	Stanton Territorial Hospital P.O. Box 10, 550 Byrne Road	Document Number: MIC20200	
		Version No: 1.0	Page: 1 of 6
NORTHWEST TERRITORIES	YELLOWKNIFE NT X1A 2N1	Distribution: Microbiology Microscopy Manual	
Health and Social Services Authority			
Services Authority	Effective:		
Document Name: Gram stain resulting in LIS –		Date Reviewed: Next Review:	
Routine specimens			
Approved By:		Status: DRAFT	

PURPOSE: To report the Gram stain results of routine specimens in a consistent manner.

SAMPLE INFORMATION:

	Wound, ear, eye, lower genital tract (excluding BV) and
Туре	male urethra gonorrhoeae specimens.
	Refer to MIC10230 – Microbiology Specimen Processing.

REAGENTS INFORMATION:

	BD™ Gram Crystal Violet, 3.8 L, B4312526	
Tupo	BD™ Gram Iodine (Stabilized), 3.8 L, B4312543	
Туре	BD™ Gram Decolorizer, 3.8 L, B4312528	
	BD™ Gram Safranin, 3.8 L, B4312531	
Source	Fisher Scientific Canada	
Storage	Store at 15° to 30°	
Stability	As per expiry date listed on bottle	

SUPPLIES:

- Frosted end glass microscope slide
- QC slide
- Methanol, absolute
- Immersion oil
- Microscope
- Slide storage tray

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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.

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PROCEDURE INSTRUCTIONS:

Step	Action			
	Pending Gram stain orders are found in the LIS Resulting Worklist:			
	Resulting Worklist \rightarrow GRM/MISC_1of2			
1	Resulting Worklist P # / ID Worklist Name 1 0305T EVERYTHING_DOWNTIME 2 040ST WOUNDS_ALL 3 050ST URINES_ALL 4 080ST BLODDS_POSITIVE 5 081ST BLODD_NEG/NO STATUS 6 090ST WATERS_PLATED 7 100ST WET PREPS 8 120ST GRM/MISC_1ot2 10 INB48 BACTEC_48 Ins 11 INBLD BACTEC_ALL 12 INOT EVERYTHING_DOWNTIME 13 INWTP WATERS PLATED IV Collected Tested From Date; 2017-10-20 To Date; 0K Cancel			
	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. Under low power (10X, 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			
2	clear or Gram negative.			
3	> 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.			

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	Under the test co	de: STGM1, use the STC	M1 keypad to report the qu	antity of		
4	epithelial cells, white blood cells, red blood cells and bacteria seen. Report cells in the order to maintain consistency with reporting. Scan approximately 20 to 40 fields.					
	Epithelial cells, w	hite blood cells and red b	lood cells are quantified as	follows under		
	LPF (10X):					
		None seen	No cells seen			
		1+	< 1 cells seen			
5		2+	1 - 9 cells seen			
5		3+	10 - 25 cells seen			
		4+	> 25 cells seen			
	NOTE: Only report "None seen" for white blood cells. If no epithelial cells or red blood cells are seen, do not report this.					
	Add one drop of i	Add one drop of immersion oil to the slide. In a representative area with predominance				
6	of inflammation o	r purulence using the oil	immersion lens (100X), exa	mine 20 to 40		
	fields to observe cell morphology and Gram reaction.					
	Bacterial and yeast cells are quantified as follows under OIF (100x):					
			No collo coor			
		None seen	No cells seen			
		None seen 1+	< 1 cells seen			
7						
7		1+	< 1 cells seen			
7		1+ 2+	< 1 cells seen 1 - 9 cells seen			
7	NOTE: If no bac	1+ 2+ 3+	< 1 cells seen 1 - 9 cells seen 10 - 25 cells seen > 25 cells seen			
		1+ 2+ 3+ 4+	< 1 cells seen 1 - 9 cells seen 10 - 25 cells seen > 25 cells seen	ate in the media		
7	If 3 - 4+ Gram-ne	1+ 2+ 3+ 4+	< 1 cells seen 1 - 9 cells seen 10 - 25 cells seen > 25 cells seen result. the smear, add " CNA-C " pla	ate in the media		
	If 3 - 4+ Gram-ne resulting plate log	1+ 2+ 3+ 4+ seria are seen, report this gative bacilli are seen in gative bacilli are seen in gand subculture original	< 1 cells seen 1 - 9 cells seen 10 - 25 cells seen > 25 cells seen result. the smear, add " CNA-C " pla			
	If 3 - 4+ Gram-ne resulting plate log Finalize STGM1 .	1+ 2+ 3+ 4+ gative bacilli are seen in and subculture original Preview instant report a	< 1 cells seen 1 - 9 cells seen 10 - 25 cells seen > 25 cells seen result. the smear, add " CNA-C " pla specimen to CNA plate.	6C1of2 worklist.		
8	If 3 - 4+ Gram-ne resulting plate log Finalize STGM1 . If finished reading prepared to be re	1+ 2+ 3+ 4+ and subculture original Preview instant report a slides, ensure Gram stat ad at a later time.	 < 1 cells seen 1 - 9 cells seen 10 - 25 cells seen > 25 cells seen > 25 cells seen result. the smear, add "CNA-C" plate specimen to CNA plate. nd save. Refresh GRM/MIS ins remaining on worklist has	SC1of2 worklist. ave been		
8	If 3 - 4+ Gram-ne resulting plate log Finalize STGM1 . If finished reading prepared to be re Gently blot exces	1+ 2+ 3+ 4+ and subculture original Preview instant report a slides, ensure Gram stat ad at a later time.	< 1 cells seen 1 - 9 cells seen 10 - 25 cells seen > 25 cells seen > 25 cells seen result. the smear, add "CNA-C" plates specimen to CNA plate. Ind save. Refresh GRM/MIS ins remaining on worklist hat er towel or gauze and save	SC1of2 worklist. ave been		

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REPORTING OF RESULTS:

IF	REPORT		
No white blood cells	Report: "No white blood cells seen"		
seen on Gram stain	Report. No write blood cens seen		
No bacteria seen on	Report: "No bacteria seen"		
Gram stain	Report. No bacteria seen		
Epithelial cells, white			
blood cells and red	- Quantitate and report using the STCM1 keyned		
blood cells seen on	 Quantitate and report using the STGM1 keypad 		
Gram stain			
Bacteria seen on	Quantitate and report using the STGM1 keypad		
Gram stain			
Bacteria resembles:			
Staphylococcus	Report: "Gram positive cocci suggestive of Staphylococci"		
spp.			
A CARE	NOTE: Use caution if in doubt. If doubt exists, report as Gram positive cocci.		
Bacteria resembles:	Report: "Gram positive cocci suggestive of Streptococci"		
Streptococcus spp.			
12 tage 4	NOTE: Use caution if in doubt. If doubt exists, report as		
一次最	Gram positive cocci.		
Bacteria resembles:	Report: "Gram positive bacilli resembling diphtheroids"		
Diphtheroids			
1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	NOTE: Use caution if in doubt. If doubt exists, report as Gram positive bacilli.		

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LIMITATIONS:

- 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.
- 2. Carefully adherence to procedure and interpretive criteria is required for accurate results. Accuracy is highly dependent on the training and skill of microscopists.
- 3. 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.).
- 4. False Gram stain results may be related to inadequately collected specimens or delays in transit.
- 5. Prior treatment with antimicrobial drugs may cause Gram-positive organisms to appear Gram-negative.

REFERENCES:

• Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016.

REVISION HISTORY:

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

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