	Stanton Territorial Hospital	Document Number: MIC20600		
NORTHWEST TERRITORIES Health and Social Services Authority	P.O. Box 10, 550 Byrne Road YELLOWKNIFE NT X1A 2N1	Version No: Distribution: Microbiology Microscop	Page: 1 of 9 y Manual	
Document Name: Gram stain resulting in LIS – Bacterial Vaginosis		Effective: Date Reviewed: Next Review:		
Approved By:		Status: DRAFT		

PURPOSE: To report the Gram stain results from Bacterial Vaginosis screens in a consistent manner.

SAMPLE INFORMATION:

Туре	Vagina.
i î he	Refer to MIC10230 – Microbiology Specimen Processing.

REAGENTS INFORMATION:

	BD™ Gram Crystal Violet, 3.8 L, B4312526
Tumo	BD™ Gram Iodine (Stabilized), 3.8 L, B4312543
гуре	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

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					
	Pending BV Gram stain orders are found in the LIS Resulting Worklist:					
	Resulting Worklist → GRM/MISC_1of2					
		Resulting Worklist	े <mark>x</mark>			
1		# / ID Worklist Na 1 030ST EVERYTHI 2 040ST WOUNDS, 3 050ST URINES_A 4 080ST BLOODS_I 5 081ST BLOODNI 6 090ST WATERS_7 7 100ST CMPTs 9 130ST GRM/MISC 10 INB48 BACTEC_4 11 INBLD BACTEC_4 12 INDTN EVERYTHI 13 INWTP WATERS © Collected From Date: 2017-10-20 Due Date/Time:	ame A ING_DOWNTIME ALL ALL ALL ALL ALL ALL ALL ALL ALL AL			
	Enter the accession nu	umber on the slide a	nd select enter to ma	rk the order.		
2	Select enter again to c	ppen Result Entry or	double click on acces	ssion number to open.		
	Under the media resul	ting log: GMBV , use	e the GMBV keypad t	to record the quantity of		
3	epithelial cells, white b	lood cells, clue cells	, yeast cells, Trichom	ionas and bacteria		
5	significant to Bacterial	Vaginosis: Lactoba	<i>cillus</i> spp., Gram vari	able bacilli resembling		
	Gardnerella vaginalis a	and Gram negative b	bacilli resembling Mo	<i>biluncus</i> spp.		
	Epithelial cells, white b	lood cells and red b	lood cells are quantif	ied as follows under		
	LPF (10X):					
		None seen	No cells seen			
		1+	< 1 cells seen			
4		2+	1 – 9 cells seer	1		
		3+	10 – 25 cells see	en		
		4+	> 25 cells seen			
	NOTE: Only record "N	None seen" for WBC				

	Document Name:	Gram stain	resulting in	LIS – Bacterial	Vaginosis
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5	Add one drop of immersion oil to the slide. Using the oil immersion lens (100X);						
J	examine 20 to 40 fields to observe cell morphology and Gram reaction.						
	Bacterial and yeast cells are quantified as follows under OIF (100x):						
		None seen	No	cells see	en		
		1+ < 1 cells seen		en			
		2+	1 –	9 cells se	en		
6		3+	10 –	25 cells s	seen		
		4+	> 2	5 cells se	en		
	l					J	
	NOTE: If no bac	teria are seen, record this	s result.				
	Calculate the Nu	gent score of the specime	en. The N	I-score is	calculate	ed by ass	essing
	the quantity of La	<i>ictobacillus</i> spp., Gram-v	ariable ba	cilli and <i>I</i>	Mobiluncı	<i>u</i> s spp. E	xamine
	20 to 40 fields an	d interpret as follows:					
		N-Sco	ore Table				
	Quantitation of Points scored per morphotype						
	Qua	ntitation of	Points s	scored p	er morpl	notype	
	Qua bacteria	ntitation of al morphotype	Points s	scored p 1+	er morpl 2+	notype 3+	4+
7	Qua bacteria Lactobacillus spp	ntitation of al morphotype	Points s	scored p	er morpl 2+ 2	notype 3+	4+
7	Qua bacteria <i>Lactobacillus</i> spp Med/large Gram	ntitation of al morphotype o.: positive bacilli	Points s None 4	scored p 1+ 3	er morpl 2+ 2	notype 3+ 1	4+ 0
7	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagii	ntitation of al morphotype o.: positive bacilli nalis:	Points s None 4	scored p 1+ 3	er morpl 2+ 2 2	3+ 3+	4+ 0
7	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagii Small, Gram varia	ntitation of al morphotype o.: positive bacilli <i>nalis</i> : able bacilli	Points s None 4 0	scored p 1+ 3 1	er morpl 2+ 2 2	3+ 1 3	4+ 0 4
7	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagin Small, Gram varia Mobiluncus spp.:	ntitation of al morphotype o.: positive bacilli nalis: able bacilli	Points : None 4 0	scored p 1+ 3 1 1	er morpl 2+ 2 2	notype 3+ 1 3 2	4+ 0 4
7	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagin Small, Gram varia Mobiluncus spp.: Curved, Gram va	ntitation of al morphotype o.: positive bacilli nalis: able bacilli riable bacilli	Points : None 4 0 0	scored p 1+ 3 1 1	er morpl 2+ 2 2 1	3+ 1 3 2	4+ 0 4 2
7	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagir Small, Gram varia Mobiluncus spp.: Curved, Gram va	ntitation of al morphotype o.: positive bacilli nalis: able bacilli riable bacilli	Points : None 4 0 0	1+ 3 1	er morpl 2+ 2 2 1	3+ 1 3 2	4+ 0 4 2
7	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagir Small, Gram varia Mobiluncus spp.: Curved, Gram va Finalize STGM1 .	ntitation of al morphotype .: positive bacilli nalis: able bacilli riable bacilli Preview instant report a	Points s None 4 0 0 nd save.	scored p 1+ 3 1 1 Refresh 0	er morpl 2+ 2 2 1 GRM/MIS	3+ 1 3 2 3 3	4+042orklist.
7	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagir Small, Gram varia Mobiluncus spp.: Curved, Gram va Finalize STGM1 . If finished reading	ntitation of al morphotype o.: positive bacilli nalis: able bacilli riable bacilli Preview instant report a g slides, ensure Gram sta	Points s None 4 0 0 nd save. ains remai	scored p 1+ 3 1 Refresh (ning on v	er morpl 2+ 2 2 1 GRM/MIS	3+ 1 3 2 3C1of2 wave been	4+ 0 4 2 orklist.
7	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagin Small, Gram varia Mobiluncus spp.: Curved, Gram va Finalize STGM1 . If finished reading prepared to be re	ntitation of al morphotype o.: positive bacilli nalis: able bacilli riable bacilli Preview instant report a g slides, ensure Gram sta ad at a later time.	Points s None 4 0 0 nd save. ains remai	scored p 1+ 3 1 Refresh (ning on v	er morpl 2+ 2 2 1 GRM/MIS	3+ 1 3 2 3C1of2 wave been	4+ 0 4 2 orklist.
8	Qua bacteria Lactobacillus spp Med/large Gram Gardnerella vagin Small, Gram varia Mobiluncus spp.: Curved, Gram va Finalize STGM1 . If finished reading prepared to be re Gently blot excess	ntitation of al morphotype o.: positive bacilli nalis: able bacilli riable bacilli Preview instant report a g slides, ensure Gram sta ad at a later time. is oil from slide using pap	Points : None 4 0 0 nd save. ains remai	scored p 1+ 3 1 1 Refresh (ning on v or gauze a	er morpl 2+ 2 2 1 GRM/MIS vorklist ha	3+ 1 3 2 SC1of2 wave been slides in	4+ 0 4 2 orklist. slide

REPORTING OF RESULTS:

IF	REPORT				
	 In SCBV test, u 	sing the SCBV keypad, select Key 1 to state:			
	"Smear NEGATIVE for Bacterial Vaginosis >SCBV2".				
	9	CBV - 1 of 1			
		Key Text			
		Smear NEGATIVE for Bacterial Vaginosis ⇒SCBV2 2 36LTE (altered indeterminate) ⇒SCBV2			
		3 Smear consistent with Bacterial Vaginosis >SCBV2			
	S	4 HNSF (scant or no cells) MIC-STAIN GRM N SCORE			
	Comment will a	innear as:			
	"Smear NEGA	TIVE for Bacterial Vaginosis"			
	Add commonts	if appropriate from SCRV2 keypad:			
	 Add comments 				
		1 }PMEN (>55yrs old)			
		2)YEAS (yeast seen)			
		4 Trichomonas vaginalis seen in smear			
		SMIC-STAIN GRM N SCORE			
N-Score is 0 - 3					
N-Score is 0 - 3	IF	ADD COMMENT			
N-Score is 0 - 3	IF	ADD COMMENT "Results may not be reliable in post-			
N-Score is 0 - 3	IF Patient is > 55 years	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the			
N-Score is 0 - 3	IF Patient is > 55 years Key 1	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture".			
N-Score is 0 - 3	IF Patient is > 55 years Key 1	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women.			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture"			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture" "Purulence suggests the presence of another			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture" "Purulence suggests the presence of another infection and/or inflammatory condition.			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2 3+ or 4+ WBC seen	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture" "Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2 3+ or 4+ WBC seen Key 3	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture" "Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for <i>N. gonorrhoeae</i> and <i>C. trachomatis</i> may be			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2 3+ or 4+ WBC seen Key 3	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture" "Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for <i>N. gonorrhoeae</i> and <i>C. trachomatis</i> may be indicated".			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2 3+ or 4+ WBC seen Key 3 Trichomonas seen	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture" "Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for <i>N. gonorrhoeae</i> and <i>C. trachomatis</i> may be indicated".			
N-Score is 0 - 3	IF Patient is > 55 years Key 1 Yeast cells seen Key 2 3+ or 4+ WBC seen Key 3 Trichomonas seen Key 4	ADD COMMENT "Results may not be reliable in post- menopausal women. Correlate with the clinical picture". "Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture" "Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for <i>N. gonorrhoeae</i> and <i>C. trachomatis</i> may be indicated". "Trichomonas vaginalis seen in smear"			

IF	REPORT			
	 In SCBV test, u "}ALTF (altere 	sing the SCBV keypad, select Key 2 to state: d indeterminate) >SCBV2".		
	 Comment will a "Gram stain sl indeterminate Add comments 	SCBV - 1 of 1 Key Text 1 Smear NEGATIVE for Bacterial Vaginosis >SCBV2 2)ALTF (altered indeterminate) >SCBV2 3 Smear consistent with Bacterial Vaginosis >SCBV2 4)INSF (scant or no cells) SMIC-STAIN GRM N SCORE appear as: hows altered vaginal flora. Results are for Bacterial Vaginosis". if appropriate_from SCBV2 keypad:		
N-Score is 4 - 6		SCBV2 1 of 1 Key Text 1 }PMEN (>55yrs old) 2 }YEAS (yeast seen) 3 }PURU (3+ or more WBC) 4 Trichomonas vaginalis seen in smear SMIC-STAIN GRM N SCORE		
	IF	ADD COMMENT		
	Patient is > 55 years Key 1	"Results may not be reliable in post- menopausal women. Correlate with the clinical picture".		
	Yeast cells seen Key 2	"Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture"		
	3+ or 4+ WBC seen Key 3	"Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for <i>N. gonorrhoeae</i> and <i>C. trachomatis</i> may be indicated".		
	Trichomonas seen Key 4	"Trichomonas vaginalis seen in smear"		

IF	REPORT			
	 In SCBV test, u 	sing the SCBV keypad, select Key 3 to state:		
	"Smear consistent with Bacterial Vaginosis >SCBV2"			
	 Comment will a "Smear consist 	SCBV - 1 of 1 Image: Amage: Amage		
	 Add comments. 	, if appropriate, from SCBV2 keypad:		
N-Score is 7 - 10		Key Text 1 }PMEN (>55yrs old) 2 }YEAS (yeast seen) 3 }PURU (3+ or more WBC) 4 Trichomonas vaginalis seen in smear SMIC-STAIN GRM N SCORE		
	IF	ADD COMMENT		
	Patient is > 55 years Key 1	"Results may not be reliable in post- menopausal women. Correlate with the clinical picture".		
	Yeast cells seen Key 2	"Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with clinical picture"		
	3+ or 4+ WBC seen Key 3	"Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for <i>N. gonorrhoeae</i> and <i>C. trachomatis</i> may be indicated".		
	Trichomonas seen Key 4	"Trichomonas vaginalis seen in smear"		

IF	REPORT					
In SCBV test, using the SCBV keypad, select Key 4 to state: "}INSF (scant or no cells)"						
	SCBV - 1 of 1					
	Key Text					
	1 Smear NEGATIVE for Bacterial Vaginosis >SCBV2					
Scant or no cells	2 }ALTF (altered indeterminate) >SCBV2					
ocant of no cens	3 Smear consistent with Bacterial Vaginosis >SCBV2					
seen on smear	4)INSF (scant or no cells)					
	SMIC-STAIN GRM N SCORE					
	Comment will appear as:					
	"Insufficient sample to assess for vaginitis. Please recollect					
	if clinically indicated"					
	Finalize order.					

LIMITATIONS:

- 1. The presence or absence of clue cells is not part of the Nugent score and not required for diagnosis.
- 2. For post-menopausal patients, laboratory diagnosis of bacterial vaginosis has not been validated and interpretation of Gram stain results needs to be taken into account. Ensure comment is added.
- For pre-pubescent girls (< 13 years), Bacterial Vaginosis should not be reported. Genital culture should be performed and Gram stain should be reported as per routine specimens. Refer to MIC MIC20200 - Gram stain resulting in LIS – Routine specimens.

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