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# Bacterial vaginosis – a laboratory and clinical diagnostics enigma

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## *Review article II*

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Diagnosing bacterial vaginosis (BV) has long been based on the clinical criteria of Amsel et al., whereby three of four defined criteria must be satisfied. Though there are other criteria and scoring methods which function well in comparison (i.e. Nugent scoring), it is not certain that they will always identify the same category of patients. Point-of-care methods based on various combinations of microbial products, presence of RNA, or more complex laboratory instrumentations such as sensor arrays, have also been introduced for the diagnosis of BV. No method for diagnosing BV can at present be regarded as the best. It could be that – based partly on tacit knowledge on the part of the clinical investigators scoring in the clinic – various scoring systems have been chosen to fit a particular BV-related problem in a particular population. In this review we critically examine these pertinent issues influencing clinical scoring and laboratory diagnostics of BV.

Key words: Bacterial vaginosis; Nugent scoring; laboratory diagnostics.

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## INTRODUCTION AND ASPECTS OF EPIDEMIOLOGY

The method of choice for diagnosing BV is Amsel's clinical criteria and in a laboratory setting Nugent scoring (1, 2). Often, however, the scoring and laboratory methods used are "in house" procedures or variants of previously published methods, which makes comparisons of methods and epidemiological surveys enigmatic. Before discussing methods for diagnosing BV it is thus necessary to review pertinent parts of the voluminous literature on BV diagnostics in various patient groups. BV is observed at many types of clinics, it is reported from primary care units,

gynecological clinics, STI clinics, and among specific patient groups such as expectant mothers, abortion applicants and sex workers. A helpful summary of the available literature was also compiled by Mead (3).

The report that corresponds most closely to a population survey deals with cytological material compiled in the Netherlands, comprising 190,000 patients and showing a BV incidence of 3.6% (4). Available figures indicate that BV is more common in developing countries than in industrialized countries. Cytological material from Brazilian reports indicates 20% BV (5), as compared with 9% from the United Kingdom (6). Among pregnant patients in Papua New Guinea, 23% are reported to be BV positive (7), as opposed to 5–16% in Europe (8, 9).

If one compares various health care units,

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16% BV-positive patients are reported from Danish primary care clinics (10) and 9% from gynecological clinics in the United Kingdom (6). In Europe as a whole, the BV incidence among pregnant women is low; for instance in Italy the figure is 5% (8) and in Finland 7% (11). Women who seek medical attention for reasons that might cause the physician to suspect high-risk sexual behavior often have a higher incidence of BV. As regards abortion applicants, a Norwegian study reports 24% incidence of BV positive (12) and a Swedish study reports 33% (13). Among patients consulting STI clinics, the incidence is as high as 40% (14). The consistently highest figures for BV cases are reported for sex workers: in Africa 40% (15) and in Asia 33% (16). As many as 47% of HIV seroprevalent patients also have BV (17).

Two relatively comprehensive and consecutively conducted Swedish studies exist, based on material collected from STI clinics during the 1980s, with a reported BV incidence of 26% and 24%, respectively (18, 19). Both studies fail to distinguish cases where BV is encountered in combination with other genital infections. Both reflect the frequency of BV at a type of clinic where the physician has a manifest interest in genital infections and not least has a patient clientele that is relatively young and sexually active. Moreover, both studies indicate that around 30% of the patients who were diagnosed with BV yielded a smear that indicated the presence of inflammation, defined as more leukocytes than epithelial cells, and are also supported by a recent study (20). With the rise during recent years of chlamydial infection, the number of cases of "pure" BV has diminished; at least this is supported by clinical material from the Swedish city of Uppsala (19). In another Swedish city, Örebro (18) this patient group had, in comparison, a BV incidence of 27%. The study comprised 100 women examined consecutively at two primary care clinics.

In summary, the studies project a picture of a situation in many countries where the BV incidence in the population at large is only a few percent. Among pregnant women, the frequency is about 10%. Around 20 to 30% of abortion applicants are diagnosed with BV, which can be compared to the figures that apply for patient groups in primary care and patient groups attending an STI clinic. Laboratory- and score-

based methods will perform with varying specificity and sensitivity in these diverse patient populations, and it can be assumed that this has influenced the choice of methods.

#### LABORATORY DIAGNOSIS OF BV IS FRAUGHT WITH PROBLEMS OF TURNAROUND TIME, COSTS AND VALIDITY

Most types of infectious disease are diagnosed by culture, by isolating an antigen or RNA/DNA from the microbe, or by serodiagnosis to determine the presence of antibodies to the microbe. Generally this type of diagnosis is not concerned with how much or how little of the microorganism is present, only that it is present. Therefore demonstration of the presence of an infectious agent is often a necessary criterion for diagnosis of the disease. This is not the case for BV, since the ultimate cause of the disease is not yet known. Therefore the patient must satisfy clinical or laboratory criteria which do not include the presence of a specific bacterium or a specified quantity thereof. However, it is probably true that if a specific bacterium (e.g. *Mobiluncus*) were the cause of BV, it would have to appear in sufficient quantities to satisfy that criterion, and conversely the bacterium might be present in minute quantities even though the clinical criteria are not satisfied. It is important to bear this in mind when comparing the various diagnostic methods. BV does not derive from a traditionally defined bacterial infection with one causative agent, but can rather be likened to the situation in other parts of the body when an anaerobic mixed flora causes infection. Therefore PCR and similar nucleic acid amplification methods have not gained ground in diagnostics. Also since BV implies a disrupted vaginal flora with a superfluity of anaerobic bacteria, anaerobic cultures cannot be employed economically to diagnose BV as they require a great deal of time and resources. For this reason, quantitative anaerobic cultures have not gained a footing in BV diagnostics. Two studies employing PCR amplification of *Mobiluncus* 16SrRNA gene segments have, however, been published in attempts to achieve a rational DNA amplification approach to BV diagnostics (21, 22). More data are, meanwhile, required to effectively explore the

usefulness of DNA amplification both as regards primer design and populations studied.

Gas chromatography and ion mobility spectrometry were used for detection of the amines produced by the mixed anaerobic vaginal flora with a view to developing diagnostic assays (23–27). There exists a clear correlation between a criteria-based diagnosis of BV and the level of amines detected in vaginal fluid by gas chromatography and ion mobility spectrometry, though none of these analytical methods has been further developed into a robust and well-validated laboratory procedure for diagnostic purposes.

### CRITERIA-BASED METHODS OF DIAGNOSING BV

Such diagnosis is based on the weighting of criteria to ensure the best possible correspondence between the criteria and the presence of BV. As is the case for all criteria-based diagnoses, the results of these methods depend on the composition of the examined population and the manner in which the criteria are validated. It is therefore important that the observer, whether a clinician or laboratory technician, is well informed and able to judge the clinical utility of the various methods available for BV diagnosis.

#### 1. Amsel criteria

The most widely recognized method is based on the Amsel criteria that were established in 1983 (1). A positive diagnosis requires that the patient satisfy three of the criteria described below.

*Discharge.* The typical BV discharge is a thin white fluid with a consistency resembling sour milk. There must not be any granular elements; the fluid must be completely homogenous. This criterion does not require that the discharge is profuse; it can be sparser than a normal vaginal discharge and yet be pathological. Many would say that frothiness is also a typical characteristic, but if the clinician adheres strictly to the original defining criteria, it is sufficient that the discharge is thin.

*pH exceeding 4.5.* Normally, vaginal pH values lie between 3.8 and 4.1. BV patients usually

have values between 4.7 and 5.5. It is unusual that BV patients have a pH value greater than 5.5. Should this occur it will in most cases be caused by cervix secretion being admixed with the vaginal fluid. This can cause a pH reading as high as 7.

*Whiff test.* A whiff test is made directly on the examination speculum even without the addition of 10% (or 20%) potassium hydroxide. The bacteria produce amines, which most noses will find malodorous, but this is quite subjective. Doping the vaginal secretion with a 10% potassium hydroxide solution can make a more objective test. The whiff test is considered positive if an odor of rotten fish is perceived after the application. The test can be made directly on the examination speculum or on a slide. When the vaginal fluid is doped with a potassium hydroxide solution, the pH will rise instantaneously from between 4 and 6 to between 12 and 13. Trimethylamine, which is produced by *Mobiluncus* when it is included in the anaerobic flora distinguishing BV cases, evaporates as gas in a matter of seconds. The human nose is very sensitive to the odor of trimethylamine and can register isolated molecules of this amine. Since the gas evaporates immediately, it is necessary to whiff at the same time potassium hydroxide is applied. If the whiff test is positive, the odor test is considered positive, but not vice versa. Some find it difficult to perceive the trimethylamine odor, which is why this test may be considered subjective (23).

*Clue cells.* Clue cells are large, asymmetrical, mature vaginal epithelial cells with a small nucleus. In cases where a large number of bacteria (thousands) adhere to the cell surface it is difficult to distinguish the edge of the epithelial cell, the epithelial cells become obscure, and will fulfil this criterion. Some of the older literature states that there must be at least 20% clue cells to fulfill the clue cell criterion, but this is not mentioned in the conventional Amsel definition (1).

#### 2. Modifying the Amsel criteria

Often diagnosis of BV is made using variants of the Amsel criteria. Since the characteristic appearance of the discharge is the most subjective criterion, this property is sometimes skipped. A BV diagnosis will in such cases re-

quire that three of three criteria are satisfied. The Center for Disease Control (CDC) in the United States suggested a few years back that it would suffice to use the pH and whiff test criteria, i.e. two of two should be satisfied, but this modification has never been fully validated.

There are even studies where only one of the Amsel criteria was used – the presence of clue cells in microscopic examinations – but in such cases a phase-contrast microscope was used (28, 29).

Several studies have indicated that the Amsel criteria are interdependent. This circumstance can be used to gauge the diagnostic acumen of the observer.

Nearly all BV patients have a pH value exceeding 4.5 (high sensitivity). If clue cells are discerned and the pH is less than 4.5, one might perhaps take a second look. When the whiff test is positive, the patient nearly always has BV (high specificity) and clue cells ought to be observable (19, 28, 30, 31).

### 3. Scoring according to Nugent

The Amsel criteria method has been greatly favored since the diagnosis can be carried out on the clinic's premises. Spiegel's method, introduced in 1983, was the first created to diagnose gram-stained vaginal smears (32). The bacteria were grouped into morphotypes; *Lactobacillus* morphotypes were called elongated bacteria and *Gardnerella* morphotypes, in analogy, were called short bacteria. These latter are small, gram-variable coccobacteria. Nugent's scoring system is a further development of Spiegel's method and includes validation of the categories of observable bacteria structures (2) (Table 1). This points to a need for experienced lab workers or that the clinical diagnostician participates in a quality assurance program.

If more than 30 lactobacilli are observed in the visual field, the score is 0 points; if no lacto-

bacilli are observed, the score will be 4 points. If no *Gardnerella*-like bacteria are observed, the score is 0 points; if more than 30 are observed, the score will be 4 points. The presence of curved rods – *Mobiluncus* – can add 2 points. All the points are added together to obtain the final score: 0–3 points indicate normal lactobacillii flora, 4–6 intermediate flora, and 7 or higher results in a diagnosis of BV. International comparisons indicate that there is a close correspondence between the Amsel criteria and the Nugent scores (33). However, since the image area in the newer microscopes is larger than the microscopic image area used to originally establish the Nugent scores, there may be considerable variation in the reported observations (34).

### 4. Scoring according to Hays/Ison

The Hays/Ison system (35, 36) is based on the observation of gram stains to estimate the ratios of the observed morphotypes rather than the exact number of the bacteria. Originally the observations were divided into three categories – normal, intermediate or BV – but in order to obtain a more precise classification, two additional categories have been introduced as compared to Nugent scoring. The new groups define those preparations that contain no bacteria at all (group 0) and those that contain large amounts of gram-positive cocci, such as *Streptococcus* or *Staphylococcus* morphotypes. These morphotypes have previously been included in the intermediate flora classification but will now make up a class of their own (class 4); the reader is referred to the discussion on intermediate flora later in this article.

### 5. Other scoring systems

Schmidt's scoring system of wet smears of vaginal fluid (wet smear criteria) resembles Nugent scoring in that it ranks the quantities of lactobacilli and cocci in the same way, although

TABLE 1. *The Nugent scoring system*

Score	Lactobacilli morphotype/per field	Gardnerella morphotype/per field	Curved bacteria (Mobiluncus)/field
0	>30	0	0
1	5–30	<1	1–5
2	1–4	1–4	>5
3	<1	5–30	
4	0	>30	

the demarcations of the intervals differ (37). Moreover, the Schmidt system does not recognize *Mobiluncus*. The Schmidt method has been validated for diagnosis of BV in primary care populations (37).

For STI populations, a combination of whiff test, pH testing, observation of clue cells and absence of lactobacilli can be applied (38).

It would be advantageous to have additional calibration tests and more validation of all the criteria-based systems, since they have obviously functioned well in diverse populations. It would also be meaningful to examine the robustness of the systems when they are applied to populations other than those for which they were developed.

#### QUALITY ASSURANCE OF CRITERIA-BASED DIAGNOSIS OF BV

Quality assurance of the criteria-based diagnoses is best done through a quality assurance program to establish benchmark criteria jointly with the affiliated microbiological laboratory or an experienced colleague.

When relying on wet smears, one can take two samples and let one air dry (29). This second smear can be gram-stained or be studied later in a rehydrated state together with an experienced colleague, thus providing a calibration of sorts.

#### DIFFICULT CASES FOR CRITERIA DIAGNOSIS – INTERMEDIATE FLORA – ALTERED VAGINAL FLORA

It is often a simple matter – whether using gram stains or wet smear specimens – to distinguish between healthy patients and patients with pronounced BV. Two other principal microscopic images of a wet smear can, however, be defined: intermediate flora and altered vaginal flora (32–35, 36, 39). These two images are moreover believed to represent other dimensions of vaginal flora than “healthy lactobacilli vaginal flora” and “BV flora”.

In the case of “intermediate flora” the question is whether this represents an intermediate group that does not truly satisfy applied criteria for BV. Alternatively, the intermediate flora may represent a transition phase between “healthy

lactobacilli-dominant vaginal flora” and “BV flora”. Such a transition phase would be significant when conducting therapy research or risk factor research and should then be analyzed independently (39). In some studies the intermediate group has been incorporated in the BV group, and this is true for studies of the risk of premature birth and of post-abort complications (40, 41).

The second principal microscopic image observable in vaginal fluid is altered vaginal flora (32, 36). The term altered vaginal flora is used to describe wet smears with few lactobacilli structures and few small rod/cocci structures, but with distinct structures that resemble large gram-positive cocci, pleomorphic gram-positive rods, which resemble enterobacteria (32). As altered vaginal flora does not satisfy the Amsel criteria it is sometimes given “normal flora” status (36); it is likely, however, that it is a transmutation of vaginal flora that cannot be characterized by the usual criteria used to diagnose BV.

#### POINT-OF-CARE TESTING FOR BV

The most important aspect of the impact of point-of-care testing in general resides in the question of the medical benefit to the patient of ordering potentially less specific or sensitive point-of-care investigations in preference to sometimes costly centralized investigations and how the quality of the ordered investigations impacts on the care of the patient. This is also true for point-of-care testing for BV, whether it is scoring by the clinician or analysis by means of a commercial system.

#### *Some aspects of metrology in relation to point-of-care testing for BV*

Outcome of point-of-care testing is often reported according to the scale on which the results are expressed. Rather than talking about quantitative, qualitative and semiquantitative results, the classification of point-of-care testing into observations and measurements is to be preferred (42). Using systematic ways of expressing outcomes of measurements (the science of metrology), examination results that are expressed on the nominal scale are observations, all other examinations are measurements. The latter need to be defined as

regards which scale, ordinal, ratio or interval is used, and also require information regarding detection value and analytical sensitivity and specificity to inform the end user about the applicability of the results (42, 43).

Results of examinations are meant to classify individuals into two groups, healthy and non-healthy, but are complicated by the fact that results of measurements may be different in equally healthy individuals and among different populations. Health or disease is not always a matter of the problem of exists/does not exist. Reports saying yes/no are therefore rarely sufficient for diagnosis and it becomes important to express results at least on an ordinal scale. There are few examinations that are purely nominal in nature; in most cases there is a cut-off below which the result may be called negative – or positive. The definition of the cut-off is critical as is the uncertainty of this value. In many cases the uncertainty is considerable although rarely known and results in a ‘grey zone’ that may be significant (44).

Many evaluations of point-of-care diagnosis of BV lack detail respecting these fundamental issues and it is thus hard to assess their usefulness and value to the patient. In general, commercially available rapid qualitative tests for BV diagnosis have not found widespread use. This is probably also due to the fact that they cannot so far compete with the criteria-based BV diagnostic methods when cost, precision and time are taken into account, nor have they been validated within clinically relevant populations. In the following only some evaluations of point-of-care testing procedures that have been published in peer-reviewed journals are discussed.

#### *Determining a rise in the pH values and the presence of trimethylamine*

Whilst these point-of-care tests are based on determining Ph and trimethylamine levels in vaginal fluid for the diagnosis of BV, ie. two of the Amsel criteria, the only published study is inconclusive as to the usefulness of the test (45).

#### *Sialidase activity*

Detection of sialidase activity from the dominant mixed anaerobic flora in BV has been proposed as a point-of-care test and validated against Amsel’s clinical criteria and Nugent scores in otherwise healthy women of childbearing age (46, 47). Acceptable-to-good values for

specificity, sensitivity and predictive values were reported.

#### *DNA probe for G. vaginalis rRNA*

The probe used is meant as a marker for BV organisms and promising results have been reported with this approach in two populations, one of pregnant women and one of otherwise healthy women of childbearing age (48, 49). Both studies conclude that there is a need to improve the criteria-based methods and that the probe can be used as a supplement. Clearly an evaluation against a strict “gold standard” method and methodology is needed.

#### *Proline aminopeptidase activity*

Promising results in detecting proline aminopeptidase activity in vaginal discharge for the diagnosis of BV were reported with respect to a possible point-of-care test but have never been confirmed in following reports in peer-reviewed journals (50).

#### *Electronic sensor array “electronic nose”*

The idea of using sensor arrays coupled with software interpretation of the resulting signals (“electronic nose”) for the diagnosis of BV is based on the assumption that the signal pattern thus detected might be an electronic counterpart to the human sensory sensation of smell and has attracted some attention. The results so far are generally disappointing (51, 52; Forsum, Wolrath & Lundström, unpublished).

#### *Self-test pH glove*

In this screening procedure one finger of a medical examination glove has an attached pH indicator paper, which the patient can introduce into her vagina and then read the measured result. If the pH level is high, it is recommended that the patient consult a physician for further evaluation. The glove is reported to be useful in studies from German populations (53). More data should be collected from other populations to determine the glove’s efficacy as a general screening test.

## CONCLUSION

In short, there are a variety of methods for BV diagnostics. None of them identifies a microbe

as the etiological agent of BV; rather at the bottom line they are all based on the criteria diagnostic principles. The various methods will not overlap exactly, but will differ in some respects. For this reason, it is not possible to say that one method is more valid than another. We therefore recommend that the Amsel criteria should be the fundamental method for practicing clinical diagnostics of BV until other well-validated methods have been published. It is important to keep this in mind when conducting comparative studies on differing populations, or when evaluating a number of therapy studies.

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