Rapid Detection of Bacterial Vaginosis (BV) by BVBlue test

Shameem Akhter1, Humayun Satter2, Shirin Tarafder2, Ruhul Amin Miah2, Sohely Sharmin3, Sharmeen Ahmed2

1Department of Microbiology, Bangladesh Institute of Health Sciences (BIHS), Mirpur-1, Dhaka. 2Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka. 3Department of Microbiology, Bangladesh Medical College, Dhanmondi, Dhaka, Bangladesh.

Abstract
Bacterial vaginosis is the commonest cause of abnormal vaginal discharge in women of reproductive age and require laboratory test for diagnosis. A total 200 women aged 15-45 years with history of abnormal vaginal discharge were included as study population. Fifty women without such history of discharge were taken as healthy control. Three vaginal swab samples were taken from each case and control. These swab samples were subjected to test by conventional methods such as Amsel clinical criteria, Gram stain Nugent method, culture and by newly developed BV Blue test. The results of the BVBlue test were compared with these methods to find out the efficacy of BVBlue test. Rate of detection of bacterial vaginosis (BV) cases was 21.5% by Amsel clinical criteria, 21.0% by Gram stain Nugent method, 21.0% by culture and 22% by BVBlue test among the study population. When comparing with the conventional test and culture, BVBlue test was 100% sensitive and 98% specific. It is rapid, technically simple and is suitable for screening large number of patient in short time where laboratory facilities are not developed.

Key words: Bacterial Vaginosis, BVBlue test, Nugent method, Abnormal vaginal discharge.

Introduction
Bacterial Vaginosis (BV) is a disorder of the vaginal ecosystem characterized by a shift in the vaginal flora from the normally predominant Lactobacillus to one dominated by sialidase enzyme-producing mixed flora including Gardnerella vaginalis, Mobiluncus, Prevotella, Bacteroides, and Mycoplasma species.1 It is the most common cause of abnormal vaginal discharge in women of reproductive age and is associated with increased susceptibility to human immunodeficiency virus and sexually transmitted infections.2

The BV is associated with several obstetrical and gynaecological conditions and disorders including spontaneous abortion, preterm labour and premature rupture of membrane, placental infection, wound infection and pelvic inflammatory diseases.3,4 Prevalence of BV varies in different population. It is higher in United States of America (38%) where as in India the rate of detection is lower 22.65%.6 Similar rate of isolation (22.65%) were reported by Bilkis et al (2003) in Bangladesh.5 Diagnosis of BV depends on both clinical and laboratory methods.

Earlier method of BV diagnosis relied on Amsel Clinical Criteria which considers factors like raised vaginal pH, positive amine odour, homogenous abnormal vaginal discharge and presence of clue cell on wet film.7 The present laboratory diagnosis of bacterial vaginosis is based on Nugent scoring system. Gradual rise of score from 0 to 7 indicates shift from normal acid-forming vaginal flora to anaerobic organism responsible for BV.8 Although Nugent Criteria is considered as gold standard for diagnosis of bacterial vaginosis, it needs skilled manpower, laboratory facilities and is prone to subjective error. As a result, it was not suitable for
rapid screening test. Later on, New Proline Amino Peptidase (NPap) Assay and BVBlue test have been introduced to overcome these problems. Among these two, BVBlue test has the advantage of being easy, rapid and bedside test. 

The BV Blue test (Gryphus Diagnostics, L.L.C.) is a chromogenic test based on the presence of elevated sialidase enzyme in vaginal discharge. In BVBlue test, vaginal discharge is treated with a chromogenic substrate (IBX 4041), incubated and the few drops of developer solution (NaOH 40 mg/ml) is added. Development of blue colour indicates the test is positive. The test is simple, rapid, reliable and can be done by unskilled person in rural setup.

Culture is another method of diagnosis of bacterial vaginosis. But by culture only Gardnerella vaginalis can be isolated because others are anaerobic and difficult to culture. Moreover, it is time consuming and need skill manpower and high grade laboratory facility. A comparative study has been done among Nugent criteria, Amsel clinical criteria, culture and BVBlue test to see the usefulness of BVBlue test as a screening test of BV.

Methods
The study was carried out in the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh during the period from July, 2004 to June, 2005. A total of 200 women, 50 pregnant and 150 non-pregnant, in the age-group of 15-45 years with history of vaginal discharge and clinically suspected of BV were enrolled for study. Fifty healthy women were taken as control. Three vaginal swab samples were collected from each patient and control and used for diagnosis of bacterial vaginosis as follows:

First sample used for Amsel test and Nugent Gram stain test, second sample used for culture and third sample used for BV Blue test.

1. Amsel test:
First sample was collected from the posterior fornix of vagina and used for assessment of consistency of vaginal discharge, wet film preparation, Amine test and Gram stain. pH was determined by placing litmus paper against the lateral vaginal wall.

1a. Amine test: 10% KOH was added to vaginal secretion. Smelling of Amine odour indicated the test as positive.

1b. Clue cells: An epithelial cell studded with Gram variable coccobacilli was detected by microscopic examination of Gram stained smear and wet film.

Positive Amsel test was assessed by presence of any three of four criteria, viz thin homogeneous abnormal vaginal discharge, pH >4, positive Amine test and presence of clue cells in wet film preparation.

2. Nugent scoring system:
Gram stained slide was assessed by using the Nugent scoring system as follows:

Morphotype were counted as the average number of bacteria in 20 oil immersion field.

\[
\begin{align*}
0 & = \text{no morphotype present} \\
1+ & = <1 \text{ morphotype present} \\
2+ & = 1 \text{ to } 4 \text{ morphotype present} \\
3+ & = 5 \text{ to } 30 \text{ morphotype present} \\
4+ & = >30 \text{ morphotype present}
\end{align*}
\]

The amount of each morphotype detected on the smear was graded and then allocated a score as shown in Table I. Scores of three columns were added and interpreted as follows:

- A slide with a total score of 7 was interpreted as “BV”.
- A slide with a total score of 4 to 6 was interpreted as “intermediate flora”.
- A slide with a total score of 0 to 3 was interpreted as “normal flora” (non-BV).

3. Culture:
Second sample was collected from posterior fornix and used for the culture of \textit{G.vaginalis}. Method of Totten et al was followed. Briefly the method was: Human blood bilayer Tween agar medium was inoculated and incubated at 37 °C in 5% CO₂ and increased humidity for 48 to 72 hours and the reading was taken by oblique lighting after 24, 48 and 72 hours. Suspected colonies were selected and identified by Gram stain and other biochemical tests.

4. BV Blue test (Gryphus Diagnostics, L.L.C.):
Third sample was collected from lower third of the vaginal wall, immersed into the tube containing IBX 4041 (chromogenic substrate), and incubated at 37 °C for 10 minutes then 1-2 drops of BV Blue developer solution (NaOH solution 40 mg/ml) was added. Development of intense blue color within 3 minutes indicated the test as positive.

Result
Incidence of BV in different age groups has been shown in Table 1.
Table 1: The amount of each morphotype detected on the smear was graded and then allocated a score as below.

<table>
<thead>
<tr>
<th>Score</th>
<th>Lactobacillus Morphotype</th>
<th>Gardnerella and Bacteroides morphotype</th>
<th>Curved Gram-variable rods</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3+</td>
<td>1+</td>
<td>1+ or 2+</td>
</tr>
<tr>
<td>2</td>
<td>2+</td>
<td>2+</td>
<td>3+ or 4+</td>
</tr>
<tr>
<td>3</td>
<td>1+</td>
<td>3+</td>
<td>4+</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

Incidence of BV among study population by different test method has been shown in Table 2. Result is also almost same among pregnant and non-pregnant group. It is to be noted that 2 % of healthy control were positive by BV Blue test method (Table 2).

Table 2: Incidence of BV among study population diagnosed by different tests (N=200)

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Amsel Clinical Criteria</th>
<th>Nugent Gram Stain Method</th>
<th>Culture of G.vaginalis Method</th>
<th>BV Blue test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>32</td>
<td>33</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>(n=150)</td>
<td>(21.3)</td>
<td>(22.0)</td>
<td>(21.3)</td>
<td>(22.6)</td>
</tr>
<tr>
<td>Pregnant</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>(n=50)</td>
<td>(18.0)</td>
<td>(18)</td>
<td>(20.0)</td>
<td>(22)</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>42</td>
<td>42</td>
<td>44</td>
</tr>
<tr>
<td>(n=200)</td>
<td>(20.5)</td>
<td>(21.0)</td>
<td>(21.0)</td>
<td>(22.0)</td>
</tr>
<tr>
<td>Healthy control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>01</td>
</tr>
<tr>
<td>(n=50)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(2.0)</td>
</tr>
</tbody>
</table>

A comparison has been shown among the results of different conventional tests and BV Blue test in Table 3. Among 44 positive BV cases 39 (88.6 %) were positive by all methods. All the cases were also positive by BV Blue test but 6.8 % and 4.6% cases were negative by Amsel criteria and Nugent criteria respectively (Table 3).

Table 3: Result of standard conventional test and BV Blue test on BV cases (n=43)

<table>
<thead>
<tr>
<th>BV positive by Amsel criteria (n=41)</th>
<th>BV positive by Nugent criteria (n=42)</th>
<th>BV positive by BV Blue test (n=44)</th>
<th>Total Cases (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>39 (88.6)</td>
</tr>
<tr>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>03 (6.8)</td>
</tr>
<tr>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>02 (4.6)</td>
</tr>
</tbody>
</table>

Incidence of BV in different age groups shows that, majority (45.5 %) of the cases were within the age group of 26-35 years followed by age group 15-25 years (41.0 %). It is to be noted that the incidence of BV was found to be increased with the increase of age (Table 4).

Table 4: Incidence of BV in different age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of Study cases</th>
<th>Amsel criteria</th>
<th>Nugent criteria</th>
<th>BV Blue test</th>
<th>G.vaginalis Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-25</td>
<td>82</td>
<td>(41.0)</td>
<td>(20.7)</td>
<td>(14.6)</td>
<td>(14.6)</td>
</tr>
<tr>
<td>26-35</td>
<td>91</td>
<td>(45.5)</td>
<td>(22.0)</td>
<td>(25.3)</td>
<td>(26.4)</td>
</tr>
<tr>
<td>36-45</td>
<td>27</td>
<td>(13.5)</td>
<td>(14.8)</td>
<td>(25.9)</td>
<td>(29.6)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>(20.5)</td>
<td>(21.0)</td>
<td>(22.0)</td>
<td>(21)</td>
</tr>
</tbody>
</table>

Discussion

Abnormal vaginal discharge is a common problem in the women of reproductive age. Most common cause of such abnormal discharge is bacterial vaginosis (BV) which is caused by mixed flora including G. vaginalis, mobiluncus, prevotella, bacteroides and mycoplasma spp. These organisms are sialidase producers and characteristically replace lactobacillus which is the predominant normal flora of vagina.1, 2 In this study a newer test, BVBlue test, has been tested for diagnosis of BV and its efficacy has been compared with Nugent Gram stain (Gold standard), Amsel clinical criteria and culture of G. vaginalis.

In our study, out of 200 suspected cases of BV, 44 (22 %) were positive for bacterial vaginosis by BV blue test. This correlates with findings of Myziuk et al 7 (21 %), Rao et al 6 (20.5 %) and Madhivanan et al 10 (19.1 %). However, Davis et al found 38% of bacterial vaginosis cases in their studies.11 In present study, detection rate of BV by Amsel clinical criteria, Nugent Gram stain, culture of G. vaginalis and BV blue test was 21.3 %, 22 %, 21.3 % and 22 % respectively. However, the difference is not statistically significant (p > 0.5). It correlates with findings of Rao et al 6 where BV was positive in 20.5 % and 17.42 % cases in Nugent gram stain and culture method respectively. However, findings of present study is in contrast to that of Myziuk et al7, who found 91.7% and 50 % cases positive by BV blue test and Amsel criteria respectively.

When standard conventional tests (Amsel criteria and Nugent criteria) are compared with BV blue tests, it is evident that 39 (88.6%) cases were positive by all tests and all these BV cases were also positive by BV Blue test (100%). When compared with culture method, all the 32 culture positive BV cases were also positive (100%) by BV Blue test. So
sensitivity of BV Blue test is 100%. However BV Blue test was positive in 2% healthy controls. So its specificity was 98%. This correlates with the study of Myziuk et al\textsuperscript{7} who found BV Blue test as 91.7% sensitive and 97.8% specific.

Majority of the patients were within the age group of 26-35 yrs (45.5%) followed by 15 to 25 yrs (41%). The difference between age group 26 to 35 and 15 to 25 were not significant (p>0.05) but difference of age groups of 36 to 45 with other groups is statistically significant (p<0.05).

When of result of BV blue test and Nugent criteria are considered, it is evident that the cases of BV increase with the increase of age. This finding correlates with that of Madhivanan et al,\textsuperscript{10} Moristal et al,\textsuperscript{13} and Jones et al,\textsuperscript{14}. However Bukshi et al,\textsuperscript{12} reported that BV is more common in younger women.

Amsel clinical criteria, Nugent scoring system and culture used for diagnosis of bacterial vaginosis is time consuming, need skilled person and high grade laboratory facility. In contrast BVBlue test is simple and rapid diagnostic test. It can be done by unskilled person in bed side and rural set up. It is rapid, technically simple and is suitable for screening large number of patients in short time where laboratory facilities are not developed.

References


