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

SAMPLE INFORMATION:

Type	• Vagina. • Refer to MIC10230 – Microbiology Specimen Processing.

REAGENTS INFORMATION:

Type	BD™ Gram Crystal Violet, 3.8 L, B4312526 BD™ Gram Iodine (Stabilized), 3.8 L, B4312543 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.

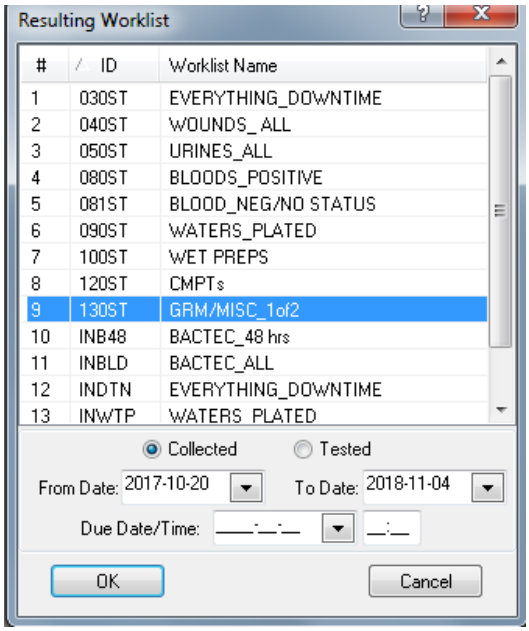
- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, 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:

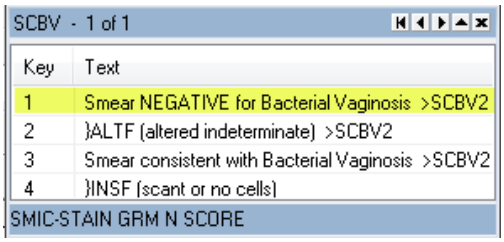
Step	Action
1	Pending BV Gram stain orders are found in the LIS Resulting Worklist: **Resulting Worklist → GRM/MISC_1of2** 
2	Enter the accession number on the slide and select enter to mark the order. Select enter again to open Result Entry or double click on accession number to open.
3	Under the media resulting log: **GMBV**, use the **GMBV** keypad to 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.
4	Epithelial cells, white blood cells and red blood cells are quantified as follows under LPF (10X): None seen — No cells seen 1+ — < 1 cells seen 2+ — 1 – 9 cells seen 3+ — 10 – 25 cells seen 4+ — > 25 cells seen **NOTE:** Only record “None seen” for WBC.

<table>
<tr><td>5</td><td>Add one drop of immersion oil to the slide. Using the oil immersion lens (100X); examine 20 to 40 fields to observe cell morphology and Gram reaction.</td></tr>
<tr><td>6</td><td>Bacterial and yeast cells are quantified as follows under OIF (100x):
<table>
<tr><td>None seen</td><td>No cells seen</td></tr>
<tr><td>1+</td><td>< 1 cells seen</td></tr>
<tr><td>2+</td><td>1 – 9 cells seen</td></tr>
<tr><td>3+</td><td>10 – 25 cells seen</td></tr>
<tr><td>4+</td><td>> 25 cells seen</td></tr>
</table>
NOTE: If no bacteria are seen, record this result.</td></tr>
<tr><td>7</td><td>Calculate the Nugent score of the specimen. The N-score is calculated by assessing the quantity of Lactobacillus spp., Gram-variable bacilli and Mobiluncus spp. Examine 20 to 40 fields and interpret as follows:
N-Score Table
<table>
<tr><th rowspan="2">Quantitation of bacterial morphotype</th><th colspan="5">Points scored per morphotype</th></tr>
<tr><th>None</th><th>1+</th><th>2+</th><th>3+</th><th>4+</th></tr>
<tr><td>Lactobacillus spp.:
Med/large Gram positive bacilli</td><td>4</td><td>3</td><td>2</td><td>1</td><td>0</td></tr>
<tr><td>Gardnerella vaginalis:
Small, Gram variable bacilli</td><td>0</td><td>1</td><td>2</td><td>3</td><td>4</td></tr>
<tr><td>Mobiluncus spp.:
Curved, Gram variable bacilli</td><td>0</td><td>1</td><td>1</td><td>2</td><td>2</td></tr>
</table></td></tr>
<tr><td>8</td><td>Finalize STGM1. Preview instant report and save. Refresh GRM/MISC1of2 worklist. If finished reading slides, ensure Gram stains remaining on worklist have been prepared to be read at a later time.</td></tr>
<tr><td>9</td><td>Gently blot excess oil from slide using paper towel or gauze and save slides in slide box marked BV.</td></tr>
</table>

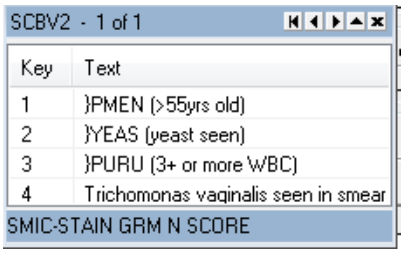
REPORTING OF RESULTS:

<table>
<tr><th>IF</th><th colspan="2">REPORT</th></tr>
<tr><td rowspan="5">N-Score is 0 - 3</td><td colspan="2">

In SCBV test, using the SCBV keypad, select Key 1 to state: “Smear NEGATIVE for Bacterial Vaginosis >SCBV2”.



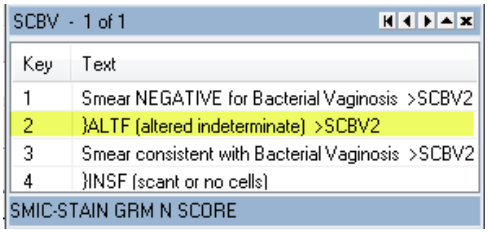
Comment will appear as: “Smear NEGATIVE for Bacterial Vaginosis”
Add comments, if appropriate, from SCBV2 keypad:



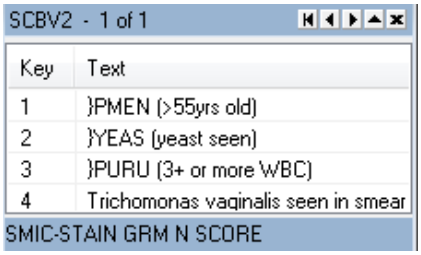
</td></tr>
<tr><td>IF</td><td>ADD COMMENT</td></tr>
<tr><td>Patient is > 55 years
Key 1</td><td>“Results may not be reliable in post-menopausal women. Correlate with the clinical picture”.</td></tr>
<tr><td>Yeast cells seen
Key 2</td><td>“Yeast Seen. Candida species are normal flora in the genital area 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>

<table>
<tr><th>IF</th><th>REPORT</th></tr>
<tr><td>N-Score is 4 - 6</td><td>

In SCBV test, using the SCBV keypad, select Key 2 to state: “}ALTF (altered indeterminate) >SCBV2”.


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

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


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
<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>
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Key 4</td><td>“Trichomonas vaginalis seen in smear”</td></tr>
</table>
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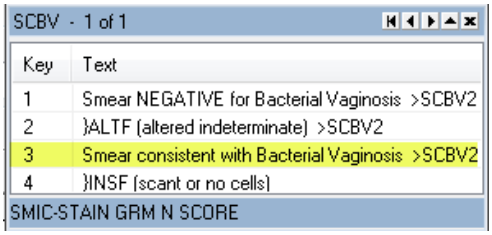
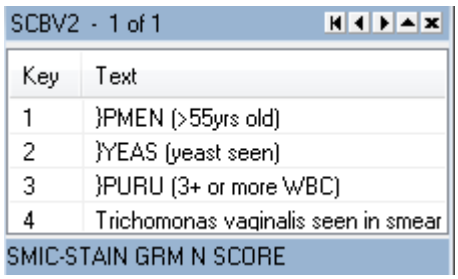
</table>

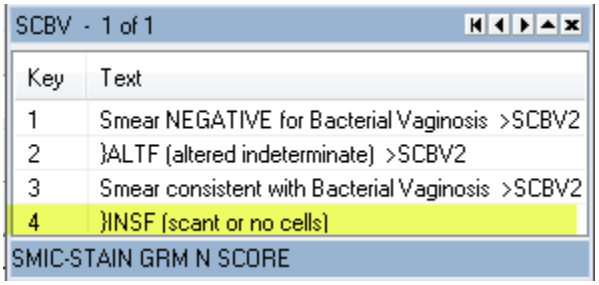
<table>
<tr><th>IF</th><th>REPORT</th></tr>
<tr><td rowspan="4">N-Score is 7 - 10</td><td>

In SCBV test, using the SCBV keypad, select Key 3 to state:
“Smear consistent with Bacterial Vaginosis >SCBV2”


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

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


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
</td></tr>
<tr><td>
<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>
</td></tr>
</table>

IF	REPORT
Scant or no cells seen on smear	• In SCBV test, using the SCBV keypad, select Key 4 to state: **"}INSF (scant or no cells)"**  • 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.
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.

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