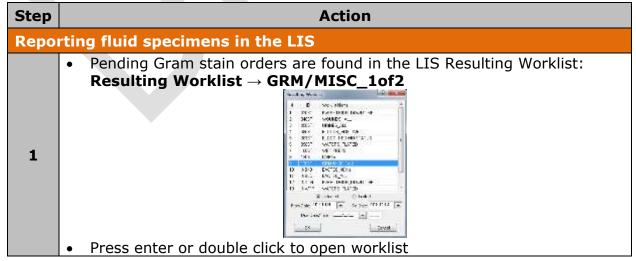
- Ensure that appropriate hand hygiene practices be used.
- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure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



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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	number to open  Under low power (X10, 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 pegative			
	<ul> <li>Determine areas representate contamination with squamou</li> </ul>		reas of	
4	Add one drop of immersion oil to the slide. In a representative area with			
	Under oil immersion (X100, OIF): quantitate epithelial cells, white blood cells, red blood cells and bacteria as follows:			
	None seen	No cells seen		
	1+	< 1 cell seen		
5	2+	1 - 9 cells seen		
	3+	10 - 25 cells seen		
	4+	> 25 cells seen		
	NOTE: Only report "None seen" for epithelial cells or red blood cells are		cteria. If no	
6	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.			

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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.

## **REPORTING INSTRUCTIONS:**

REPORTING INSTR		
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 <b>STGM1</b> keypad	
Bacteria seen on Gram stain	<ul> <li>Quantitate and report using the STGM1 keypad</li> <li>Bacteria seen in the Gram stain of sterile fluids are considered a critical result. Phone ordering location to give result</li> <li>Document call in the "Call" box</li> <li>If unable to reach ordering location, consult the hospital wide policy 15-10-V1-Laboratory Critical Results Procedure</li> </ul>	
Bacteria		
resembles:		
Staphylococcus		
spp.	Report: "Gram positive cocci suggestive of	
St 150 027 .	Staphylococci"	
	NOTE: Use caution. If doubt exists, report as Gram positive cocci.	
Bacteria resembles: <b>Streptococcus</b>	Report: "Gram positive cocci suggestive of Streptococci"	
spp.	*If sample location is Stanton Territorial Hospital or Inuvik Regional Hospital, copy appropriate infection control (SIPAC or IIPAC)*	
100	NOTE: Use caution. If doubt exists, report as Gram positive cocci.	

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Step	Action		
Comp	Complete reading of sterile fluid slides		
1	<ul> <li>If the specimen is routine, save the gram stain and do not finalize STGM1</li> <li>If the specimen is STAT, finalize STGM1</li> <li>Preview instant report and save</li> <li>If finished reading slides, ensure Gram stains remaining on worklist have been prepared to be read at a later time</li> </ul>		
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.		

#### **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.
- 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
- MIC60060-Microbiology Stain Quality Control
- 15-10-V1-Laboratory Critical Results Procedure

#### **REFERENCES:**

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

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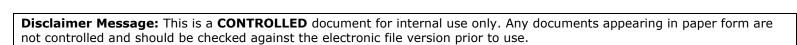
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APPROVAL:	
Date	

# **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



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