## Comparison between different diagnostic tests used for bacterial vaginosis

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## Abstract

Background: Bacterial vaginosis is the primary reason for unusual vaginal expulsion in females of fertile age resulting in poor pregnancy ending, pelvic seditious disease, chorioamnioitis, endometritis and increased susceptibility to HIV. As the prevalence is high, and usual investigative tests require laboratory expertise, a future study was therefore performed to compare different diagnostic methods for making an precise diagnosis of bacterial vaginosis. Materials and Methods: This study was conducted in Department of Microbiology which included females of reproductive age group, attending Obstetrics and Gynaecology OPD of TMMC & RC, Moradabad, over a period of one year (March 2017- March 2018). A total of 400 patients with complaints of vaginal discharge and other associated complaints like pruritus, pain lower back, primary or secondary infertility were included in the study. Diagnosis of Bacterial vaginosis was done by using an array of both conventional techniques which included Gram's staining, Nugent's score, vaginal pH etc., and a newer diagnostic technique (OSOM BV Blue test). The patients were also examined PV and the relevant clinical findings were also noted. Results: Out of a total of 400 patients listed for the study, 240 were diagnosed as having bacterial vaginosis. Comparison of the different methods for the diagnosis of bacterial vaginosis revealed that bacterial vaginosis could be detected by 'clue cells' in 100% patients, by OSOM BV Blue test in 97.6% patients, by Amsel's criteria in 67.1% patients, and by evaluation of vaginal pH and vaginal amines in 63.4% and 62.2% patients respectively. Conclusion: Different diagnostic techniques have varied sensitivity and specificity in diagnosis of bacterial vaginosis. OSOM B.V. blue test is found to be highly sensitive and can be performed easily in clinical settings where microscopic facilities are not available.

Keywords: Diagnosis, Bacterial Vaginosis, Gram's Staining, Nugent's Score, Vaginal pH, OSOM BV Blue Test.

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#### Introduction

Bacterial vaginosis (BV) is a regular clinical condition in women of fertile age [1]. It is conventionally appreciated as a temporary community disturbance of the vaginal microbiota, though really involving self-organization as a resilient biofilm community [2]. BV is a condition where the normal flora of the vagina is disturbed and replaced by an extra-growth of certain microbes or it is associated with an imbalance in the bacteria that are usually present in a woman's vagina. The vagina normally contains mostly "healthy" bacteria, and fewer "harmful" bacteria, BV develops when harmful bacteria outnumbers healthy bacteria [1]. The etiological bacteria are Anaerobic bacteria (Prevotella, Mobiluncus, Gardnerella vaginalis, Ureaplasma, Mycoplasma) [3]. It depicts an exceptional and difficult change in the flora of the vagina, which is characterized by a reduction in the occurrence and the

Manuscript received: 10<sup>th</sup> October 2019 Reviewed: 20<sup>th</sup> October 2019 Author Corrected: 26<sup>th</sup> October 2019 Accepted for Publication: 30<sup>th</sup> October 2019 numbers of lactobacilli and a rise in the concentration of *Gardnerella vaginalis* and resident anaerobic bacteria [1]. The most common types of lactobacilli in the vagina include Lactobacillus crispatus, L. jensenii, L. gasseri, and L. iners. Most of these lactobacilli act as important host-defense mechanisms against BV by secreting substances that inhibit the growth of microbial pathogens and indigenous anaerobes [4]. Clinical features were first described by Gardner and Dukes [5] and range from asymptomatic to an increased thin vaginal discharge with or without a fishy odour. Some women with bacterial vaginosis have a foul smelling, thin, uniform frothy, vaginal exonerate [1,6].

There are a number of pregnancy and/or gynecological complications associated with BV. Specifically, BV has been associated with both STI and non-chlamydial, nongonococcal pelvic inflammatory disease [7]. The contributing risk factors for BV comprises of low socioeconomic status, vaginal douching, smoking, new or multiple sex partners, unprotected intercourse and women who have sex with women [3]. BV increases chances of receptiveness to HIV infection and other Sexually transmitted diseases, such as herpes simplex virus (HSV), chlamydia and gonorrhoea.

As it is just an extra-growth of the unhealthy flora of the vagina devoid of swelling, there is no single best method for the diagnosis of bacterial vaginosis [1,6] This condition is defined by a composite of four clinical criteria: (1) Vaginal discharge is homogeneous; (2) vaginal discharge had a pH  $\geq$ 4.7; (3) vaginal discharge had an amine-like odor when mixed with 10% potassium hydroxide; (4) vaginal discharge contained clue cells representing  $\geq$ 20% of vaginal epithelial cells [8].

Although Gram stain is considered the diagnostic standard, bacterial vaginosis is traditionally diagnosed using the Amsel criteria [3] Criteria include thin, homogenous discharge; a positive whiff test; the presence of clue cells on microscopy; and a vaginal pH greater than 4.5. However, many a times, multiple criteria are used for the conclusion of bacterial vaginosis.

Even though, bacterial vaginosis (BV) is a common but highly enigmatic condition which is associated with adverse outcomes for women and their neonates [9] As the prevalence is high, and regular diagnostic tests require laboratory expertise, in view of this, the present prospective study was therefore performed to evaluate different diagnostic methods for making an exact diagnosis of bacterial vaginosis.

## **Methods and Materials**

The study was conducted among 400 females of reproductive age group with complaints of vaginal discharge attending OPD of Gynaecology and Obstetrics, in association with Department of Microbiology, TMMC & RC, Moradabad, over a period of one year (March 2017-March 2018).

**Study setting:** OPD of Gynaecology and Obstetrics, TMMC & RC, Moradabad Duration of study: 18 months. **Duration**: Over a period of one year (March 2017-March 2018).

Study description: Design: Prospective, observational study

Sample size: 400 selected randomly

**Sample size justification:** Sample size of the study was determined using SAS 9.2 package.

Minimum sample required to have 80% power to detect change of mean 5 and SD 3.5 at alpha=0.05.

**Ethical approval:** This study was approved by Institutional Ethics Committee (IEC) of a tertiary center in a teaching hospital.

**Inclusion criteria:** Patients with complaints of vaginal discharge and other associated complaints like pruritus, pain lower back, primary or secondary infertility were included in the study.

**Exclusion criteria:** Women with history of receiving systemic antibiotic therapy or local vaginal antimicrobial therapy within the preceding 15 days, who were menstruating at the time of the examination, vaginal bleeding, placenta previa, spermicide use, recent douching, or sexual intercourse within 24 hour.

**Methodology:** Diagnosis of BV was done using an array of conventional techniques which included Gram's staining, Nugent's score, vaginal pH and a newer diagnostic technique (OSOM BV Blue test). The patients were also examined PV and the relevant clinical findings were also noted.

## Data collection

## Specimen collection

Following a history and physical examination, vaginal fluid was collected from posterior fornix of the patient's vagina by using sterile swabs. Sample was taken from vagina of patient using sterile cotton swabs. Two swabs were taken from every patient.

- First swab: used for gram staining
- Second swab: used for OSOM BV BLUE test.

1) On Gram staining Clue cells i.e. epithelial cells with adhering gram-negative small rods and gram variable cocco-bacilli were seen. In bacterial vaginosis usually, there were very few or no pus cell and lactobacilli were not seen. Gram-positive cocci were also seen.

**2) The OSOM BV BLUE Test**<sup>®</sup> is an enzyme activitybased test based on activity of sialidase enzyme in vaginal fluid specimens, an enzyme produced by bacterial pathogens, such as, *Gardnerella vaginalis, Bacteroides spp., Prevo tella spp., and Mobiluncus spp.* 

This test procedure contains a color producing substrate for bacterial sialidase enzyme. In this method, a vaginal fluid sample is placed in the BV Test. The sample then reacts with the color producing substrate. A Developer Solution is poured after the reaction. If the sample has increased level of sialidase activity (positive), a blue or green colour will be seen in BV Test container or in the swab head. If the sample has no sialidase activity (negative), or has very low levels, a yellow colour will be seen in the BV Test container. **pH Test:** Vaginal pH was tested with a pH paper held with a forcep and dipped into the vaginal secretion. Colour change was noted. pH > 4.5 is considered positive for bacterial vaginosis [10].

#### Data analysis

- Sensitivity and specificity were calculated for each testing method using the following formulas:
- Sensitivity: (number of true positives/(number of true positives + number of false negatives))\*100;
- Specificity: (number of true negatives/(number of true negatives + number of false positives))\*100.

**Statistical analysis:** Data were analysed using SPSS V 15.0 (Statistical Package for Social Sciences, Version 15.0) package. Data were given as Mean and SD for quantitative variables and Number (Percentage %) for qualitative variables.

Chi square tests were applied to compare percentages of more than 2 groups and Fisher Exact Probability tests were applied to compare percentages between 2 groups.

All tests were 2 tailed. Level of Significance was taken as  $P \leq 0.05$ .

#### Result

Out of a total of 400 patients enrolled for the study, 240 were diagnosed as having bacterial vaginosis (Table 1). The maximum number of patients (51.3%) were in the age group 21-30 years, followed by 154 (38%) patients in the 31-40-year age group. The highest prevalence of bacterial vaginosis was also seen in these age groups, 27.7% in the 31-40-year age group as compared to 26.4% in the age group 21-30 years.

he most usual presenting complaints in patients in whom bacterial vaginosis was present were discharge PV (64.7%), discharge PV with pruritus (83.3%), and discharge PV with pain lower abdomen (91.2%). It was also noted that bacterial vaginosis was significantly more common in pregnant females (86.6%) as compared to non-pregnant females (13.4%), and this difference was clinically significant (p<0.001).

#### Table-1: Distribution of total cases as diagnosed by OSOM BV Blue Test.

Bacterial vaginosis	Number of patients (%)	
Absent	160 (40)	
Present	240 (60)	
Total patients	400 (100)	

Comparison of the different methods for the diagnosis of bacterial vaginosis revealed that bacterial vaginosis could be detected by 'clue cells' in 100% patients, by OSOM BV Blue test in 96.7% patients, by Amsel's criteria in 68.8% patients, and by evaluation of vaginal pH and vaginal amines in 65% and 60% patients respectively (Table 2). It is important to note that the 'clue cells' were consistently observed in all (100%) patients, and they were absent in all the patients who were negative for bacterial vaginosis. Similarly, the OSOM BV Blue test<sup>®</sup> was also very accurate, giving a sensitivity of 96.7% and a specificity of 97.5%. The difference between the sensitivity and specificity of the OSOM BV Blue test and Gram's staining was statistically significant (p<0.001) (Table 2). Out of a total of 400 patients, 42.5% were detected by Amsel's criteria, whereas Gram's staining detected 60% patients.

Table-2: Cross tabulation showing patients with bacterial vaginosis as detected by various tests and their co-relation with
Gram's staining.

Test done	Results in patients with bacterial vaginosis by Gram's staining (n = 240)	Results in patients without bacterial vaginosis by Gram's staining (n = 160)	Total patients (n = 400)
OSOM BV Blue test	232(96.7)	6 (3.8)	238 (59.5)
Vaginal pH >4.5	156 (65)	19 (11.9)	175 (43.8)
Amine test	149 (60)	22 (13.8)	171 (42.6)
Clue cells	240(100)	0 (0)	240 (60)
Amsel's criteria	102 (42.5)	15 (9.4)	117 (42.8)

## Discussion

Bacterial vaginosis is the most common cause of vaginal discharge among women of reproductive age group. It is an extremely common health problem for women, occurring in 35% of women attending sexually transmitted infections clinic, 15-20% of pregnant women and 5-15% of women attending gynaecology clinics [11]. In the present study, 240 (60%) out of 400 patients were diagnosed as having bacterial vaginosis. This high prevalence may be due to inclusion of only symptomatic group in the present study.

In India different workers reported varying prevalence of bacterial vaginosis. In a study done in Karnataka, India, prevalence of bacterial vaginosis using Nugent's criteria was reported to be 20.5% [6]. Prasad et al [12] found the rate of bacterial vaginosis in young married women in Tamil Nadu to be 18%, Agarwal et al [13] reported the prevalence of bacterial vaginosis in 21.7% cases, Bhalla et al [14] reported the occurrence, of bacterial vaginosis in 32.8% cases whereas, Dadhwal et al [15] reported bacterial vaginosis in 8.6% cases. In similar studies done by Saleem et al [10] and Naqvi et al [16] the prevalence of bacterial vaginosis as 3.5% in Vietnam whereas Jones et al reported the prevalence of bacterial vaginosis in Peru as 27% [18].

In the present study, based on Nugent's score by gram's staining, bacterial vaginosis (score > 7) was found in 60% of women, and deviation from normal commensal flora (score 4-6) was found in 26.9% cases. Bhalla et al [14] reported bacterial vaginosis in 32.8% and intermediate flora in 16.9% women, whereas Sewankambo et al [19] reported bacterial vaginosis in 50.8% and intermediate flora in 31.7% women.

A study by Coleman JS et al [20] reported bacteria that were frequently detected in women with BV that included Gardnerella vaginalis, Atopobium vaginae, Megasphaera types, Leptotrichia amnionii, Sneathia sanguinegens, Porphyromonas asaccharolytica, a bacterium related to Eggerthella hongkongensis, and bacteria related to Prevotella species. Less frequently detected bacteria included Peptostreptococcus spp., Aerococcus, Anaerococcus, Gemella, and Veillonella genera.

Gram's Method of staining is simple, inexpensive, sensitive, specific and reproducible way to diagnose bacterial vaginosis [21,22] Conventional diagnostic methods such as gram staining based on Nugent scoring systemare popular [23] Amsel's criterion is also a standard method which provides accurate rapid diagnosis and has been used in many studies evaluating bacterial vaginosis [22]. However, recently, OSOM BV blue test with almost equally efficacious, less time consuming and minimal technical expertise requiring is emerged for rapid diagnosis of bacterial vaginosis. OSOM BV blue test is based on the principal of detection of bacterial sialidase activity (an enzyme produced by bacterial pathogens such as Gardnerella vaginalis, Bacteroides spp., Prevotella spp., and Mobiluncus spp.) in vaginal discharge [24].

Bacterial vaginosis as diagnosed by OSOM BV Blue test<sup>®</sup> in the present study was 58% (232 out of 400 patients). In the present study, sensitivity and specificity of OSOM BV Blue test<sup>®</sup> as compared to Gram's staining was found to be 97.6% and 97.5% respectively which is quite similar as reported by Myziuk et al [25] who demonstrated sensitivity and specificity of BV blue test<sup>®</sup> as 91.7% and 97.8% respectively. A study was conducted by Mahajan G et al [26] on 200 cases complaining of vaginal discharge. Of these, 68 (34%) were diagnosed by BV blue test. Shujatullah F et al [24] in a similar study observed that OSOM BV blue test showed good efficacy, as compared with gram staining in diagnosing BV and thus, the performance of OSOM BV blue was better than the methods based on Amsel's criteria.

Madhivanan P et al [27] examined the performance of the BVBlue POC test as compared to Amsel's criteria and Nugent scoring of Gram stains among women with and without symptoms of BV. Their results showed that BVBlue test had poor sensitivity in detecting BV (38.1%) but was highly specific (92.7%) in a population of women attending a reproductive health clinic in Mysore, India. In a study by Khatoon R et al [28] bacterial vaginosis was detected in 60.8% of patients. OSOM BV Blue test detected maximum number of cases with sensitivity and specificity of 95.3 and 92.1%, respectively.

There is a great need for an inexpensive diagnostic method that is both reliable and unifies clinical and microbiological parameters to make it more sensitive while retaining its specificity [26] Thus, B. V. blue procedure can be used as a point-of-care test useful in making rapid and accurate diagnosis of bacterial vaginosis in setups lacking microscopic facilities or technical expertise.

The limitation of the present study is that it is an institutionbased study; further cross-sectional surveys with larger populations are required. As with most diagnostic tests, BV Blue has limitations. Since mixed vaginal infections may occur, the presence of sialidase activity does not rule out the presence of yeast, *T. vaginalis*, or other organisms. To prevent adverse performance of BV Blue, it should not be used in women who have recently douched, engaged in vaginal sexual intercourse, or used spermicides, vaginal lubricants, or feminine deodorant sprays within 72 hours prior to testing. BV Blue is a quick and easy test that detects the presence of sialidase activity by utilizing a chromogenic substrate of bacterial sialidase to produce a color reaction when a color developer solution is added to the reaction vessel. BV Blue appears to be a useful point-of-care tool to provide presumptive diagnostic information for women with BV when used in conjunction with clinical and patient information [25].

## Conclusion

Different diagnostic techniques have varied sensitivity and specificity in diagnosis of bacterial vaginosis. OSOM B.V. blue procedure is a point of care procedure which can be easily done without expertise and lab facilities, still showed high sensitivity and specificity.

## What this study adds to existing knowledge?

The study adds with most diagnostic tests, BV Blue has limitations. Since mixed vaginal infections may occur, the presence of sialidase activity does not rule out the presence of yeast, T. vaginalis, or other organisms.

## Author's contribution

**Dr. Vasundhara:** Contributed in design of this work, collection of data and editing.

**Dr. Farooq U:** Conception of the idea and collect data. **Dr. Rehana Najam:** Contributed in writing manuscript and analyze the data.

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