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| Gram Stain for Bacterial Vaginosis |
| **Purpose** | This procedure provides instruction for reading Gram stains for Bacterial Vaginosis. |
| **Principal and Clinical Significance** | Bacterial Vaginosis is a clinical syndrome in women during childbearing years characterized by an abnormal vaginal discharge. In bacterial vaginosis, the predominant microbiota of the vagina shifts from normally predominant lactobacilli to a mixture of *Gardnerella vaginalis, Prevotella spp, Mobiluncus spp* and other anaerobes. G. vaginalis organism appear as small Gram-variable pleomorphic bacilli, varying from coccobacilli to longer forms up to 2 to 3 µm that palisade or appear coryneform. Mobiluncus spp. are curved Gram negative rods. The comparison of relative numbers of lactobacilli to Gram negative curved rods and Gram variable coccobacilli allows for the diagnosis of BV to be made. Interpretation of these Gram stains with Nugent’s criteria remains the definitive laboratory method for diagnosis. (1) |
| **Policy Statements** | This procedure applies to day shift Microbiologists who read Gram stains in Minneapolis. |
| **Test Code****Materials** | GSBV

|  |  |  |
| --- | --- | --- |
| **Reagents** | **Supplies** | **Equipment** |
| BBL™* Gram Crystal Violet (primary stain) Cat. No. 212525
* Gram Iodine (mordant) Cat. No. 212542
* Gram decolorizer (decolorizer)

Cat. No. 212527* Gram Safranin (counterstain) Cat. No. 212531

Store at 15-30ºC | * Glass slide
* Frosted glass slide
* 0.85% sterile saline
* Sterile disposable pipette
 | * Incinerator
* Inoculating loop
* Microscope
* Slide warmer
* Vortex mixer
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| **Specimen** | * Acceptable specimen: vaginal secretions on a sterile cotton or Dacron swab.
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| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling.1. [*Biohazard Containment*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20201%20%20%20Biohazard%20Containment.doc)
2. [*Biohazardous Spills*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20204%20%20%20Biohazardous%20spills.doc)
3. [*Safety in the Microbiology Laboratory*](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20202%20%20%20Safety%20in%20the%20Microbiology%20Lab%20Policy.doc)
 |
| **Procedure** | 1. Roll the swab gently across the slide or place swab in saline, vortex, place 1-2 drops of saline on slide.
2. Perform Gram stain following instruction from [MC 2.0 Gram Stain](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMC%202%20Staining%5CMC%202.0%20Gram%20stain.docx) Procedure.
3. Quantitate and report WBCs and Clue cells under low power averaging 10-20 fields using the following table:

Table 1 WBCs

|  |  |  |  |
| --- | --- | --- | --- |
| Grade | WBCs observed under low power | Result | Key |
| 4+ | >25 WBCs  | 4+ WBCS | 4 tab W |
| 3+ | 10-25 WBCs | 3+ WBCS | 3 tab W |
| 2+ | 1-9 WBCs | 2+ WBCS | 2 tab W |
| 1+ | <1 WBCs | 1+ WBC | 1 tab W |
|  | 0 WBCs | NWBC | ‘ |

Table 2 Clue Cells

|  |  |  |  |
| --- | --- | --- | --- |
| Grade | Clue cells observed under low power | Result | Key |
| 4+ | >25 Clue cells | 4+ CLUE | 4 tab 8 |
| 3+ | 10-25 Clue cells | 3+ CLUE | 3 tab 8 |
| 2+ | 1-9 Clue cells | 2+ CLUE | 2 tab 8 |
| 1+ | <1 Clue cells | 1+ CLUE | 1 tab 8 |
|  | 0 Clue cells | NCLU | 7 |

1. Quantitate and report Yeast under high power averaging 20-40 fields using the following table:

Table 3 Yeast

|  |  |  |  |
| --- | --- | --- | --- |
| Grade | Yeast observed under high power | Result | Key |
| 4+ | >30  | 4+ Yeast | 4 tab U |
| 3+ | 6-30 | 3+ Yeast | 3 tab U |
| 2+ | 1-5 | 2+ Yeast | 2 tab U |
| 1+ | <1 | 1+ Yeast | 1 tab U |
|  | 0 | No yeast present | Y |

1. Evaluate the Gram stain to determine score for bacterial vaginosis. Determine the number of bacteria in 20-40 field using same criteria in table in section D. Circle in each row the number that corresponds to the quantitation visualized in the smear. Add circled numbers to get the total score.

Table 4 Standardized scoring method for evaluation of Gram stain for BV

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Quantitation of bacterial morphology | None | 1+ | 2+ | 3+ | 4+ |
| Medium to large Gram positive rods (*Lactobacilli*) | 4 | 3 | 2 | 1 | 0 |
| Small Gram negative or variable rods (*Gardnerella vaginalis)* | 0 | 1 | 2 | 3 | 4 |
| Curved Gram negative rods (*Mobiluncus spp)* | 0 | 1 | 1 | 2 | 2 |

Interpret score as follows using the following table:Table 5 score codes, key and interpretation

|  |  |  |  |
| --- | --- | --- | --- |
| Code | Score | Key | Interpretation |
| NVM | 0-3 | [ | morphology consistent with normal vaginal microbiota  |
| TVMM | 4-6 | ] | mixed morphology consistent with transition from normal vaginal microbiota |
| BVM | 7-10 | , | Mixed morphology consistent with bacterial vaginosis  |

**Example 1:** 4+ medium to large Gram positive rods seen = 0  no small Gram negative rods seen = 0 no curved Gram negative rods seen = 0  Total score = 0 =NVM**Example 2:** 2+ medium to large Gram positive rods seen = 2  3+ small Gram negative rods seen = 3  2+ curved Gram negative rods seen = 1 Total score = 6 =TVMM**Example 3:** no medium to large Gram positive rods = 4  4+ small Gram negative rods seen = 4 4+ curved Gram negative rods seen = 2 Total score = 10 =BVM  |
| **Method Performance Specifications** | * Reagent Preparation
1. Decolorizer
2. Add 500 ml 95% alcohol to 500 ml of acetone.
3. Mix thoroughly.
* Reagent Storage
1. Gram Crystal Violet / Gram Safranin / Gram Iodine
2. Store reagents at room temperature until expiration date.
3. The expiration date is for unopened bottles.
4. Do not open until ready to use.
5. Decolorizer
6. Store at room temperature.
7. 1 year expiration
 |
| **Result Reporting** | 1. Report in Sunquest Microbiology Result Entry, Direct Exam tab.
2. Report WBCs, Clue cells and yeast
3. Report result determined by score:

NVM 0-3: morphology consistent with normal vaginal microbiotaTVMM 4-6: mixed morphology consistent with transition from normal vaginal microbiotaBVM 7-10: Mixed morphology consistent with bacterial vaginosis Example: 3+ Clue Cells Present 3+ CLUE 1+ WBCs 1+ WBCS 1+ Yeast 1+ Yeast  Mixed morphology consistent with bacterial vaginosis BVM 1. Final Result. Key / on keyboard.
 |
| **References** | 1. Leber, A. L. Clinical Microbiology Procedures Handbook. (3.2.1) 4th Edition, 2016 ASM Press Washington, DC
 |
| **Appendices** |  |
| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Susan DeMeyere | 9/15/2020 | Initial Version |
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