

## Challenge G103

November 2010

Gram: Vaginal swab – Positive Nugent score consistent with Bacterial vaginosis (BV)

### HISTORY

The sample simulated a vaginal smear from a 19 year old woman with discharge.

### CMPT QA

The smear, reviewed at CMPT, presented 2+ (1-5/oif) neutrophils, 2+ (1-5/oif) epithelial cells 4+ (>50/oif) gram variable bacilli (see Figure 1).

### SURVEY RESULTS

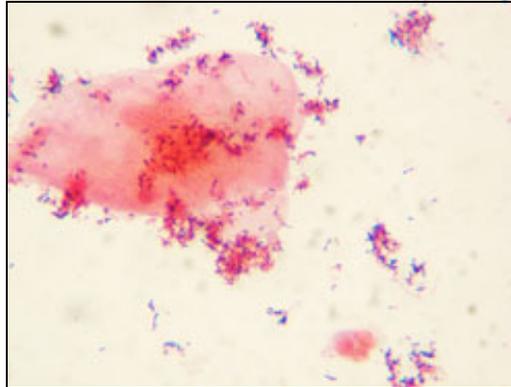
**Reference Labs:** 11/15 (73%) laboratories reported cell and bacterial component; 15/15 (100%) laboratories reported BV comment.

**Cell component:** 11/15 (73%) labs reported 1+/2+ neutrophils and 1+ to 4+ epithelial cells (2 labs reported Clue cells).

**Bacteria component:** 9 (60%) labs reported BV positive and 4+ gram variable bacilli/suggestive of *G. vaginalis*/*Gardnerella*-like organisms/*Gardnerella* or *Bacteroides* sp. +/- 1+ gram positive bacilli; 1 lab reported 2+ gram variable bacilli, 4+ gram positive bacilli (pleomorphic), 1+ gram positive bacilli (*Lactobacillus*) and reported BV inconclusive; 1 lab reported 4+ gram positive bacilli and reported BV equivocal.

**BV comment:** 11/15 labs reported the smear as BV positive, 2 reported BV inconclusive/equivocal, and 2 reported BV negative.

**Participants:** Although most participants reported details of the cellular and bacterial com-



**Figure1:** epithelial cell, and neutrophils; numerous gram variable bacilli (no lactobacilli).

ponent of this smear, these results were not individually graded. Only the BV comments that were reported by the laboratories were graded as the clinical interpretation of what was seen on microscopic examination of the vaginal smear.

Table 1 provides a summary of the reporting comments. Only 57% of the laboratories processing the sample reported the sample as positive for bacterial vaginosis (BV). Of those labs, 11% reported “altered vaginal flora” but did not state if it was BV positive or not; 9% of the laboratories reported indeterminate or inconclusive results. Twenty-one (18%) laboratories reported negative for BV, AVF, not consistent with BV or normal bacterial vaginal flora even though this slide only contained *Gardnerella*

### Grading

#### Maximum grade: 4

Although consensus was not reached amongst the reference laboratories on the reporting of the cellular and bacterial component of this specimen, the absence of lactobacilli and a predominance of Gram-variable bacilli suggestive of *Gardnerella* indicate gross alteration in the normal vaginal flora.

Laboratories were given a grade of 4 for reporting the presence of BV, while those that reported either ‘altered vaginal flora’ or an equivocal/inconclusive, indeterminate result were given a grade of 3. One laboratory reported a Nugent score of 5 and was given an unacceptable grade because of the absence of a clinically relevant interpretive comment.

All laboratories that provided ‘no report’, negative for BV, of the presence of normal vaginal flora were given a grade of 0.

**Table –1:** Reported results for G103 smear – Bacterial vaginosis comment -

BV comment	A	B	C	C1	Total	Grade
positive	44	13	2	7	66	4
AVF +/- snnp	10	1	1	1	13	3
equivocal/inconclusive, indeterminate	5	4		1	10	3
AVF, not consistent with bacterial vaginosis	3	1			4	0
negative	9	4	2	1	16	0
no report	2		2	2	5	0
normal bacterial vaginal flora	1				1	0
Nugent score = 5	1				1	0
snnp, refer		2	6	9	17	ungraded
<b>Total</b>	<b>74</b>	<b>25</b>	<b>13</b>	<b>21</b>	<b>133</b>	

AVF: altered vaginal flora; snnp: sample not normally processed.

(Gram-variable bacilli) and no lactobacilli. Five participants did not report any comments.

## LABORATORY DIAGNOSIS

**Bacterial Vaginosis:** Gram stain of vaginal fluid is an objective method of diagnosis of bacterial vaginosis that correlates well with the clinical criteria described by Amsel and colleagues.<sup>1</sup>

Nugent then established specific microscopic grading system 20-years ago for the diagnosis of BV and this procedure is still used by most clinical laboratories for the microscopic evaluation of vaginal smears.<sup>2</sup> The Nugent score (N-Score) is determined by evaluating the presence of gram variable coccobacilli with vacuoles suggestive of *Gardnerella/Bacteroides*, curved anaerobic rods suggestive of *Mobiluncus* species, and a corresponding decrease in the normal amounts of lactobacilli present (see Table 2).<sup>2,3</sup>

### Reporting of Vaginal Smears for BV

N-Score ≤3 = Report “Smear negative for Bacterial Vaginosis”.

N-Score 4-6 = Report “Gram stain shows altered vaginal flora. Results are indeterminant for Bacterial Vaginosis”.

N-Score ≥7 = Report “Smear consistent with Bacterial Vaginosis”.

Post-Menopausal Women (i.e., ≥ 45 years)<sup>4</sup> should have an additional comment added to all vaginal smear reports: “Results may not be reliable in post-menopausal women. Correlate with the clinical picture.”

### Reporting of Other Cellular Components

Typically, in uncomplicated BV, the immunological response is very scarce or absent.<sup>5</sup>

Finding increased number of PMNs (3+ to 4+) in a patient with BV suggests the presence of coexisting sexually transmitted infection, notably cervicitis due to *Neisseria gonorrhoeae* or

*Chlamydia trachomatis* or vaginitis due to *Trichomonas vaginalis* or yeast.<sup>5,6</sup> Women with desquamative inflammatory vaginitis (DSIV) may also present with symptoms similar to those of BV (increased number of PMNs) but with little alteration in their vaginal flora.<sup>7</sup> It is important to differentiate women with DSIV from BV because the treatment is different.

The presence of 3+ to 4+ PMNs should be reported. An additional comment, “Presence of purulence suggests the presence of another infection and/or inflammatory condition. Correlate with clinical picture. Testing for *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis* may be indicated” should be included with the report.

Clue cells, which are vaginal epithelial cells studded with tiny coccobacilli, best appreciated at the edges of the cell and may be dense enough to partially obscure the nucleus, are seen in more than 90% of patients with BV.<sup>8</sup> Although the presence of clue cells are not included in the Nugent scoring system, their presence are synonymous with the presence of BV when altered vaginal flora is present and should be reported when found.

The presence of Clue cells is reported if the N-Score ≥4. If the N-Score is indeterminant (i.e., 4-6) then additional fields should be examined for Clue cells before reporting. If the N-Score is indicative of BV (i.e., 7-10) then only Clue cells should be reported, if found as part of the routine microscopic exam. An additional comment —“Presence of Clue cells suggest transition of vaginal flora towards Bacterial vaginosis, repeat testing of another vaginal smear is recommended.” — should be added where the N-Score is indeterminant.

## Reporting of Other Agents

The presence of yeast, particularly when present in moderate to heavy amounts (3+ to 4+) should also be routinely assessed and reported. Also vaginal smears are an insensitive method for diagnosing the presence of *Trichomonas vaginalis*. If *T.vaginalis* is found, they should always be reported.

## CLINICAL SIGNIFICANCE

Vaginitis is one of the most common infections seen in primary care. Effective treatment of vulvovaginal infections requires accurate diag-

**Table-2:** Scoring Criteria for the Microscopic Diagnosis of Bacterial Vaginosis

Number of <i>Lactobacillus</i>	Score	Number of <i>Gardnerella/Bacteroides</i>	Score	Number of CGNB	Score	N-Score
≥30	0	0	0	0	0	0
5-20	1	<1	1	<1	1	3
1-4	2	1-4	2	1-4	1	5
<1	3	5-20	3	5-20	2	8
0	4	≥30	4	≥30	2	10

**N-Score** = The sum of the scores for the presence/absence of *Lactobacillus*, *Gardnerella/Bacteroides* and curved Gram-negative bacilli (CGNB).

nosis based on the clinical history and physical exam, and the laboratory testing of a vaginal sample.<sup>1,2,6,9</sup> Bacterial vaginosis (~25-30% prevalence) and *Candida* (~20-25% prevalence) overgrowth are by far the most common infections diagnosed in adolescent girls and women (i.e., >13 yrs. to elderly), while *Trichomonas vaginalis* infection occurs much less often (i.e., ~1% prevalence).

Women with BV have an increased susceptibility to sexually transmitted infections (STIs) including human immunodeficiency virus (HIV) and some also have adverse pregnancy outcomes (i.e., pelvic inflammatory disease, premature labor)<sup>6,9,10-12</sup> A shift in the normal vaginal flora is caused due to a decline in the levels of 'beneficial' lactic acid and hydrogen peroxide producing lactobacilli. BV is diagnosed by this shift in the normal vaginal flora towards an overgrowth of a wide variety of fastidious and anaerobic bacteria, most notably *Gardnerella vaginalis*, *Mycoplasma hominis*, *Mobiluncus*, *Prevotella*, *Peptostreptococcus*, and *Porphyromonas* spp.<sup>1-3,10</sup>

Recent molecular studies of vaginal flora in women with BV have also found a new bacterium, *Atopobium vaginae*.<sup>13</sup> It was present in the majority of patients, but rarely in BV negative women.<sup>13</sup> A mixture of these organisms is usually present in concentrations 100-1000 times greater in women with BV than in the healthy vagina.<sup>1-3,10</sup> Although the pathogenesis of BV and the triggers that causes this alteration in the vaginal ecosystem are not understood, several factors have been identified that may predispose women to the development of BV including sexually intercourse, broad-spectrum antibiotic use and more recently Vitamin D deficiency in pregnant women. In addition, antibiotic treatment of BV results in only a temporary shift in the microbial flora in many cases, and greater than 30% of BV patients will have a recurrence by 3 months.

BV is clearly a heterogenous disorder based on distinct immunological profiles. Vaginal levels of IL-8 and IgA anti-hemolysis are inversely correlated with local concentrations of sialidase and prolidase.<sup>4,14</sup> Clinical evidence is accruing that only the sub-group of women with elevated levels of these enzymes suffer the sequel of BV such as adverse pregnancy outcomes.<sup>4,14</sup> Measurement of sialidase or prolidase activity may therefore be better markers than a clinical or Gram-stained vaginal smear assessment for

women with an increased risk of adverse BV-related pathology.

## REFERENCES

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The Committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.