

<b>PROGRAM Standard Operating Procedure – Laboratory Services</b>	
Title: MIC20600 – Gram stain reporting in LIS-Bacterial Vaginosis Screens	Policy Number: 15-162-V1
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 15/05/2024	Next Review Date: 15/05/2026
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 15/05/2024
Accreditation Canada Applicable Standard: NA	

**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 sequelae 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 standard operating procedure applies to Medical Laboratory Technologists (MLTs) reporting bacterial vaginosis screens in the LIS.

**SAMPLE INFORMATION:**

Type
<ul style="list-style-type: none"><li>• Vagina</li><li>• Refer to MIC10100-Microbiology Specimen Processing</li></ul>

**REAGENTS and/or MEDIA:**

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

**Disclaimer Message:** This is a **CONTROLLED** document for internal use only. Any documents appearing in paper form are not controlled and should be checked against the electronic file version prior to use.

**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 potentially infectious materials or cultures:

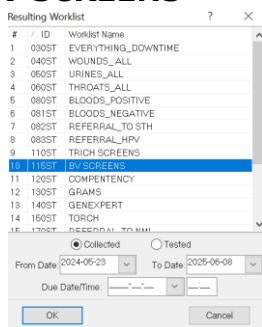
- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure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

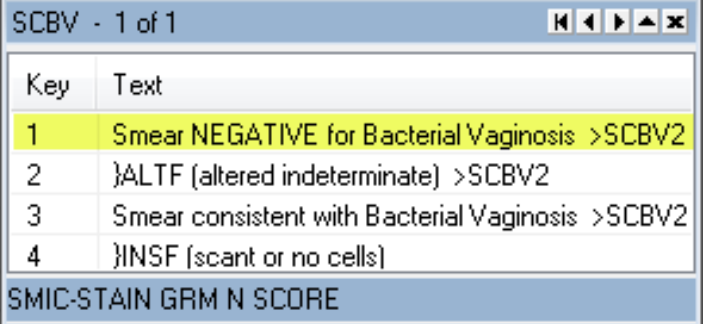
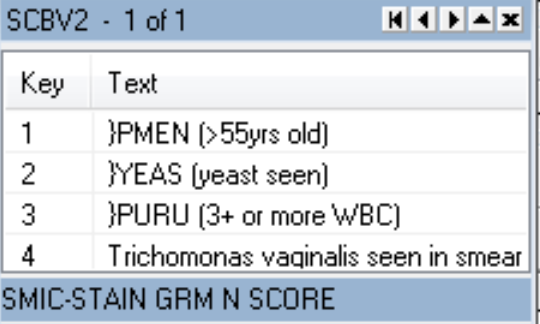
Step	Action
<b>Reporting Bacterial Vaginosis Screens in the LIS</b>	
<b>1</b>	<ul style="list-style-type: none"> <li>• Pending BV gram stain orders are found in the LIS Resulting Worklist: <b>Resulting Worklist → BV SCREENS</b></li> </ul>  <ul style="list-style-type: none"> <li>• Press enter or double click to open worklist</li> </ul>

**Disclaimer Message:** This is a **CONTROLLED** document for internal use only. Any documents appearing in paper form are not controlled and should be checked against the electronic file version prior to use.

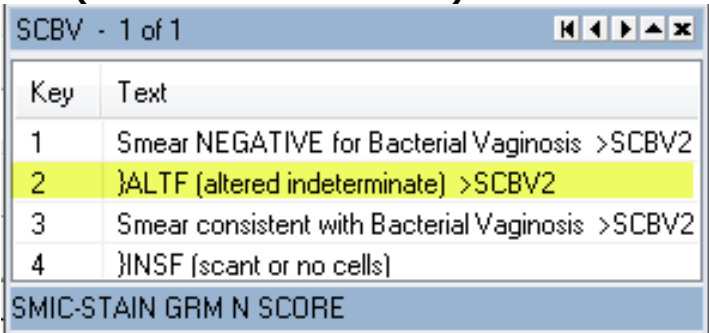
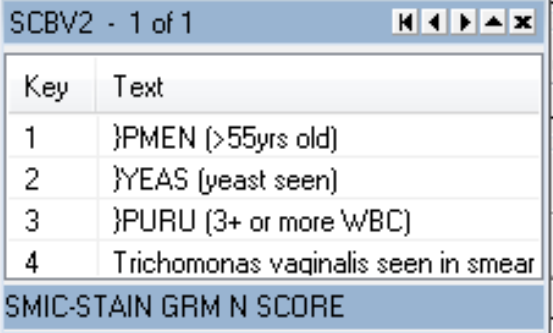
<b>2</b>	<ul style="list-style-type: none"> <li>Enter the accession number on the slide and select enter to mark the order</li> <li>Select enter again to open Result Entry or double click on accession number to open</li> </ul>																																			
<b>3</b>	<ul style="list-style-type: none"> <li>Check the age of the patient. BV Screen is not performed on patients <math>\leq 13</math> years of age</li> <li>Refer to MIC10110-Vaginal Swab Processing Job Aid</li> </ul>																																			
<b>4</b>	Add one drop of immersion oil to the slide. In a representative area with predominance of inflammation or purulence using the oil immersion lens (100X), examine 20 to 40 fields to observe cell morphology and gram reaction.																																			
<b>5</b>	<p><u>Under oil immersion (X100, OIF)</u>: quantitate epithelial cells, white blood cells, red blood cells and bacteria as follows:</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th style="text-align: center;">None seen</th> <th style="text-align: center;">No cells seen</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;"><b>1+</b></td> <td style="text-align: center;"><b>&lt; 1 cell seen</b></td> </tr> <tr> <td style="text-align: center;"><b>2+</b></td> <td style="text-align: center;"><b>1 - 9 cells seen</b></td> </tr> <tr> <td style="text-align: center;"><b>3+</b></td> <td style="text-align: center;"><b>10 - 25 cells seen</b></td> </tr> <tr> <td style="text-align: center;"><b>4+</b></td> <td style="text-align: center;"><b>&gt; 25 cells seen</b></td> </tr> </tbody> </table>	None seen	No cells seen	<b>1+</b>	<b>&lt; 1 cell seen</b>	<b>2+</b>	<b>1 - 9 cells seen</b>	<b>3+</b>	<b>10 - 25 cells seen</b>	<b>4+</b>	<b>&gt; 25 cells seen</b>																									
None seen	No cells seen																																			
<b>1+</b>	<b>&lt; 1 cell seen</b>																																			
<b>2+</b>	<b>1 - 9 cells seen</b>																																			
<b>3+</b>	<b>10 - 25 cells seen</b>																																			
<b>4+</b>	<b>&gt; 25 cells seen</b>																																			
<b>6</b>	Under the media code: <b>GMBV</b> , use the <b>GMBV 1 of 2</b> keypad to report the quantity of epithelial cells, white blood cells, clue cells, yeast cells, <i>Trichomonas</i> and bacteria significant to Bacterial Vaginosis: <i>Lactobacillus</i> spp., gram variable bacilli resembling <i>Gardnerella vaginalis</i> and gram negative bacilli resembling <i>Mobiluncus</i> spp.																																			
<b>7</b>	<ul style="list-style-type: none"> <li>Use the <b>GMBV 2 of 2</b> keypad to enter the N-score of the sample:</li> </ul> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th colspan="6" style="text-align: center;"><b>N-Score Table</b></th> </tr> <tr> <th rowspan="2" style="text-align: center;">Quantitation of bacterial morphotype</th> <th colspan="5" style="text-align: center;">Points scored per morphotype</th> </tr> <tr> <th style="text-align: center;">None</th> <th style="text-align: center;">1+</th> <th style="text-align: center;">2+</th> <th style="text-align: center;">3+</th> <th style="text-align: center;">4+</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;"><i>Lactobacillus</i> spp.: Med/large gram positive bacilli</td> <td style="text-align: center;">4</td> <td style="text-align: center;">3</td> <td style="text-align: center;">2</td> <td style="text-align: center;">1</td> <td style="text-align: center;">0</td> </tr> <tr> <td style="text-align: center;"><i>Gardnerella vaginalis</i>: Small, gram variable bacilli</td> <td style="text-align: center;">0</td> <td style="text-align: center;">1</td> <td style="text-align: center;">2</td> <td style="text-align: center;">3</td> <td style="text-align: center;">4</td> </tr> <tr> <td style="text-align: center;"><i>Mobiluncus</i> spp.: Curved, gram variable bacilli</td> <td style="text-align: center;">0</td> <td style="text-align: center;">1</td> <td style="text-align: center;">1</td> <td style="text-align: center;">2</td> <td style="text-align: center;">2</td> </tr> </tbody> </table>	<b>N-Score Table</b>						Quantitation of bacterial morphotype	Points scored per morphotype					None	1+	2+	3+	4+	<i>Lactobacillus</i> spp.: Med/large gram positive bacilli	4	3	2	1	0	<i>Gardnerella vaginalis</i> : Small, gram variable bacilli	0	1	2	3	4	<i>Mobiluncus</i> spp.: Curved, gram variable bacilli	0	1	1	2	2
<b>N-Score Table</b>																																				
Quantitation of bacterial morphotype	Points scored per morphotype																																			
	None	1+	2+	3+	4+																															
<i>Lactobacillus</i> spp.: Med/large gram positive bacilli	4	3	2	1	0																															
<i>Gardnerella vaginalis</i> : Small, gram variable bacilli	0	1	2	3	4																															
<i>Mobiluncus</i> spp.: Curved, gram variable bacilli	0	1	1	2	2																															
<b>8</b>	Under the test code: <b>SCBV</b> , use the <b>SCBV</b> keypad to report the BV result.																																			

**Disclaimer Message:** This is a **CONTROLLED** document for internal use only. Any documents appearing in paper form are not controlled and should be checked against the electronic file version prior to use.

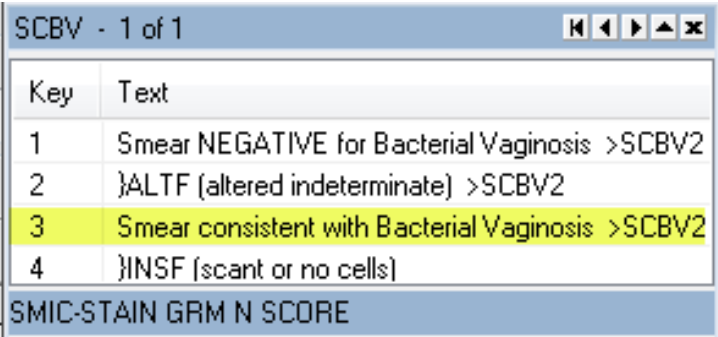
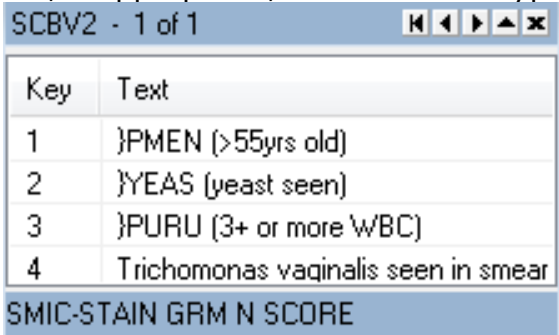
**REPORTING INSTRUCTIONS:**

IF	REPORT	
<p><b>N-Score is 0 - 3</b></p>	<p>1. In SCBV test, using the SCBV keypad, select Key 1:  <b>"Smear NEGATIVE for Bacterial Vaginosis &gt;SCBV2"</b></p>  <p>2. Comment will appear as:  <b>"Smear NEGATIVE for Bacterial Vaginosis"</b></p> <p>3. Add comments, if appropriate, from <b>SCBV2</b> keypad:</p> 	
	IF	ADD COMMENT
	Patient is >55 Key 1 }PMEN	<b>"Results may not be reliable in post-menopausal women. Correlate with the clinical picture."</b>
	Yeast cells seen Key 2 }YEAS	<b>"Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. Correlate with the clinical picture."</b>
	3+ or 4+ WBC Key 3 }PURU	<b>"Purulence suggests the presence of another infection and/or inflammatory condition. Testing for N. gonorrhoeae and C. trachomatis may be indicated. Correlate with the clinical picture."</b>
	Trichomonas Key 4 <b>"Trichomonas vaginalis seen in smear"</b>	
	<p>4. Finalize <b>SCBV</b>                      5. Preview instant report and save</p>	

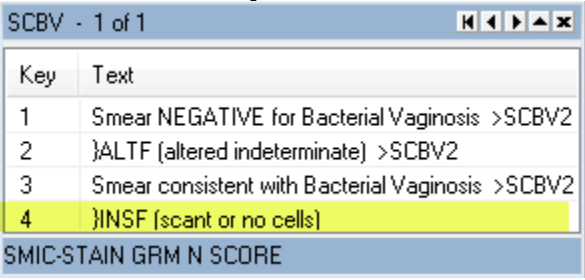
**Disclaimer Message:** This is a **CONTROLLED** document for internal use only. Any documents appearing in paper form are not controlled and should be checked against the electronic file version prior to use.

IF	REPORT		
<b>N-Score is 4 - 6</b>	1. In SCBV test, using the SCBV keypad, select Key 2: <b>"}ALTF (altered indeterminate) &gt;SCBV2"</b> . 		
	2. Comment will appear as: <b>"Gram stain shows altered vaginal flora. Results are indeterminate for Bacterial Vaginosis"</b>		
	3. Add comments, if appropriate, from SCBV2 keypad: 		
	IF	ADD COMMENT	
	Patient is >55 Key 1 }PMEN	<b>"Results may not be reliable in post-menopausal women. Correlate with the clinical picture."</b>	
Yeast cells seen Key 2 }YEAS	<b>"Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. Correlate with the clinical picture."</b>		
3+ or 4+ WBC Key 3 }PURU	<b>"Purulence suggests the presence of another infection and/or inflammatory condition. Testing for N. gonorrhoeae and C. trachomatis may be indicated. Correlate with the clinical picture."</b>		
Trichomonas seen Key 4	<b>"Trichomonas vaginalis seen in smear"</b>		
4. Finalize <b>SCBV</b> 5. Preview instant report and save			

**Disclaimer Message:** This is a **CONTROLLED** document for internal use only. Any documents appearing in paper form are not controlled and should be checked against the electronic file version prior to use.

IF	REPORT	
<b>N-Score is 7 - 10</b>	<p>1. In SCBV test, using the SCBV keypad, select Key 3:  <b>"Smear consistent with Bacterial Vaginosis &gt;SCBV2"</b></p>  <p>2. Comment will appear as:  <b>"Smear consistent with Bacterial Vaginosis"</b></p> <p>3. Add comments, if appropriate, from SCBV2 keypad:</p> 	
	IF	ADD COMMENT
	Patient is >55 Key 1 }PMEN	<b>"Results may not be reliable in post-menopausal women. Correlate with the clinical picture."</b>
	Yeast cells seen Key 2 }YEAS	<b>"Yeast Seen. Candida species are normal flora in the genital area of 30-40% of women. Correlate with the clinical picture."</b>
	3+ or 4+ WBC Key 3 }PURU	<b>"Purulence suggests the presence of another infection and/or inflammatory condition. Testing for N. gonorrhoeae and C. trachomatis may be indicated. Correlate with the clinical picture."</b>
Trichomonas seen Key 4	<b>"Trichomonas vaginalis seen in smear"</b>	
	<p>4. Finalize <b>SCBV</b></p> <p>5. Preview instant report and save</p>	

**Disclaimer Message:** This is a **CONTROLLED** document for internal use only. Any documents appearing in paper form are not controlled and should be checked against the electronic file version prior to use.

IF	REPORT
<b>Scant or no cells seen on smear</b>	<ol style="list-style-type: none"> <li>In SCBV test, using the SCBV keypad, select Key 4 to report:  <b>"}INSF (scant or no cells)"</b>  </li> <li>Comment will appear as:  <b>"Insufficient sample to assess for vaginitis. Please recollect if clinically indicated"</b></li> <li>Finalize <b>SCBV</b></li> <li>Preview instant report and save</li> </ol>

Step	Action
<b>Complete reading of BV slides</b>	
<b>1</b>	<ul style="list-style-type: none"> <li>Refresh <b>BV SCREENS</b> worklist</li> <li>If finished reading BV slides, ensure BVs remaining on worklist have been prepared to be read at a later time</li> </ul>
<b>2</b>	Slides can be discarded in the sharps container. BV slides are not saved.

**LIMITATIONS:**

- The presence or absence of clue cells is not part of the Nugent score and not required for diagnosis.
- 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.
- For pre-pubescent girls ( $\leq 13$  years), Bacterial Vaginosis should not be reported. Genital culture should be performed.  
 Refer to MIC10110-Vaginal Swab Processing Job Aid

**CROSS-REFERENCES:**

- MIC10100-Microbiology Specimen Processing
- MIC10110-Vaginal Swab Processing Job Aid
- MIC60060-Microbiology Stain Quality Control

**REFERENCES:**

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

**Disclaimer Message:** This is a **CONTROLLED** document for internal use only. Any documents appearing in paper form are not controlled and should be checked against the electronic file version prior to use.

**APPROVAL:**

May 15, 2024

Date



Director, Laboratory and Diagnostic Imaging Services

**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
3.0	20 Feb 24	Procedure reviewed	L. Steven
4.0	02 Mar 26	Procedure reviewed	L. Steven

**Disclaimer Message:** This is a **CONTROLLED** document for internal use only. Any documents appearing in paper form are not controlled and should be checked against the electronic file version prior to use.