Optimization of Quantitative PCR Assays for Rapid and Sensitive Detection of Gardnerella Vaginalis and Associated Anaerobes in Bacterial Vaginosis

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Abstract : The disadvantages of conventional procedures for diagnosing bacterial vaginosis (BV), such as Nugent scoring and Amsel's criteria, include a lack of accessibility, the need for specialist expertise, and a high degree of subjectivity. The most prevalent gynaecological infection in the world today is still bacterial vaginosis (BV). The purpose of this work is to enhance quantitative polymerase chain reaction (qPCR) tests in order to identify the presence of Gardnerella vaginalis and other anaerobes that are linked with bacterial vaginosis (BV), such as Atopobium vaginae, BVAB2 (a member of the Clostridiales family that has not been cultivated), and Megasphaera phylotypes 1 and 2. The objective is to surmount these obstacles. The test was fine-tuned, primer-probe efficiency was increased, and quantitative cutoffs were established for more accurate identification with the aid of an analysis of vaginal samples. The improved quantitative polymerase chain reaction (qPCR) assays performed better than traditional diagnostic approaches and shown good sensitivity and specificity in diagnosing symptomatic bacterial vaginosis (BV). Two significant advantages of the streamlined method are the reduction of dependency on subjective rating systems and the acceleration of turnaround times. According to the findings, optimised molecular tests have the potential to be a quick, accurate, and clinically useful substitute for the present techniques of bacterial vaginosis diagnosis.

Keywords: Bacterial vaginosis (BV), *Gardnerella vaginalis*, Anaerobes, *Atopobium vaginae*, BVAB2, *Megasphaera*, Quantitative PCR (qPCR)

INTRODUCTION

Worldwide, between 23.2% to 29.1% of reproductive-aged women have bacterial vaginosis (BV), making it the most common vaginal infection. Bacteroide vaginosis (BV) occurs at an alarmingly high rate in certain demographics and geographic regions. Yeast infections and trichomoniasis are not as frequent as bacterial vaginosis, which ranks first among gynaecological diseases in the US. In addition to being an irritating, stinky, and sometimes embarrassing cause of vaginal discharge, bacterial vaginosis (BV) has serious medical consequences [1]. It has many adverse reproductive consequences, including as an increased risk of HIV and other STDs, pelvic inflammatory illness, premature labour, low birth weight babies, and miscarriage. These findings emphasise the need of getting a correct diagnosis as soon as possible.

Microbial Dynamics in BV

In most vaginal environments, Lactobacillus crispatus, gasseri, jensenii, and iners prevail. Lactic acid-producing bacteria maintain vaginal homeostasis, lower pH, and prevent opportunistic infections [2]. BV is caused by an imbalance of Lactobacilli and anaerobic organisms such Gardnerella vaginalis, Atopobium vaginae, Prevotella, Mobiluncus, Megasphaera, and BVAB2. G. vaginalis is crucial to BV formation. It may form a biofilm on vaginal epithelial cells that other anaerobes may colonise. Biofilm formation affects antibiotic resistance, BV recurrence following treatment, and infection persistence. This biofilm's anaerobes synergise, making detection and treatment harder.

Limitations of Conventional Diagnostic Methods

For a long period of time, the Nugent score and Amsel's clinical criteria were the two methods that were used to diagnose BV. An elevated vaginal pH level of more than 4.5, the 1983 technique devised by Amsel, a thin homogenous discharge, a positive "whiff test," and clue cells are all required. Despite its simplicity, subjectivity and clinical skill hamper reproducibility. Nugent scoring instead evaluates Gram-stained vaginal smears by relative quantities of Mobiluncus, Gardnerella/Prevotella, and Lactobacillus [3]. Despite its use in research, Nugent scoring is too time-consuming and requires microscopy skill for point-of-care application.

Subjectivity and observer variability are fundamental drawbacks of both techniques.

- Laboratory homogeneity is lacking.
- Poor for resource-constrained settings.
- Bacterial species number uncertainty.

Due to these limitations, traditional methods may misdiagnose, underdiagnose, or postpone treatment.

Molecular Approaches to BV Diagnosis

Molecular biology advances like qPCR have made BV diagnosis more accurate, fast, and reproducible. Using species-specific microbial DNA detection and quantification, qPCR may distinguish symptomatic from asymptomatic states depending on bacterial burden [4]. Unlike

Nugent scoring, multiple investigations have indicated that A. vaginae and G. vaginalis loading strongly correlate with BV diagnosis. Multiplex qPCR can detect various Megasphaera phylotypes and BVAB2 simultaneously, improving BV-associated organism discovery.

Molecular testing provide several benefits, including great sensitivity for low-abundance microorganisms.

- Health-related taxa specificity.
- Ability to quantify diagnostic load thresholds.
- Reduced subjectivity and observer bias.
- Fast processing improves healthcare choices.

Despite these advantages, standardising thresholds, assay design inconsistencies, and finding the right balance between multiplex detection and assay performance are remain challenges.

Rationale for Optimization of qPCR Assays

Despite its potential, qPCR must be optimised for consistent, clinically effective BV diagnosis. Optimizing includes amplification efficiency, cut-off settings, primer and probe design, and adding numerous target species to multiple platforms without compromising test sensitivity. Practical testing must be inexpensive, fast, and adaptable to varied clinical circumstances [5]. Optimized qPCR assays targeting anaerobes such Atopobium vaginae, BVAB2, Megasphaera species, and Gardnerella vaginalis, a biofilm starter and BV pathogen, show promise for sensitive and fast detection. These diagnostics may enhance diagnostic accuracy, early intervention, and recurrence rates by guiding patients to the best therapies.

OBJECTIVES

1. To assess the diagnostic accuracy of the optimized qPCR assays in relation to traditional techniques like Amsel's criteria and Nugent scoring;
2. To optimize quantitative PCR tests for the quick and accurate detection of Gardnerella vaginalis and important BV-associated anaerobes.

MATERIAL AND METHOD

In India, a group of researchers conducted a cross-sectional clinical assessment, collecting data over the course of eleven months, from March 2023 to January 2024. The research project included females who were between the ages of eighteen and forty-five, who attended the outpatient clinic for gynaecology, and who were suffering symptoms of vaginal discharge.

Inclusion Criteria

Women who are at least eighteen years old and willing to provide their informed permission.

Exclusion Criteria

- Pregnancy or menstruation at the time of sampling.
- Antibiotic or antifungal use within the last 30 days.
- Known immunocompromised status or ongoing treatment for other urogenital infections.

One hundred and twenty females were recruited. All of the participants supplied their informed consent, and the Institutional Ethics Committee granted its official approval.

Clinical Evaluation

The Nugent grading of Gram-stained vaginal smears and Amsel's criteria (≥3 of 4 symptoms) were used to detect bacterial vaginosis (BV).

- Scores 0-3: Normal.
- Scores 4–6: Intermediate.
- Scores 7-10 imply positive BV.

Sample Collection and Storage

Swabs were collected from the vagina using aseptic techniques on two separate occasions. Two distinct tubes were used. One was used for molecular analysis, while the other was used for Gramme staining (Nugent scoring). Prior to the processing step, the swabs for polymerase chain reaction (PCR) were placed in sterile transport medium, and the samples were stored at -20°C.

DNA Extraction

The Qiagen, India QIAamp DNA Mini Kit was used to extract DNA from the sample following the manufacturer's protocol. A NanoDrop spectrophotometer was used in order to verify both the concentration and the purity.

Quantitative PCR Assays

The following organisms were chosen for species-specific qPCR assay optimization:

- *Gardnerella vaginalis*
- *Atopobium vaginae*
- *Megasphaera* phylotypes 1 and 2
- BVAB2

During the experiments conducted on a Bio-Rad CFX96 real-time PCR system, the reactions were carried out using the following cycling conditions:…

- Denaturation at a temperature of 95°C for two minutes
- Repeat forty times, each time alternating between 60 and 95 degrees Celsius for 45 and 10 seconds, respectively.

The concentrations of the primers and probes were in the ranges of 200–500 nanomoles per litre and 100–200 nanomoles per litre, respectively. The threshold for positive outcomes was set at ten gene copies per responder. Plasmids that contained the target sequences in the range of 10^2 to 10^6 copies were employed in order to generate standard curves. Negative controls were included for each and every one of the runs.

Statistical Analysis

This research made use of the Indian-made statistics program IBM SPSS version 26. Quantitative PCR (qPCR) assays that have been recently upgraded were assessed for their diagnostic efficacy by determining their sensitivity, specificity, PPV, and NPV. As a point of comparison, we used Nugent scores and Amsel's criteria. The best bacterial load cut-off values were determined by generating Receiver Operating Characteristic (ROC) curves.

RESULT

Clinical specimen characteristics

A total of 385 vaginal specimens collected from 146 women were analysed, as shown in Table 1. Patients from longitudinal studies were included because bacterial vaginosis (BV) evaluations are necessary in many therapeutic settings, including initial diagnosis, test-of-cure, and short- and long-term follow-ups. Only 23 of the 123 women who fulfilled Amsel's BV criterion were in good health in any other way. Antibiotics were often prescribed to women who tested positive for BV. Out of 103 women who came back for follow-up after the first week of therapy, 97 had fully resolved BV and 6 still had some. During the 40-45 day short-term follow-up, 32 out of the 84 women who were checked again had BV. Collecting 51 specimens weekly for up to five months, 24 women were part of the long-term follow-up. While 744 samples showed normal flora (Nugent scores 0-3) among BV-free women, 41 samples showed intermediate flora (Nugent scores 4-6). Mixed flora was found in 20 samples, and aberrant flora (Nugent scores 7–10) was seen in 151 specimens from BV women. Recent research has shown that people in both the BV and non-BV groups may have intermediate Nugent scores, and that those with intermediate flora are more prone to show a variety of clinical BV symptoms. Creating a diagnostic tool to identify BV in treatment-needing women was the major objective of this investigation. Asymptomatic women with aberrant flora should not be treated, hence Amsel's criteria were used for BV identification.

Table 1. Features Of The Specimens Assessed For The Research

Characteristic	First visit	One week after therapy	40–45 days short-term follow-up	Monthly long-term follow-up	Total
Number of women	146	103	85	24	147a
Number of samples	146	103	85	51	385
Amsel's criteria 0–2, n (%)	23 (16%)	97 (94%)	53 (62%)	41 (80%)	214 (56%)

Amsel's criteria 0–3b, n (%)	16 (70%)	84 (85%)	41 (77%)	33 (80%)	174 (81%)
Nugent score 4–6b, n (%)	7 (30%)	13 (13%)	12 (23%)	8 (20%)	40 (19%)
Nugent score 3–4, n (%)	123 (84%)	6 (5.8%)	32 (38%)	10 (20%)	171 (44%)
Nugent score 4–6c, n (%)	16 (13%)	0 (0%)	4 (13%)	0 (0%)	20 (12%)
Nugent score 7–10c, n (%)	107 (87%)	6 (86%)	28 (88%)	10 (100%)	151 (88%)

Assessment of qPCR tests for the diagnosis of BV.

A battery of qPCR testing identified vaginal-healthy Lactobacillus spp. and BV-linked species. The disease has been most typically associated to G. vaginalis [9]. Cultured vaginal tissues revealed a fussy bacterium associated to BV, A. vaginae [10]. DNA sequencing has revealed several uncultured BV-linked organisms. We focused on BVAB2 and Megasphaera phylotypes since BV-positive patients had a significant risk of BV (116:1). L. crispatus, L. gasseri, and L. jensenii are commensal Lactobacillus species that are lost when BV begins [11], but L. iners is uncommon since it may be present in both healthy and BV patients. L. iners is the most common vaginal species in some persons [12], despite its unclear role in BV. Besides BV-associated microbes, we tested a novel multiplex PCR assay for Lactobacillus spp. on vaginal tissues.

These qPCR tests were performed on DNA samples collected from vaginas that tested positive for BV (n 171) or negative for BV (n 214). By BV-associated organism, the following are the percentages of positive and negative specimens: The following phyla of Megasphaera were found: BVAB2 (80% and 18%), A. vaginae (94% and 43%), G. vaginalis (98% and 57%). In contrast to BV specimens, which only had 1%, 6%, and 9% of these strains of Listeria, non-BV specimens had 41% of these strains. In contrast to 82% of non-BV specimens, 94% of BV specimens included the common bacteria L. iners. Table 2 shows the data classification

process that began with BV presence/absence. Since G. vaginalis, A. vaginae, and BVAB2 were so common in samples that did not have BV, their diagnostic specificity is poor. Megasphaera phylotypes 1 and 2 demonstrated a sensitivity of 81% for BV identification, which is rather low considering its high specificity. When used alone, Lactobacillus spp. are ineffective as BV negative indicators (74% specificity vs. 70% sensitivity). Hence, a BV diagnostic test that is very accurate could not be developed in this research by only looking for organisms without taking their abundance into account. Both BV and non-BV samples included G. vaginalis, A. vaginae, and BVAB2, although the median quantities of these bacteria were much greater in the BV samples. We aimed to find out whether these creatures could consistently signal BV at certain concentration levels. An accurate way to diagnose BV was by measuring the amounts of A. vaginae or G. vaginalis in specimens. We used ROC analysis on the qPCR data (Fig. 1) and found the best cut-off values (Table 3) to optimise the diagnostic accuracy. As shown in Table 2, our method outperformed categorical assessments of these species in terms of specificity. The specificity was enhanced from 42% to 84% and the sensitivity was reduced from 98% to 84% when a threshold of 1.10×10^5 copies per response for G. vaginalis was implemented. Specificity increased from 58% to 91% and sensitivity decreased from 94% to 87% while using the same cut-off for A. vaginae. Using a lower cutoff of 1.00×10^3 copies per response for BVAB2 improved specificity from 82% to 92% and sensitivity from 79% to 80%. After accounting for multiple comparisons, A. vaginae showed a higher ROC AUC than BVAB2 (P = 0.025), as shown in Table 4.

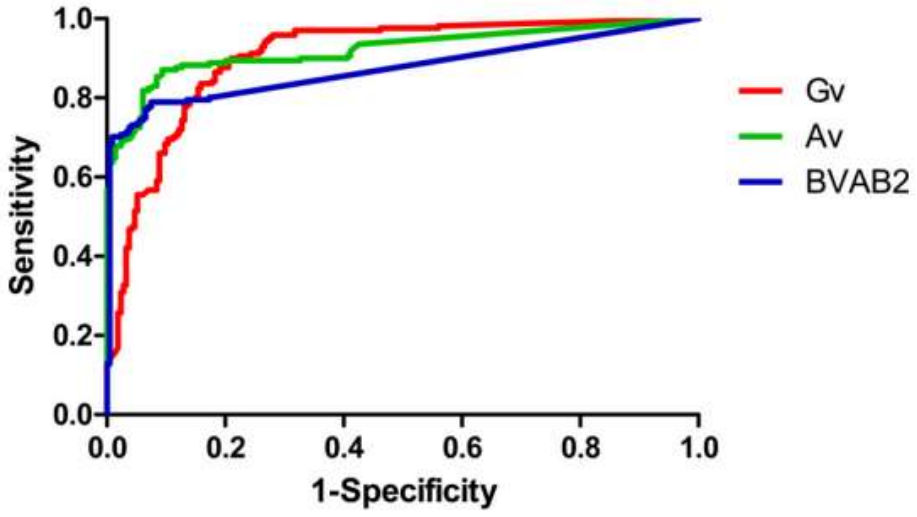

Figure 1. ROC analysis of each BV-associated organism's qPCR results. A. vaginae (Av); G. vaginalis (Gv).

Table 2. Assessment of qPCR tests for BV diagnosis based on organisms detected nonquantitatively

Organism / Marker	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Likelihood Ratio (95% CI)
G. vaginalis	98.3 (95.0–99.6)	42.5 (35.8–49.5)	1.71 (1.52–1.92)
A. vaginae	93.6 (88.8–96.7)	57.5 (50.6–64.2)	2.20 (1.87–2.58)
BVAB2	80.1 (73.3–85.8)	82.2 (76.5–87.1)	4.32 (3.23–5.76)
Megasphaera phylotype 1	68.4 (60.9–75.3)	98.1 (95.3–99.5)	36.6 (13.8–97.2)
Megasphaera phylotype 2	31.0 (24.2–38.5)	100 (98.3–100)	∞
Megasphaera phylotype 1 or 2	80.7 (74.0–86.3)	98.1 (95.3–99.4)	43.2 (16.3–114)
L. iners	94.2 (89.5–97.2)	17.8 (12.9–23.6)	1.14 (1.06–1.23)
Absence of L. crispatus	82.5 (75.9–87.8)	40.7 (34.0–47.6)	2.32 (1.61–3.33)
Absence of L. gasseri	94.2 (89.5–97.2)	14.5 (10.1–19.9)	1.10 (1.03–1.18)
Absence of L. jensenii	90.6 (85.3–94.6)	39.3 (32.7–46.1)	1.49 (1.33–1.68)
Absence of L. crispatus, L. gasseri, L. jensenii	70.1 (63.5–76.1)	73.7 (66.4–80.1)	2.46 (1.97–3.08)

Table 3. Diagnostic efficacy of specific qPCR tests using ROC analysis-derived cutoffs

Model / Organism	AUC (95% CI)	Cutoff	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Likelihood Ratio (95% CI)
G. vaginalis	0.90 (0.87–0.94)	1.10×10^5	83.6 (77.2–88.8)	84.1 (78.5–88.7)	5.26 (3.84–7.21)
A. vaginae	0.92 (0.89–0.95)	1.10×10^5	87.1 (81.2–91.8)	90.7 (85.9–94.2)	9.32 (6.12–14.2)
BVAB2	0.87 (0.84–0.91)	1.00×10^3	78.9 (72.1–84.8)	92.5 (88.1–95.7)	10.6 (6.55–17.0)
G. vaginalis + A. vaginae	0.96 (0.94–0.98)	1.26×10^6	87.1 (81.2–91.6)	93.9 (89.8–96.7)	14.3 (8.44–24.4)
G. vaginalis + BVAB2	0.93 (0.91–0.96)	6.41×10^5	85.4 (79.2–90.3)	90.7 (85.9–94.2)	9.14 (5.99–13.9)
A. vaginae + BVAB2	0.95 (0.93–0.98)	1.53×10^5	92.4 (87.4–95.9)	90.2 (85.4–93.8)	9.42 (6.26–14.2)
G. vaginalis + A. vaginae + BVAB2	0.96 (0.94–0.98)	1.17×10^6	91.2 (85.9–95.0)	93.0 (88.7–96.0)	13.0 (7.97–21.2)
BIC model	0.98 (0.97–0.99)	0.20	91.8 (86.6–95.5)	95.3 (91.6–97.7)	19.7 (10.7–36.1)

Next, we calculated the combined titers of G. vaginalis and A. vaginae using the BVAB2 assay. When compared to the ROC AUC (Table 3), the ROC AUC for combinations of these animals was higher (Fig. 2). Although G. vaginalis and A. vaginae had a greater sensitivity (87%), they improved specificity to 94% from 84% to 87%. But the combination was less specific than BVAB2 alone (91% vs. 93%) and only slightly more sensitive than either organism alone (84% to 85%). When combined, A. vaginae and BVAB2 increased sensitivity to 92% (from 79% to 87% when used alone) but decreasing specificity to 90% (from 91% to 93% when used alone) [14, 15]. The sensitivity and specificity of the three species were 91% and 93%, respectively, which was second only to G. vaginalis and A. vaginae, which achieved 94%. Table 4 shows that, with the exception of the coupling of G. vaginalis and BVAB2, no organism combination increased the AUC in the ROC analysis compared to the performance of any species alone. We tested the efficacy of different combinations of the three species. Compared to BVAB2 ($P = 0.001$) or G. vaginalis and A. vaginae ($P = 0.039$), the trio worked better ($P = 0.039$) [16]. We did not quantify Megasphaera phylotypes 1 and 2 because of their low frequency in non-BV specimens, which is 0.0 percent and 1.9%, respectively. The ROC analysis of both individual and composite species showed that non-BV specimens had a low occurrence of Lactobacillus spp. (74%), which resulted in extremely low AUC values (0.46) (Table 2). Based on these findings, it seems that BV diagnosis might be improved by combining data from many species.

Developing and using a logistic regression model to diagnose BV

Logistic regression described the complicated relationships between several variables and a categorical outcome (BV existence or absence). Selecting model variables was the initial step. More variables increase the likelihood of the model, but overfitting—when the model reflects data set error rather than true connections between variables and outcomes—increases [17]. The Bayesian information criterion (BIC) was used to assess models containing every conceivable combination of variables. It is a maximum-likelihood metric that penalises model complexity, meaning the incorporation of more variables. A lower BIC score is preferable when there is less unexplained variance and more variables [18]. All 512 possible permutations of the variables were examined using this approach, as seen in Figure 3. Part of the best model for prediction were Megasphaera phylotypes 1 and 2, as well as A. vaginae.

Megasphaera phylotypes 1 and 2 (1.00), A. vaginae, G. vaginalis (0.88), L. iners (0.38), L. jensenii (0.21), BVAB2 (0.09), L. crispatus (0.06), and L. gasseri (0.05) were the top contributors to the model. Using k-fold cross-validation (CV), a method that divides the dataset into k subsamples, variable selection was further optimised. One subsample is utilised for testing, while the other k-1 subsamples train the model. In phylogenetic investigations, a 10-fold CV is often used. According to the data that was not provided, this method yielded the best logistic regression model. The Bayesian Information Criterion (BIC) model was shown to have superior performance by ROC analysis (Fig. 2). Table 3 shows that the BIC-selected model has a better specificity (95%) and comparable sensitivity (92%), in comparison to models based on simple additive organism titers. In addition to a high positive predictive value (PPV) of 94% and a negative predictive value (NPV) of 94%, Table 4 shows that this model also provides a high level of diagnostic accuracy (94%). The model identified 214 specimens as non-BV and 157 specimens as BV out of 385 total specimens. Therefore, a more accurate PCR-based BV diagnosis was made possible by logistic regression modelling.

Table 4. The DeLong test's P values indicate how significant the difference between the ROC curves' AUCs.

Comparison	G. vaginalis	A. vaginae	BVAB2	G. vaginalis + A. vaginae	G. vaginalis + BVAB2	A. vaginae + BVAB2	G. vaginalis + A. vaginae + BVAB2	BIC model
G. vaginalis + A. vaginae	0.001	0.014	—	0.001	—	—	—	—
G. vaginalis + BVAB2	0.001	NS	0.0017	0.0012	—	—	—	—

A. vaginae + BVAB2	0.0032	0.0015	0.001	NS	NS	—	—	—
G. vaginalis + A. vaginae + BVAB2	0.001	0.0046	0.001	NS	0.001	NS	—	—
BIC model	0.001	0.001	0.001	0.0012	0.001	0.0028	0.0048	—

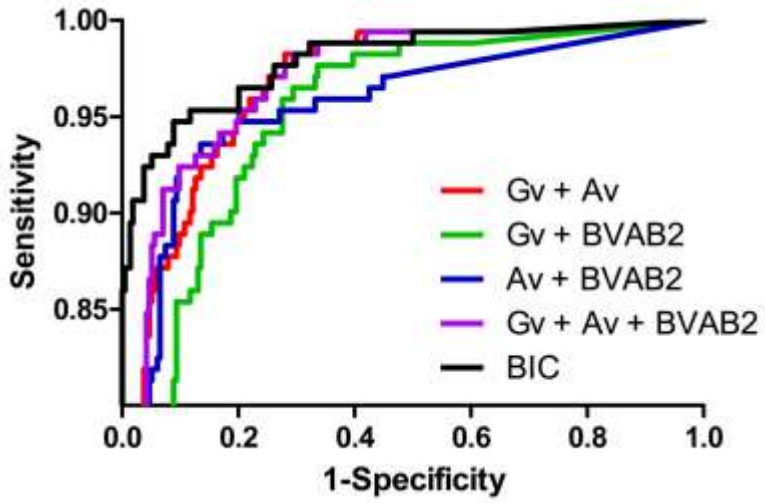

Figure 2. BIC logistic regression and ROC analysis of quantitative polymerase chain reaction for bacterial vaginosis organisms (Av, Gv)

It should be mentioned that the findings could have been impacted by the fact that the majority of research participants were sampled many times. During the first three visits of each patient, we used the BIC-selected model to evaluate the accuracy of individual specimens (Table 5). The model's 91% sensitivity and specificity across all time points showed that it was reliable in various clinical situations, and the results were consistent between visits. With a sensitivity of 91% and specificity of 96%, the model was able to identify 84% of women who tested positive for BV on the initial visit. At the second appointment, sensitivity was 100% and

specificity was 96%; 94% of the women tested negative. Sensitivity and specificity were 91% at the third visit, when 38% of women tested positive for BV. According to these results, the effect of repeated sampling on model performance was small. Table 6 shows the results of comparing the accuracy of the BIC-selected model in women with normal or abnormal flora to those with intermediate flora. In the case of women with BV and aberrant flora, the model accurately recognised 144 out of 151 specimens (95%) while in the case of women without BV and normal flora, 171 out of 174 specimens (98%) were properly identified. The model performed worse when applied to specimens collected from women with intermediate flora; it was able to correctly identify 65% of BV-positive samples and 83% of BV-negative samples.

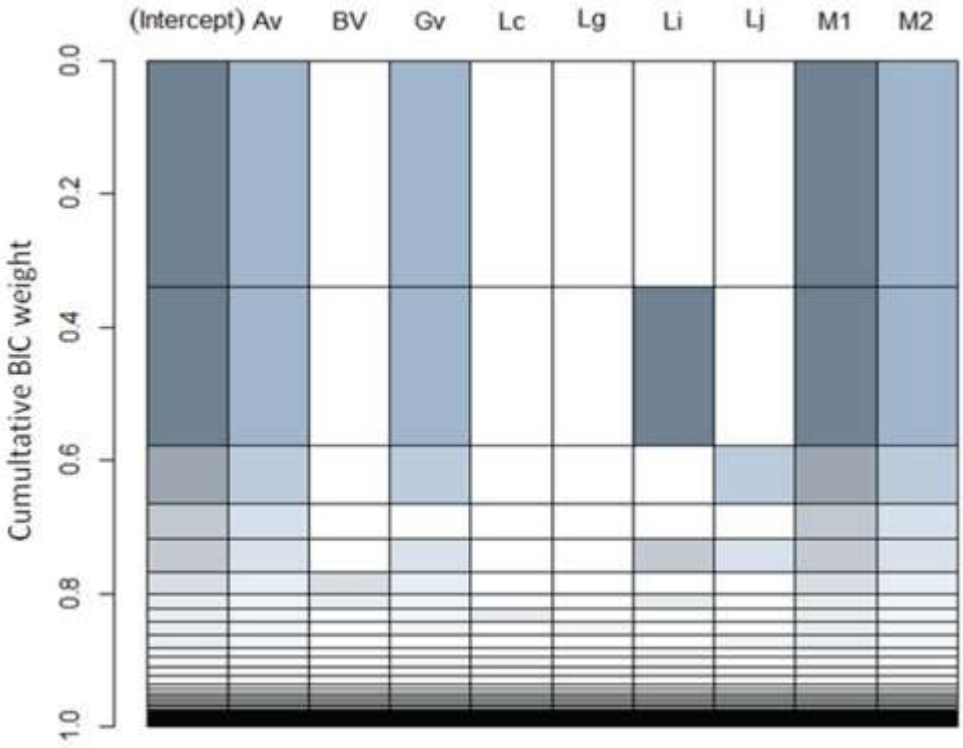

Figure 3. BIC Model Weights and Variable Importance for BV-Associated Organisms"

Table 5. Model performance as determined by BIC on specimens during site visits

Subgroup / Evaluation	Initial visit	Post-treatment (1 week)	Short-term follow-up (40–45 Days)	Long-term follow-up (monthly)	Total
No. of women	146	103	85	24	148
No. of samples	146	103	85	51	385
Amsel's criteria 0–2 (non-BV), n	23	97	53	41	214
Correct (%)	22 (96%)	93 (96%)	48 (91%)	41 (100%)	204 (95%)
Incorrect (%)	1 (4.3%)	4 (4.1%)	5 (9.4%)	0 (0%)	10 (4.7%)
Amsel's criteria 3–4 (BV), n	123	6	32	10	171
Correct (%)	112 (91%)	6 (100%)	29 (91%)	10 (100%)	157 (92%)
Incorrect (%)	11 (8.9%)	0 (0%)	3 (9.4%)	0 (0%)	14 (8.2%)
Sensitivity (%) (95% CI)	91 (85–95)	100 (54–100)	91 (75–98)	100 (69–100)	92 (87–95)
Specificity (%) (95% CI)	96 (78–100)	96 (90–99)	91 (79–97)	100 (91–100)	95 (92–98)

PPV (%) (95% CI)	99 (95–100)	60 (26–88)	85 (69–95)	100 (96–100)	94 (89–97)
NPV (%) (95% CI)	67 (48–82)	100 (96–100)	94 (84–99)	100 (91–100)	94 (89–96)

Table 6. The accuracy of the BIC model in detecting BV in specimens categorised using the Nugent score

Specimen Group	Nugent Score	BIC Model Accuracy (%) for BV vs Non-BV
Non-BV (Amsel's 0–2)	Normal (0–3)	98
Non-BV (Amsel's 0–2)	Intermediate (4–6)	83
BV (Amsel's 3–4)	Intermediate (4–6)	65
BV (Amsel's 3–4)	Abnormal (7–10)	95

CONCLUSION

The development and optimisation of a quantitative polymerase chain reaction (qPCR) test were the goals of this study in order to rapidly detect Gardnerella vaginalis and other significant anaerobes linked to bacterial vaginosis in Indian women. We were able to achieve good sensitivity and specificity by the use of quantitative analysis using ROC-guided cutoffs by including specimens with intermediate Nugent scores, which are often removed in previous studies. The best set of species to include in the panel were BVAB2, G. vaginalis, A. vaginae, and Megasphaera phylotypes 1 and 2, as determined using logistic regression with Bayesian

Information Criterion (BIC) and cross-validation. The diagnostic accuracy of this panel was greater than that of individual targets or mixtures of Lactobacillus species. Clinical diagnosis, patient treatment, and research in the Indian population might greatly benefit from this technique because of its reliability in classifying bacterial vaginosis (BV) cases, whether they are symptomatic or intermediate ones.

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