PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC20600 – Gram stain reporting in LIS-Bacterial Vaginosis Screens	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director, Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
Bacterial vaginosis (BV) is a clinical syndrome in women during their childbearing years characterized by an abnormal vaginal discharge. BV is a common condition, but it is particularly important in pregnant women. It is a risk factor for obstetric sequalae such as low birth weight and premature delivery and is associated with preterm birth, miscarriage, amniotic infections, and postpartum endometritis. In BV, the predominant microbiota of the vagina shifts from normally predominant lactobacilli to a mixture of *Gardnerella vaginalis*, *Prevotella* spp., *Mobiluncus* spp. and other anaerobes.

PURPOSE/RATIONALE:
This standard operating procedure describes how to report the gram stain results of bacterial vaginosis screens in the LIS in a consistent manner.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) reporting bacteria vaginosis screens in the LIS.

SAMPLE INFORMATION:

Type	• Vagina • Refer to MIC10100-Microbiology Specimen Processing

REAGENTS and/or MEDIA:
- Methanol
- Gram Crystal Violet
- Gram Iodine (Stabilized)
- Gram Decolorizer
- Gram Safranin

SUPPLIES:

- Glass microscope 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:

Step	Action
Reporting Bacterial Vaginosis Screens in the LIS	
1	• Pending BV gram stain orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRM/MISC_1of2**  • 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

3

- Check the age of the patient. BV Screen is not performed on patients ≤13 years of age
- Genital culture is performed on vaginal specimens if patient is ≤13 years of age
- If patient is ≤13 years of age, cancel the test SCBV and order CXGEN

4

- Add one drop of immersion oil to the slide. Under oil immersion (X100, OIF) result the media **GMBV** using the **GMBV 1 of 2** keypad
- Record the quantity of epithelial cells, white blood cells, clue cells, yeast cells, Trichomonas and bacteria significant to Bacterial Vaginosis: *Lactobacillus* spp., gram variable bacilli resembling *Gardnerella vaginalis* and gram negative bacilli resembling *Mobiluncus* spp.

None seen	**No cells seen**
1+	**< 1 cell seen**
2+	**1 - 9 cells seen**
3+	**10 - 25 cells seen**
4+	**> 25 cells seen**

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- Use the **GMBV 2 of 2** keypad to enter the N-score of the sample:

N-Score Table					
Quantitation of bacterial morphotype	**Points scored per morphotype**				
	None	**1+**	**2+**	**3+**	**4+**
Lactobacillus spp.: Med/large gram positive bacilli	4	3	2	1	0
Gardnerella vaginalis: Small, gram variable bacilli	0	1	2	3	4
Mobiluncus spp.: Curved, gram variable bacilli	0	1	1	2	2

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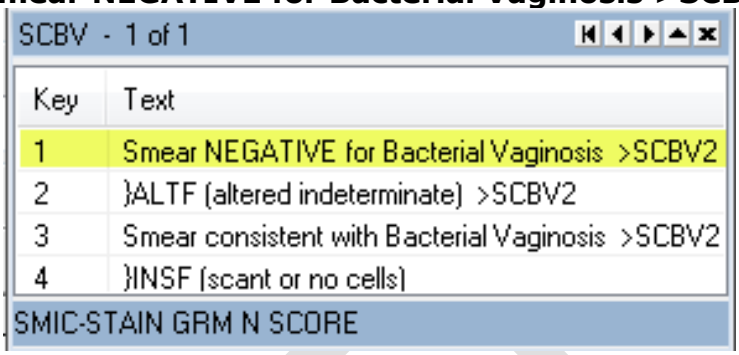
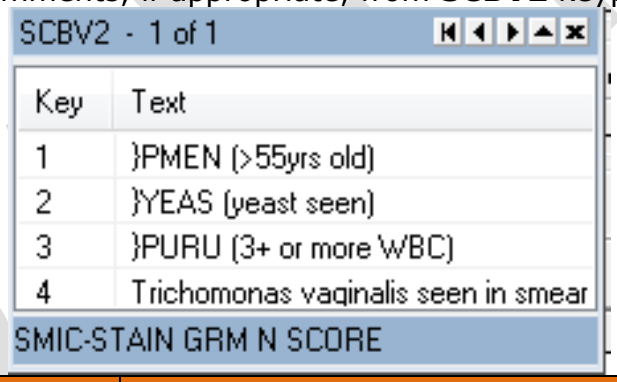
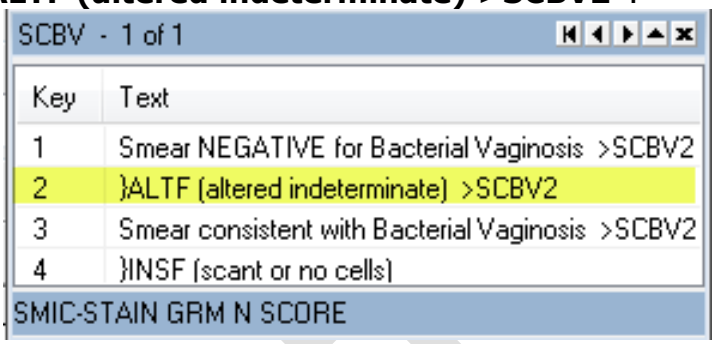
Under the test code: **SCBV,** use the **SCBV** keypad to report the BV result.

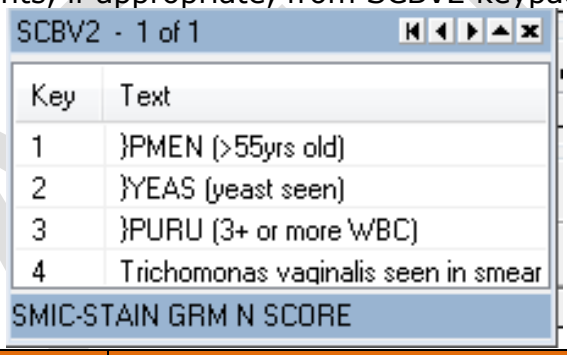
REPORTING INSTRUCTIONS:

<table>
<tr><th>IF</th><th>REPORT</th></tr>
<tr><td>N-Score is
0 - 3</td><td>
1. In SCBV test, using the SCBV keypad, select Key 1:
“Smear NEGATIVE for Bacterial Vaginosis >SCBV2”


2. Comment will appear as:
“Smear NEGATIVE for Bacterial Vaginosis”
3. Add comments, if appropriate, from SCBV2 keypad:


<table>
<tr><th>IF</th><th>ADD COMMENT</th></tr>
<tr><td>Patient is >55
Key 1</td><td>“Results may not be reliable in post-menopausal women. Correlate with the clinical picture”.</td></tr>
<tr><td>Yeast cells seen
Key 2</td><td>“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”</td></tr>
<tr><td>3+ or 4+ WBC
Key 3</td><td>“Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for N. gonorrhoeae and C. trachomatis may be indicated”.</td></tr>
<tr><td>Trichomonas
Key 4</td><td>“Trichomonas vaginalis seen in smear”</td></tr>
</table>
4. Finalize SCBV
5. Preview instant report and save
</td></tr>
</table>

<table>
<tr><th>IF</th><th>REPORT</th></tr>
<tr><td>N-Score is
4 - 6</td><td>
1. In SCBV test, using the SCBV keypad, select Key 2: "}ALTF (altered indeterminate) >SCBV2".


2. Comment will appear as: "Gram stain shows altered vaginal flora. Results are indeterminate for Bacterial Vaginosis"
3. Add comments, if appropriate, from SCBV2 keypad:


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<tr><th>IF</th><th>ADD COMMENT</th></tr>
<tr><td>Patient is > 55 years
Key 1</td><td>"Results may not be reliable in post-menopausal women. Correlate with the clinical picture".</td></tr>
<tr><td>Yeast cells seen
Key 2</td><td>"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"</td></tr>
<tr><td>3+ or 4+ WBC seen
Key 3</td><td>"Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for N. gonorrhoeae and C. trachomatis may be indicated".</td></tr>
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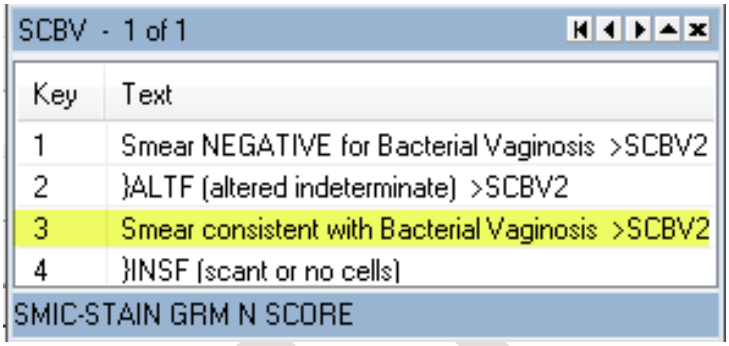
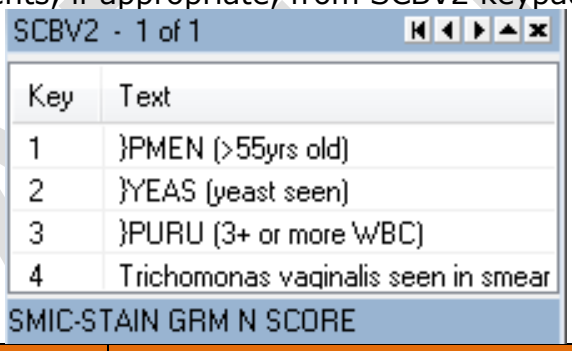
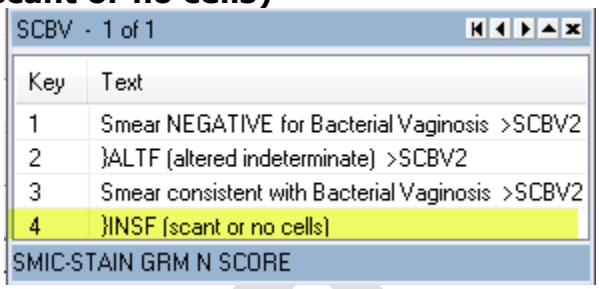
<table>
<tr><th>IF</th><th>REPORT</th></tr>
<tr><td>N-Score is
7 - 10</td><td>
1. In SCBV test, using the SCBV keypad, select Key 3: “Smear consistent with Bacterial Vaginosis >SCBV2”


2. Comment will appear as: “Smear consistent with Bacterial Vaginosis”
3. Add comments, if appropriate, from SCBV2 keypad:


<table>
<tr><th>IF</th><th>ADD COMMENT</th></tr>
<tr><td>Patient is > 55 years
Key 1</td><td>“Results may not be reliable in post-menopausal women. Correlate with the clinical picture”.</td></tr>
<tr><td>Yeast cells seen
Key 2</td><td>“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”</td></tr>
<tr><td>3+ or 4+ WBC seen
Key 3</td><td>“Purulence suggests the presence of another infection and/or inflammatory condition. Correlate with the clinical picture. Testing for N. gonorrhoeae and C. trachomatis may be indicated”.</td></tr>
<tr><td>Trichomonas seen
Key 4</td><td>“Trichomonas vaginalis seen in smear”</td></tr>
</table>
4. Finalize SCBV
5. Preview instant report and save
</td></tr>
</table>

IF	REPORT
Scant or no cells seen on smear	1. In SCBV test, using the SCBV keypad, select Key 4 to report: **"}INSF (scant or no cells)"**  2. Comment will appear as: **"Insufficient sample to assess for vaginitis. Please recollect if clinically indicated"** 3. Finalize **SCBV** 4. Preview instant report and save

Step	Action
Complete reading of BV slides	
1	• Refresh **GRM/MISC1of2** worklist • If finished reading BV slides, ensure BVs remaining on worklist have been prepared to be read at a later time
2	Slides can be discarded in the sharps container. BV slides are not saved.

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 considered. Ensure comment is added.
3. 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.

CROSS-REFERENCES:

- MIC10100-Microbiology Specimen Processing
- MIC20200-Gram stain resulting in LIS – Routine specimens.
- MIC60060-Microbiology Stain Quality Control

REFERENCES:

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

APPROVAL:

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Date

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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