ORIGINAL ARTICLE

Comparison of the BD Max™ vaginal panel, against standard methods, for the detection of common vaginitis conditions

Julie A Creighton and Bethany J Mills

ABSTRACT

Objectives: Bacterial vaginosis, vulvovaginal candidiasis and *Trichomonas vaginalis* infections are common causes of vaginitis and vaginal discharge conditions that frequently affect women, particularly during reproductive years. The aim of this study was to compare the performance of the BD MAX™ Vaginal Panel (MAX VP), a qualitative molecular test against routine laboratory procedures for the detection of these conditions. The primary focus was for the detection of bacterial vaginosis as Gram stain assessment is a particularly challenging area for microbiology laboratories, due to subjective interpretation.

Methods: Vaginal swabs, collected in Amies transport medium from 96 females, between November to December 2022, were included in the study. Routine methods including Gram stain and culture were performed before the swabs were transferred into AlinityM UVE Sample Buffer Tubes for PCR analysis with the BD MAX™ Vaginal Panel, using the BD MAX system.

Results: The performance of BD MAX™ Vaginal Panel was assessed in comparison to routine diagnostic tests. Overall, BD MAX™ Vaginal Panel performed with high sensitivity and specificity. For the diagnosis of bacterial vaginosis (BV), BD Max™ Vaginal Panel achieved a sensitivity of 94.1% and specificity of 95.2%; for the diagnosis of candidiasis, 96.0% and 97.2% respectively; and for diagnosis of *T. vaginalis* a sensitivity of 100% and specificity could not be calculated.

Conclusion: The results of our study demonstrated that the BD MAX™ Vaginal Panel provided high sensitivity and specificity for the diagnosis BV, candidiasis and *T. vaginalis* and correlated well with Gram stain interpretation. The overall results of our study are in line with other evaluations of BD MAX™ Vaginal Panel. We suggest that BD MAX™ Vaginal Panel would be a useful adjunct to Gram stain testing when the Gram is inconclusive or indeterminate.

Keywords: Bacterial vaginosis, Vulvovaginal candidiasis, *Trichomonas vaginalis,* molecular test, BD MAX™ Vaginal Panel.

NZ J Med Lab Sci 2023; 77(3): 124-127

INTRODUCTION

Vaginal swab analysis, commonly including culture and Gram stain, represents one of the high-volume sample processes in a clinical microbiology laboratory. The laboratory receives samples from both symptomatic and asymptomatic women. Vaginitis and vaginal discharge conditions are frequent complaints among women, especially during the reproductive years, and are most commonly caused by bacterial vaginosis (BV), vulvovaginal candidiasis, and *Trichomonas vaginalis* infections (1,2). Sexually transmitted infectious agents such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* can also be responsible for abnormal vaginal discharge, as can non-infectious syndromes such as cytolytic vaginosis, atrophic vaginosis and desquamative vaginitis. In addition to vaginal discharge investigations, the laboratory receives vaginal swabs for antenatal screening, rupture of membranes, post-partum infections, and sexual-abuse or toxic shock investigations; all adding to the complex array of clinical settings.

Normal vaginal flora during the reproductive years is characterised by a predominance of aerobic lactobacilli, including *Lactobacillus crispatus, L. jensenii* and *L. gasseri,* which helps to confer a protective environment against the development of BV partly due to the production of lactic acid and hydrogen peroxide. In contrast, symptomatic BV is associated with a reduction or absence of lactobacilli with a corresponding increase of a diverse group of facultative and anaerobic bacteria, most frequently including *Gardnerella vaginalis, Atopobium vaginae*, BV-associated bacterium-2 (BVAB-2), *Prevotella species* and *Megasphaera-1* (2,3). *G. vaginalis* is the precursor for the formation of a polymicrobial biofilm, which adheres to the vaginal epithelial cells, and may contribute to therapy resilience and BV recurrence (2).

Diagnosis of vaginitis consists of a physical examination by a clinician, followed by laboratory analysis of a vaginal swab. Bacterial vaginosis has the highest prevalence in vaginitis, ranging from 6% - 60%, depending on the population studied (3,4). BV can be asymptomatic in many women, perhaps reflecting day-to-day variations in vaginal flora during a monthly hormonal cycle, rather than a true dysbiosis (3). Gram stain testing of vaginal smears, is low cost and only requires a microscope, has become the standard for diagnosing BV (2). However, Gram stain interpretation can be subjective, even with an experienced operator, and the correct distinction of bacterial morphotypes may be difficult or impossible by microscopy. In addition, swabs collected after a physical examination may contain gel lubricant residue which can result in very poor-staining smears, often with insufficient material on the slide, making interpretation challenging. Difficult Gram stains can also be time consuming, especially if they require review by one or more senior scientist.

The diagnosis of a *Candida* infection consists of Gram stain and culture for yeasts on selective media such as chromogenic *Candida* agar or Sabouraud-Dextrose Agar. Gram stain analysis for yeast cells and pseudohyphae can be insensitive and yeast cells can be difficult to visualise if they are over decolourised. The presence of yeast, either found in a Gram stain or from culture, does not always differentiate candidiasis from colonisation, as asymptomatic vaginal colonisation by *Candida* species can be found in 20% of women (1).

T. vaginalis is a sexually transmitted protozoan parasite, which usually elicits an offensive smelling purulent vaginal discharge; although it can be asymptomatic in some women. Laboratory diagnosis of *T. vaginalis* can range from an insensitive, but low-cost, wet-mount microscopy analysis, to more sensitive but progressively more expensive assays such as immunochromatographic rapid antigen strips or nucleic acid amplification methods.

As an alternative to Gram stain or wet-mount, molecular analysis of the vaginal microbiome is non-subjective and rapid, potentially enabling an improved service for the clinicians. The BD MAX™ Vaginal Panel (MAX VP) is an automated qualitative multiplex molecular test for the direct detection of BV, *Candida* species (including *C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Pichia kudriavzevii* (formally known as *C. krusei*), *C. glabrata* and *T. vaginalis*, from vaginal swabs, performed using the BD MAX System.

The aim of this study was to compare the performance of the BD MAX™ Vaginal Panel against routine laboratory procedures for the detection of BV, *Candida* spp., and *T. vaginalis*. The primary focus was for the direct detection of BV-associated bacteria, as Gram stain assessment is a particularly challenging area for microbiology laboratories.

METHODS

Study design and setting

This comparative study was conducted at Canterbury Health Laboratories (CHL) to compare the performance of the BD MAX™ Vaginal Panel for the direct detection of BV, and to

compare the *Candida* spp., yeast culture, and AlinityM molecular detection of *Trichomonas vaginalis*.

Sample population and specimen selection

The study included vaginal swabs collected in Amies Transport medium and AlinityM multi-collect tubes from patients attending hospitals, sexual health clinics, and after-hours clinics in the Canterbury area. From a pool of specimens received between November to December 2022, 96 vaginal swabs in Amies Transport medium were selected for inclusion in the study. The majority of specimens were randomly selected, while approximately 20% were retrospectively included based on a Gram stain result inconclusive for BV during routine testing.

BD MAXTM Vaginal Panel and comparator methods

The BD MAX™ Vaginal Panel (MAX VP) is a qualitative diagnostic test that uses real-time PCR and fluorogenic target-specific hybridization probes. This enables the detection and differentiation of DNA from bacteria associated with BV (*Gardnerella vaginalis, Atopobium vaginae*, BV-associated bacterium-2 (BVAB-2) and *Megasphaera-1*), *Candida* spp., and *T. vaginalis*. Testing of the vaginal swabs in Amies Transport medium with the BD MAX Vaginal Panel was performed following completion of routine laboratory procedures, including a Gram stain and culture on Sabouraud-Dextrose Agar. At CHL, a modified qualitative version of the Ison-Hay's method is used for Gram stain diagnosis of BV (5). A selective protocol is enforced for testing of *T. vaginalis,* with diagnosis made by nucleic acid amplification testing (NAAT) of AlinityM multi-collect swabs, therefore only 27/96 samples were available for the comparison of *T. vaginalis* detection, which limited our ability to fully evaluate the performance of MAX VP. As vaginal swabs could not be inoculated into BD MAX UVE Sample Buffer Tubes at the time of collection, the vaginal swabs were transferred from Amies Transport medium and snapped off into BD MAX UVE Sample Buffer Tubes, as recommended by BD MAX support staff. The tubes were vortexed and processed within 2 hours or refrigerated overnight and vortexed again. Testing of samples was then performed on the BD MAX System, in line with the instructions provided in the MAX VP package insert.

Interpretation of results and statistical methods

The performance of the MAX VP was determined based on concordance with results obtained by Gram staining analysis, yeast culture, and AlinityM *T. vaginalis* NAAT. For the purpose of the study and statistical analysis, a true BV was defined by the presence of classic or indeterminate/intermediate BV defining features in a Gram stain, using Ison-Hay's criteria. In the absence of features typical of normal vaginal flora or BV, such as when mixed skin flora or no organisms were seen, cases were classed as BV negative. In some instances, re-evaluation of a Gram stain was triggered by discordance between the reported Gram and MAX VP. In these cases, classification as a true BV was determined using the above criteria under the guidance of an experienced Senior Scientist. Where the initial recorded Gram stain differed from the senior scientist's subsequent Gram decision, a false positive or negative Gram stain result was recorded for statistical analysis. A true *Candida* positive result was defined by the isolation of yeast from culture, and true positive *T. vaginalis* by a positive AlinityM NAAT result. It is of note that routine testing for *T. vaginalis* is only performed on high-risk patients or on request and so there was limited data on the true *T. vaginalis* rates of the 96 samples collected. Using these comparative methods to define true positives and negatives, the data was extrapolated to calculate the sensitivity, specificity, positive predictive value, and negative predictive values for both the BD MAX™ Vaginal Panel and Gram stain analysis.

RESULTS

A total of 96 vaginal swabs were included in this study for the detection of BV and yeast, with a subset of 27/96 samples available for the comparison of *T. vaginalis* detection. Results are shown in Table 1. MAX VP was negative for BV, *Candida* spp. and *T. vaginalis* in 41 samples; Gram stain was negative for BV and/or yeast in 53 samples; and yeast culture was negative in 73 samples.

Overall the MAX VP performed with high sensitivity and specificity compared to routine methods. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are shown in Table 2.

There were 34 (35.4%) patients determined to be a true positive for BV, based on final Gram stain review and consensus. MAX VP was positive in 35 samples, which included 3 false positive results and 2 false negative results, achieving a sensitivity of 94.1% and specificity of 95.2%. Initial Gram stain evaluation found 31 samples with BV-associated flora, one of which was determined to be a false positive and there were 4 false negative results. The respective sensitivity and specificity of Gram stain was 88.2% and 98.4%.

Regarding *Candida* infection, there were 25/96 (26%) patients determined to be true positives, based on a combination of culture and molecular results. Of note were two samples which were positive by MAX VP, but negative by both Gram stain and culture, but the patients had recurrent *Candida* infections and were on fluconazole treatment at the time of testing. The samples were regarded as true positives. MAX VP was positive for 26 samples, which included 2 false positive results and 1 false negative result, achieving a sensitivity of 96.0% and specificity of 97.2%. Conversely, the sensitivity of Gram stain for the detection of yeast was only 56.0%, with a specificity of 100%.

With respect to the detection of *T. vaginalis*, all 96 samples were tested using MAX VP; however, an evaluation of the performance of MAX VP, compared to AlinityM PCR, was limited to 27 samples from a subgroup of high-risk patients. Five samples returned a positive MAX VP result for the detection of *T. vaginalis*, compared with only 2 positive samples on the AlinityM. One case where MAX VP was positive, but AlinityM negative, immediately preceded a true positive sample and was presumed to be contaminated during processing. The AlinityM results on the two remaining samples were not able to be accessed due to selective testing for *T. vaginalis*. Hence, the sensitivity of MAX VP was 100%, but specificity and PPV cannot be determined.

Incidence of co-infection for both BV and yeast was 8.3% (8/96). Two patients who tested positive for TV also tested positive for BV by MAX VP and Gram stain, possibly reflecting altered vaginal flora due to the presence of *T. vaginalis*. No patients had a dual infection with yeast and *T. vaginalis*.

DISCUSSION

Analysis of female genital samples is commonly performed in a clinical laboratory, with Gram stain interpretation considered the laboratory standard for the diagnosis of BV. However, the subjective and widely accepted challenging nature of Gram stain assessment prompted us to investigate a molecular alternative.

While there is some controversy over what constitutes a true BV disease state, the laboratory needs to alert the clinician to the possibility of BV due to the association with serious outcomes such as preterm birth, spontaneous abortion, or acquisition of sexually transmitted diseases. BV involves complex vaginal flora dynamics, only recently revealed with molecular analysis (3,6). Importantly, many of the microorganisms involved may not be culturable, easily distinguished or even seen in a Gram stain, strengthening the potential for improved diagnosis using molecular analysis.

The results of our study demonstrated that the MAX VP provided high sensitivity and specificity and correlated well with Gram stain interpretation for the diagnosis of BV. The prevalence of BV in this study was 35.4%, which is likely higher than our routine sample baseline due to some retrospective selective sample testing bias based on a difficult-to-classify Gram stain.

Table 1. Results for 96 vaginal samples, showing the number of positives tests for each method.

Result	Max VP	Gram (BV, Yeast)	Culture (Yeast)	Alinitym (T. vaginalis)
BV	41	53	73	25
BV (Indeterminate)	24	21	-	-
BV + Yeast	-	8	-	-
BV + *T. vaginalis*	9	2	2	-
T. vaginalis	2	-	-	-
Yeast	3	-	-	2
BV + Yeast	17	12	21	-

BV = bacterial vaginosis

Table 2. Performance results for BD MAX™ Vaginal Panel compared with routine testing.

	Patients with Bacterial vaginosis							
	POS =34		NEG = 62		**Sensitivity**	**Specificity**	**PPV**	**NPV**
	TP	**FN**	**TN**	**FP**				
BD MAX	32	2	59	3	**94.1%**	**95.2%**	**91.4%**	**96.7%**
GRAM	30	4	61	1	**88.2%**	**98.4%**	**96.8%**	**93.8%**
	Patients with Yeast							
	POS =25		NEG = 71		**Sensitivity**	**Specificity**	**PPV**	**NPV**
	TP	**FN**	**TN**	**FP**				
BD MAX	24	1	69	2	**96.0%**	**97.2%**	**92.3%**	**98.6%**
GRAM	14	11	71	0	**56.0%**	**100%**	**100%**	**86.6%**
	Patients with *T. vaginalis*							
	POS =2		NEG = 94		**Sensitivity**	**Specificity**	**PPV**	**NPV**
	TP	FN	TN	FP				
BD MAX	2	0	91	3	**100%**	**ND**	**ND**	**100%**

BV = bacterial vaginosis, TP = True Positive, FN = False Negative, TN = True Negative, FP = False Positive, PPV = Positive Predictive Value, NPV = Negative Predictive Value, ND = Not Determined.

The prevalence of *Candida* species group in our study was 26%, with the MAX VP achieving superior sensitivity and higher NPV compared to Gram stain. The MAX VP can separately detect and identify *C. glabrata* and *Pichia kudriavzevii (C. krusei)* from other *Candida* spp. While none of our samples tested positive or grew these yeast types, the ability to differentiate *C. glabrata* and *Pichia kudriavzevii* is clinically important due to their inherent fluconazole resistance.

The overall results of our study are in line with other evaluations of MAX VP (1,7,8). A trial by Hillier et al, of 290 vaginal samples from symptomatic women, determined that the detection of BV by Gram stain was similar to MAX VP with frequencies of 36% and 37% respectively (1). For the detection of yeast, they found culture to be more sensitive than MAX VP, resulting in a concordance of 90% for *Candida* spp. Group and 98% for C. glabrata. In Hillier's study, the incidence of *T. vaginalis* was 7%, returning 100% concordance between MAX VP and GeneXpert PCR. The authors concluded that the utilisation of standardised testing, as provided by MAX VP, would improve patient care compared to in-clinic diagnosis of vaginitis.

Schwebke et al, compared the performance of MAX VP against a clinician's assessment and various in-clinic tests for the diagnosis of vaginitis attributed to BV, *Candida* spp. And *T. vaginalis*. In their study of >1,500 symptomatic females, with prevalence rates of BV 58%, *Candida* spp. 32% and *T. vaginalis* 8%, the MAX VP out-performed clinical assessment, especially with multiple-cause vaginitis, achieving high rates of sensitivity and specificity (7).

The Ison-Hay's Gram stain method for BV diagnosis was also used in a United Kingdom study of 196 symptomatic women attending a sexual health service (8). MAX VP was compared to standard tests, revealing a high sensitivity of 94.4% for the detection of BV, but a lower specificity of 79.0%, which the authors attributed to possible vaginal dysbiosis due to a high rate of sexually transmitted diseases in the study group. The sensitivity and specificity of MAX VP for the detection of all *Candida* species was 86.4% and 86.0% respectively. The lower performance level for *Candida* spp. Was considered to be due to asymptomatic colonisation.

Vaginal flora from asymptomatic females may contain BV-associated flora, therefore the true value of molecular technology, particularly for the diagnosis of BV, may be best utilised when the patient is symptomatic. Molecular detection of multiple etiologic-specific organisms, combined with clinical symptoms, could decrease inappropriate treatment and improve patient care (1,2,7).

Limitations of this study include a lack of correlation of laboratory results against clinical diagnosis, as no clinical records were able to be accessed. The Nugent criteria is the recognised gold-standard method for the determination of BV by Gram stain. Our laboratory uses a qualitative Ison-Hay's method, which has been found to have excellent agreement with Nugent criteria; however, there may be some discrepancies in sensitivity and specificity from using this alternative assessment. Other limitations involve those which are recognised as being intrinsic to molecular detection including non-viable organisms may test positive, the diagnosis of BV may not always be linked to a disease state, and the presence of yeast in small numbers may constitute normal flora. Finally, we performed MAX VP testing on swabs which had already been used for routine testing, which

could potentially negatively affect sensitivity. In our opinion, these limitations would have only a minor effect on the overall performance of MAX VP.

CONCLUSION

The BD™ Max Vaginal Panel is a sensitive and specific molecular test for the determination of BV, yeast and *T. vaginalis* from vaginal swabs. The test is easy to perform, can be used with vaginal swabs collected in Aimes transport medium, and is complete in approximately three hours. Clinicians should be aware that there is a potential for overdiagnosis of either BV, when a patient is transiently colonised with bacteria other than *Lactobacillus* species, or candidiasis, when yeast are present as commensal organisms. A Gram stain smear would still have to be examined for uncommon syndromes such as atrophic vaginitis, cytolytic vaginosis and desquamative vaginitis. Clinical laboratories could benefit from using the BD MAX as a reflex test when the Gram stain is inconclusive or indeterminate.

ACKNOWLEDGMENTS

BD Max™ Vaginal Panel kits and UVE Sample Buffer Tubes used in this trial were supplied by BD.

AUTHOR INFORMATION

Julie A Creighton, DipMLT, FNZIMLS, Senior Medical Laboratory Scientist[1] and Clinical Lecturer[2]
Bethany J Mills, BMLSc, Medical Laboratory Scientist1

[1]Department of Microbiology, Canterbury Health Laboratories, Christchurch, New Zealand
[2]University of Otago, Christchurch, New Zealand.

Correspondence: Julie Creighton, Microbiology Laboratory, Canterbury Health Laboratories
email: julie.creighton@cdhb.health.nz

REFERENCES

1. Hillier SL, Austin M, Macio I, et al. Diagnosis and treatment of vaginal discharge syndromes in community practice settings. *Clin Infect Dis* 2021; 72: 1538-1543.
2. Coleman JS, Gaydos CA. Molecular diagnosis of bacterial vaginosis: an update. *J Clin Microbiol* 2018; 56: e00342-18.
3. Diop K, Dufour JC, Levasseur A, Fenollar F. Exhaustive repertoire of human vaginal microbiota. *Hum Microb J* 2019; 11: 100051.
4. Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. *Am J Obstet Gynecol* 2013; 209: 505-523.
5. Ison CA, Hay PE. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. *Sex Transm Infect* 2002; 78: 413-415.
6. Srinivasan S, Morgan MT, Liu C et al. More than meets the eye: associations of vaginal bacteria with gram stain morphotypes using molecular phylogenetic analysis. *PloS One* 2013; 8: e78633.
7. Schwebke JR, Gaydos CA, Nyirjesy P, et al. Diagnostic performance of a molecular test versus clinician assessment of vaginitis. *J Clin Microbiol* 2018; 56: e00252-18.
8. Sherrard J. Evaluation of the BD MAXTM Vaginal Panel for the detection of vaginal infections in a sexual health service in the UK. *Int J STD AIDS* 2019; 30(4): 411-414.