The laboratory diagnosis of bacterial vaginosis

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Bacterial vaginosis (BV) is an extremely common health problem for women. In addition to the troublesome symptoms often associated with a disruption in the balance of vaginal flora, BV is associated with adverse gynecological and pregnancy outcomes. Although not technically a sexually transmitted infection, BV is a sexually associated condition. Diagnostic tests include real-time clinical/microbiological diagnosis, and the current gold standard, the standardized evaluation of morphotypes on Gram stain analysis. The inappropriate use of vaginal culture can be misleading. Future developments into molecular-based diagnostics will be important to further understand this complex endogenous flora disruption.

Key Words: *Bacterial vaginosis; Infection; Vaginitis*

Diagnostic de la vaginose bactérienne en laboratoire

La vaginose bactérienne est un problème de santé extrêmement courant chez la femme. En plus des symptômes ennuyeux souvent associés à ce déséquilibre de la flore vaginale, la vaginose bactérienne est associée à des problèmes gynécologiques et de fertilité. Bien qu'il ne s'agisse pas à proprement parler d'une infection transmissible sexuellement, la vaginose bactérienne est une maladie qui a une composante sexuelle. Les analyses diagnostiques reposent notamment sur l'examen clinique et les tests microbiologiques en temps réel, et sur la norme diagnostique actuelle, l'analyse standardisée des morphotypes par coloration de Gram. L'utilisation inappropriée de la culture vaginale est parfois trompeuse, c'est pourquoi les développements à venir au chapitre du diagnostic moléculaire seront importants si nous voulons mieux comprendre cette perturbation endogène complexe de la flore.

Bacterial vaginosis (BV) is one of the most common lower genital tract conditions, occurring in 35% of women attending sexually transmitted infection (STI) clinics, 15% to 20% of pregnant women, and 5% to 15% of women attending gynecology clinics (1). Clinical features were first described by Gardner and Dukes (2), and range from asymptomatic to an increased thin vaginal discharge with or without a fishy odour. There are a number of pregnancy and/or gynecological complications associated with BV (3). Specifically, BV has been associated with both STI and nonchlamydial, nongonococcal pelvic inflammatory disease (4,5). Of importance, BV may be associated with an increased risk of acquisition of HIV (6-8). In addition, BV has been associated with infections following termination of pregnancy, insertion of intrauterine devices, and hysterectomy, both vaginal and abdominal (7-10). This syndrome has been associated with serious pregnancy complications, including premature rupture of the membranes, preterm delivery and postpartum endometritis (11-16). Given the varied and important conditions associated with this common disorder, an accurate and clearly understood diagnosis is critical.

The diagnosis of BV has been problematic due to its complex polymicrobial nature (17). Vaginal cultures were often used as a primary laboratory test in the past, but this was found to be of little value. Organisms classically associated with BV, including *Gardnerella vaginalis*, can be recovered on laboratory media from 83% to 94% of women with clinical signs of BV, but are also recovered in 36% to 55% of asymptomatic women without clinical features (18). In addition, culture and identification of other bacteria from vaginal specimens such as *Bacteroides* species, *Peptostreptococcus* species and *Mycoplasma hominis* has been evaluated, and found to be specific but insensitive and costly to the laboratory (19). Other anaerobic bacteria strongly associated with BV, such as *Mobiluncus* species, are very difficult to recover by culture (20). At the same time, normal vaginal lactobacilli are significantly reduced or absent. As a consequence, clinical diagnosis must rely on methods that identify proportions of bacterial morphotypes in the vaginal specimen (21).

SPECIMEN CHOICE, COLLECTION AND TRANSPORT

The collection of material for diagnosis is ideally performed during a comprehensive pelvic examination using a speculum. At the time of speculum examination, an evaluation of the nature of the discharge is made by the clinician, and a specimen from the lateral vaginal wall and posterior fornix can be taken with a sterile swab. The classical BV discharge is thin, homogeneous and grey/yellow in colour. However, absence of the classic discharge does not rule out disturbed vaginal flora. In certain clinical circumstances, where STI pathogens have been ruled out and there is no other reason for a speculum examination, the swab can be taken as a blind vaginal swab taken by the clinician or the patient (22). Two basic methods

TABLE 1
Scoring system (0–10) for the Gram-stained vaginal smears

Score	Lactobacillus morphotypes	*Gardnerella* and *Bacteroides* species morphotypes	Curved Gram-variable rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

1+ ≤ 1/1000× field; 2+ = 1–5/1000× field; 3+ = 6–30/1000× field; 4+ ≥ 30/1000× field

of diagnostic testing can be used: laboratory based and clinical 'bedside' testing. For the purposes of laboratory based testing, the swab can be placed in a standard bacterial culture transport medium to maintain moistness or can be smeared onto a slide and air dried for later Gram stain. Transportation for either of these transport systems (culturette or dried slide) can be at room temperature or 4°C.

DIAGNOSTIC TESTS
Performance related to specimen type, and diagnostic test limitations
There are two main categories of diagnostic tests for BV: clinical criteria and laboratory-based testing.

The most widely accepted clinical criteria are 'Amsel's criteria' (23). This clinical diagnosis requires that three of the following four criteria be met: first, a vaginal pH of greater than pH 4.5; second, the presence of clue cells in the vaginal fluid; third, a milky, homogeneous vaginal discharge; and finally, the release of an amine (fishy) odour after addition of 10% potassium hydroxide to the vaginal fluid (23). The pH can be determined directly with the use of pH sticks placed on the vaginal wall or with the use of a swab which is touched on pH paper in the range covering pH 4.0 to pH 6.5. The swab is then extracted into 0.2 mL of physiological saline either on a glass slide or in a test tube; a drop of the extract is then placed on a glass slide. A drop of 10% potassium hydroxide is placed on another glass slide. The swab is then stirred in the 10% potassium hydroxide and immediately evaluated for the presence of a fishy odour. Both drops are then covered with a coverslip and examined at 400× magnification with a light microscope. Clue cells are identified as vaginal epithelial cells with such a heavy coating of bacteria that the peripheral borders are obscured. If three of four criteria are met, then a clinical diagnosis of BV can be made.

For the laboratory testing method, the preferred specimen is an unfixed vaginal smear sent to the laboratory to be Gram stained by standard methods. The stained slide is read, and the number of morphotypes are evaluated based on a standardized scoring method. The diagnostic criteria developed by Spiegel et al (24) and later modified by Nugent et al (25) has been a well-reproduced standardized Gram stain scoring method (Table 1).

In the methodology by Nugent et al (25), the swab was obtained from the lateral vaginal wall and rolled on a glass slide. The smears were then heat fixed and Gram stained using safranin as the counterstain. The smear was then evaluated for the following morphotypes under oil immersion (1000× magnification): large Gram-positive rods (lactobacillus morphotypes), small Gram-variable rods (*G vaginalis* morphotypes), small Gram-negative rods (*Bacteroides* species morphotypes), curved Gram-variable rods (*Mobiluncus* species morphotypes) and Gram-positive cocci. Although Gram-positive cocci are not part of the scoring system, some laboratories will report them if they are present in significant numbers. Increased numbers of Gram-positive cocci are not part of the pattern of the normal vaginal flora. Of note, the Nugent scoring system yielded an improvement in intercentre agreement compared with the previously published criteria (24), but a standardized scoring method is the most important approach.

A score of zero to three is considered to be normal, four to six is considered intermediate, and seven to ten is defined as BV. Intermediate vaginal flora is reported to the clinician for management based on the clinical context. Thirty two per cent of patients with an intermediate score will proceed to BV and 30% to normal flora. Many authors feel that an intermediate score should be included as abnormal given the high rate of transition to BV. The decision to recheck or treat is based on the clinical risk of proceeding to BV (26). This scoring system correlates well with clinical disease (18). The clinical methodology is useful because it allows for an immediate answer in certain urgent clinical situations, but the Gram stain method appears to be more accurate (27-29). However, in pregnancy in the setting of rupture of membranes, it has good negative predictive value (83%) but poor sensitivity (30).

There have been alternative diagnostic methods suggested, but none are currently better than the standardized Gram stain methodology. The use of gas-liquid chromatography, vaginal cultures and liquid preparation Papanicolaou smears have been proposed as alternative methods of diagnosis due to the practical advantage of sampling and common transportation to the laboratory. At the time of writing, this has only been done in research settings and would require significant changes in the approach to reading smears. There are variable reports of a general lack of sensitivity but reasonable specificity (31,32). To date, nucleic acid techniques have not proven to be useful for the clinical diagnosis of the complex microbial imbalance, but may prove useful in the future (33). Many researchers are exploring a genetic basis for evaluation of the complex microbial flora of the vagina; there is some preliminary promise in the use of chaperonin 60-based evaluations, while others are using an RNA-based approach. None of these techniques are currently useful in the clinical setting due to complexity and cost, but they may be highly valuable in the future (34,35). In summary, the most useful current diagnostic method is the vaginal Gram stain.

Proficiency and quality assurance
The laboratory diagnosis of BV is mainly achieved by microscopy. Quality assurance should therefore ensure good practice in preparing and reading Gram stains, competency in the microscopists, and correct maintenance and set up of the microscopes. The laboratory should have ongoing communication with its clinicians to ensure that the smear being submitted is vaginal and not cervical. This should be indicated on the specimen requisition. A negative result for BV on a cervical smear could lead to inappropriate patient management. Good practice requires that the report on the Gram smear should mention the presence or absence of yeast cells.

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