ELEVATING THE STANDARD OF CARE FOR WOMEN'S HEALTH: THE BD MAX™ VAGINAL PANEL AND MANAGEMENT OF VAGINAL INFECTIONS

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SUMMARY

Misdiagnosis, leading to inappropriate treatment of vaginal infections, can prolong symptoms and increase an affected woman's risk of developing serious complications. Clinical procedures and traditional diagnostic techniques are insufficient to accurately diagnose the most common types of vaginal infections. The BD MAX™ Vaginal Panel is a validated in vitro diagnostic (IVD) molecular solution designed to simplify, standardize, and improve the accuracy of vaginal infection diagnosis. Performed on the BD MAX™ System, the panel is an automated assay for the direct detection of DNA targets from bacteria associated with bacterial vaginosis (BV; qualitative results reported based on detection and quantitation of targeted organism markers), *Candida* species associated with vulvovaginal candidiasis (VVC), and *Trichomonas vaginalis* (TV). The assay is indicated for use on vaginal swabs from women who are symptomatic for vaginal infections and uses real-time polymerase chain reaction (PCR) for the amplification of specific DNA targets and fluorogenic target-specific hybridization probes to detect and differentiate DNA from relevant organisms with clinical accuracy, potentially leading to improvements in diagnosis. With improved diagnosis, patients may receive appropriate treatment sooner while avoiding the expense and burden of unnecessary care.

INTRODUCTION

Vaginal infections, including bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis (TV), are among the most common reasons for which women in the United States seek medical care. Approximately 30% of women in the country test positive for BV at any given time, and an estimated 75% of women will have at least one episode of VVC, TV or BV in their lifetimes.[1] *Trichomonas vaginalis* infection is present in more than 11% of women age 40 years or older, with higher prevalence among incarcerated women and women being treated for other sexually transmitted infections (STIs). Together, vaginal infections result in approximately 10 million visits to physician offices annually.[1-4]

UP TO 75% of women experience at least one episode of VVC, TV, or BV in their lifetimes.[1]

Many women who experience symptoms of vaginosis or vaginitis assume they have a simple yeast infection and attempt to self-treat with over-the-counter antifungals. However, 50% to 66% of these women self-diagnose incorrectly, leading to incorrect treatment.[5,6] Up to 40% of patients cannot be diagnosed at the initial office visit of a primary care provider using standard currently available office procedures.[7] This pattern of inaccurate and inconsistent diagnosis, due in part to variations in clinical practice, can result in serious complications. Improper treatment can result in continuation of irritating symptoms that disrupt quality of life. Ineffective treatment of the infection can also increase the risk of serious complications, such as delivery of preterm or low-birth-weight babies, late-term miscarriage, HIV acquisition or transmission, and development of pelvic inflammatory disease (PID).[2,3,5,8,9]

Inaccurate diagnosis may also lead to incorrect treatment and complications that may include unnecessary adverse effects, associated healthcare system costs, and potential spread of antibiotic-resistant pathogens.[7] Poor antifungal stewardship for VVC is especially concerning because *Candida* is listed as a "serious threat" on the list of resistant organisms published by the Centers for Disease Control and Prevention (CDC).[10]

LIMITATIONS OF TRADITIONAL DIAGNOSTIC TECHNIQUES

Clinicians traditionally assess vaginal infections using a combination of symptoms, physical examination results, pH of vaginal fluid, microscopy, and the *whiff test* (for fishy odor from vaginal discharge before or after addition of 10% KOH).[2,11] Specifically, BV is commonly assessed using criteria developed by Richard Amsel, which require 3 of the following 4 signs or symptoms for a positive diagnosis.[12]

- Thin, homogenous vaginal discharge
- Presence of clue cells (vaginal epithelial cells studded with adherent coccobacilli on microscopic examination)
- Vaginal pH >4.5
- Positive whiff test results

Due in part to their subjectivity, these so-called Amsel criteria have significant limitations in terms of sensitivity and specificity when used in routine clinical practice.[2,13,14] For example, in a prospective observational study of 269 women, the sensitivity and specificity of Amsel criteria were 69% and 93%, respectively.[15] In another study by Schwiertz et al, who examined 220 specimens from women with relapsing vaginitis, a clinical diagnosis of BV based on Amsel criteria was incorrect in 61% of cases.[13] Another current standard for laboratory diagnosis of BV is the Nugent score (NS), which is based on a quantitative estimate of the relative concentration of the following bacteria observed in a Gram stain[4,16] :

- *Lactobacillus*
- Small gram-negative and Gram-variable rods (e.g., *G. vaginalis*, *Bacteroides*, *Prevotella*, *Porphyromonas*, *Peptostreptococcus*)
- Curved gram-variable rods (e.g., *Mobiluncus*)

Although NS is considered the criterion standard for diagnosis of BV, its use by clinical laboratories is limited by its complexity and subjectivity. Specifically:

- The test requires a painstaking, non-standardized method, which results in an intermediate (or inconclusive) outcome in approximately 8% to 22% of specimens.
- Gram staining does not permit identification of several bacteria involved in BV, such as *Atopobium vaginae*, which has variable morphologic characteristics in histology preparations.[17]

Women with VVC are commonly diagnosed by microscopic examination of vaginal secretions, when wet-mount results (saline or 10% KOH) or a Gram stain reveal budding yeasts, hyphae, or pseudohyphae. Vaginal cultures for *Candida* are recommended for women with negative result on wet-mount testing but existing signs or symptoms of fungal infection.[2,4,5]

As with traditional diagnostic methods for BV, microscopy for VVC has relatively low sensitivity (61%) and specificity (77%) in clinical practice.[2] For example, in the Schwiertz et al study, a diagnosis of *Candida* infection was incorrect in 77% of cases, including 75% of cases in which microscopy was used for the diagnosis.[13]

LOW SENSITIVITY AND SPECIFICITY microscopy for VVC has relatively low sensitivity (61%) and specificity (77%) in clinical practice.[2]

TV has traditionally been diagnosed microscopically, with sensitivity ranging from 50% to 70% with high specificity (100%).[2] With the availability of FDA-cleared molecular tests, microscopy is no longer recommended.[4]

In summary, clinical procedures and traditional diagnostic techniques are insufficient to diagnose the most common types of vaginal infections. Overall, traditional methods tend to be subjective, lack sensitivity and specificity,[2,15,18] often are highly manual, and require a special skill set. This can result in incorrect diagnosis, increasing the risk that women with vaginal infections will not receive appropriate treatment.[15]

THE BENEFITS OF MOLECULAR METHODS

Increasing evidence indicates that the use of nucleic acid amplification tests (NAATs) for detection of BV, VVC, and TV can improve accuracy and is more objective compared with traditional methods, potentially leading to better patient management.[4,8,18,19]

BV: In an analysis of vaginal specimens from 163 women, Shipitsyna et al found that quantitative determination of the presence of *G. vaginalis, Atopobium vaginae, Eggerthella, Prevotella, BVAB2,* and *Megasphaera* type 1 by nucleic acid sequencing was highly accurate for BV diagnosis.[17] Similarly, depletion of *Lactobacillus* combined with the presence of either *G. vaginalis* or *A. vaginae* at diagnostic levels was also highly predictive of BV. As such, NAAT measurements of the abundance of normal and BV microbiota relative to total bacteria in vaginal fluid may provide a more accurate BV diagnosis, rather than qualitative detection or absolute counts of BV-related microorganisms.[18]

VVC: For VVC detection, studies have found that multiplex PCR methods provide a rapid, simple, and reliable alternative to conventional methods to identify common clinical fungal isolates.[19]

TV: The Centers for Disease Control and Prevention (CDC) have published 2015 STD guidelines to recommend NAAT technology as a highly sensitive and specific, preferred method for TV detection.[4]

BD MAX™ VAGINAL PANEL PERFORMANCE CHARACTERISTICS

The BD MAX™ Vaginal Panel is an in vitro diagnostic (IVD) molecular solution that has the potential to improve diagnosis of vaginitis/vaginosis. With improved diagnosis, patients may receive appropriate treatment sooner, while avoiding the expense and burden of unnecessary care. The panel runs on the BD MAX™ System, which can perform 2 to 24 tests simultaneously. The system offers a proven path to improved laboratory efficiency by combining and automating real-time PCR extraction, amplification, and detection into a single platform capable of running FDA-cleared/-authorized and open-system assays.

The panel is designed for the direct detection of BV, *Candida* species associated with VVC, and TV. It is indicated for use on vaginal swabs from women exhibiting symptoms of vaginal infection and utilizes real-time PCR for the amplification of specific DNA targets and fluorogenic target-specific hybridization probes to detect and differentiate DNA from the organisms listed in **Table 1**.[20]

TABLE 1 Organisms Detected by the BD MAX™ Vaginal Panel[20]

Test Name	Organisms Detected	Reports (Positive or Negative)
Bacterial Vaginosis	• *Lactobacillus* species (*L. crispatus* and *L. jensenii*) • *Gardnerella vaginalis* • *Atopobium vaginae* • *Bacterial vaginosis associated bacteria–2 (BVAB-2)* • *Megasphaera-1*	Bacterial vaginosis

Candidiasis	• *Candida* group
	  (*C. albicans, C. tropicalis,*
	  C. parapsilosis, C. dubliniensis)
	
	• *C. glabrata*
	• *C. krusei*
		Candida group
		
		• *C. glabrata*
		• *C. krusei*

Trichomonas vaginalis	• *T. vaginalis*	*T. vaginalis*

The panel provides an objective positive or negative result for BV based on a quantitative algorithm that determines the ratio of vaginal bacteria (i.e., the assay detects BV as a syndrome, rather than identification based on the presence or absence of specific bacterial species alone). The panel also reports results for *Candida* group species (*C.* group), as well as two separate results for *C. glabrata* and *C. krusei*, and TV.[20] *C. glabrata* and *C. krusei* are detected separately because these strains have intrinsic resistance and knowledge of their presence may influence different treatment, leading to improved antimicrobial stewardship.

The BD clinical evaluation of the vaginal panel assay used a more robust reference method than the current standard of care to maximize the sensitivity of the Reference Method (RM) against which the panel's performance was established. This optimized RM combined best-practice methods and used modified Amsel criteria to diagnose BV when an intermediate NS value was obtained.[20-22] During the course of a preclinical study, Amsel criteria were assessed. Individual components of Amsel criteria other than vaginal discharge were more closely correlated to the NS standard **(Table 2)**.[21] Additionally, the accurate diagnosis of vaginal infections is often difficult because different causative agents can result

in similar clinical symptoms and women may have more than one microorganism infecting simultaneously (a so-called "mixed infection"). Specifically, vaginal itching and discomfort, as well as abnormal vaginal discharge, are examples of symptoms that can be related to *Trichomonas vaginalis, Candida* species associated with vulvovaginal candidiasis (VVC), or bacterial vaginosis (BV).[2,4] Mixed infections can also be common. In the preclinical study, BV was found in 54.1% (269/497) of all the specimens tested. For 37.2% (100/269) of the BV positives, TV and/or *Candida* spp. were also found by the study reference methods.[21] When these mixed infections were present, the accuracy of the individual Amsel criteria and of the clinical diagnosis decreased even further **(Table 2)**.[21] Different Amsel criteria combinations previously described in the literature were assessed. The modified Amsel criteria, in which 2 of 3 criteria (vaginal pH, Whiff test, and clue cells) represented a positive result, showed the best agreement with the NS **(Table 3)**.[22] For the clinical study, the criteria were modified by removing "vaginal discharge" as a criterion to reduce subjectivity (i.e., avoiding inaccurately decreasing overall agreement) of the Amsel criteria when combined with NS. Therefore the modified Amsel criteria was defined such that two of three remaining criteria (vaginal pH, whiff test and clue cells) was interpreted as "positive".

TABLE 2 Amsel Criteria Sensitivity Against NS of 7 to 10 Regarding Absence or Presence of TV and/or *Candida* Species (preclinical data)[21]

Patient Infected Status	% (No.)				
	Conventional Amsel	Clue Cells	Whiff Test	Vaginal pH>4.5	Vaginal Discharge
BV only	84.6% (143)[a]	86.3% (145)[b]	82.7% (139)[b]	91.1% (154)[a]	71.0% (120)[a]
BV mixed with TV and/or *Candida* spp.	60.0% (60)[c]	63.6% (63)[d]	64.0% (64)[c]	75.0% (75)[c]	42.0% (42)[c]

NS, Nugent Score.
[a]*n* = 169
[b]*n* = 168
[c]*n* = 100
[d]*n* = 99

TABLE 3 Amsel Criteria Variations vs Nugent Score (Preclinical Data)[22]

Reference Method	Criteria	Symptoms					Agreement with Nugent Score			
		Original Description	Clue Cells	Thin, Homogenous Abnormal Discharge	pH >4.5	Whiff Test	Any Abnormal Discharge with Odor	PPA %(n)	NPA % (n)	Overall Agreement % (n)
Conventional Amsel	3/4	(1)	X	X	X	X		75.5 (206)	88.4 (152)	80.4 (358)
Amsel Variations	2/3	(2)	X		X		X	80.6 (220)	85.5 (147)	82.5 (367)
		(3)	X		X	X		81.0 (221)	86.0 (148)	82.9 (369)
		(4)	X	X	X			79.5 (217)	80.8 (139)	80.0 (356)
	2/2	(5)			X	X		74.7 (204)	91.3 (157)	81.1 (361)
Individual Criterion	1/1	(6)	X					78.0 (213)	81.4 (140)	79.3 (353)
		(7)			X			85.3 (233)	76.7 (132)	82.0 (365)

(1) Conventional Amsel (=3/4)
(2) Mod. Amsel (abnormal discharge with odor) (=2/3)
(3) Mod. Amsel (no discharge) (=2/3)
(4) Mod. Amsel (no whiff) (=2/3)
(5) Mod. Amsel (Whiff and pH) (=2/2)
(6) Clue cells only (=1/1)
(7) Mod. Amsel (pH only)

BD MAX™ Vaginal Panel assay performance characteristics were evaluated in a clinical study for which specimens were collected from 10 geographically diverse sites. Two additional sites performed BD MAX™ Vaginal Panel and/or reference-method testing. Subjects included women with symptoms of vaginitis or bacterial vaginosis. For each patient, a vaginal swab was collected by the clinician or patient (in a clinical setting) using the BD MAX™ UVE Specimen Collection Kit and tested. Three additional vaginal swabs were collected for reference-method testing, as follows[20]:

- **BV** status was determined using a combination of NS and Amsel criteria, a combined reference method that is more robust than that used in current clinical practice. Results from specimens with normal flora as determined by NS were considered negative. Specimens with BV flora per NS were considered positive, whereas those with intermediate flora were further sub-divided into positive or negative categories using Amsel criteria. Specimens positive for 2 out of the 3 following criteria were considered Amsel positive: vaginal pH >4.5, presence of clue cells, and positive whiff-test result.[20]

- ***Candida*** status was determined by selective (*Candida*) CHROMagar™ chromogenic medium and Sabouraud Dextrose agar, Emmons plate culture followed by PCR amplification and bidirectional sequencing of the ITS-2 gene for *Candida* species identification.[20]

- **TV** status was determined by microscopic visualization of motile trichomonads in saline wet mount of vaginal secretion and by culture using the InPouch™ TV (Biomed Diagnostics, Inc.) culture for *T. vaginalis*. Any positive result was sufficient to consider the specimen reference method positive for *T. vaginalis*.[20]

A total of 1,763 subjects were enrolled in the study. Of those, 1,740 specimens came from eligible subjects. The number of evaluable specimens with the reportable reference method and BD MAX™ Vaginal Panel results were 1,559/1,582 for BV, 1,618/1,628 for *Candida* and 1,600/1,610 for TV for clinician- and self-collected swabs respectively. The performance of the assay is shown in **Table 4**.[20]

TABLE 4 Summary of BD MAX™ Vaginal Panel Performance Characteristics (Clinical Trial Data)[20]

Target	Collection Type	Sensitivity % (No.)	Specificity % (No.)
BV	Clinician Self	90.5% (797) 90.7% (803)	85.8% (582) 84.5% (589)
Candida group	Clinician Self	90.9% (462) 92.2% (470)	94.1% (1045) 91.9% (1028)
C. glabrata	Clinician Self Simulated	75.9% (22)[a,b] 86.7% (26)[c,d] 100% (50)[e]	99.7% (1584) 99.6% (1592) 100% (50)
C. krusei	Clinician Self Simulated	... ... 100% (50)[e]	99.8% (1614) 100% (1628) 100% (50)
TV	Clinician Self	93.1% (121)[f] 93.2% (124)[f]	99.3%(1459)[g] 99.3% (1466)[g]

[a]Out of 7 *C. glabrata* false-negative results, 6 showed chromagar results consistent with low *C. glabrata* load (1+ to 2+ growth level) and 1 showed chromagar result consistent with high *C. glabrata* load (3+ growth level).
[b]The BD MAX™ Vaginal Panel detected BV and/or *Candida* group signals in 6 out of 7 specimens with *C. glabrata* false-negative results.
[c]Out of 4 *C. glabrata* false-negative results, 3 showed chromagar results consistent with low *C. glabrata* load (1+ to 2+ growth level) and 1 showed chromagar result consistent with high *C. glabrata* load (3+ growth level).
[d]The BD MAX™ Vaginal Panel detected BV and/or *Candida* group signals in the 4 specimens with *C. glabrata* false-negative results.
[e]Out of 50 positive panel members, 25 were tested at ≥1 and <2× the level of detection (LoD).
[f]9 false-negative results were recorded. Of those, 7 were found negative with an FDA-cleared molecular method.
[g]11 false-positive results were recorded. Of those, 10 were found positive with an FDA-cleared molecular method.

Nonreportable rates for the panel are shown in **Table 5**. Of all the clinician-collected specimens initially evaluated with the BD MAX™ Vaginal Panel, 3.0% were initially reported to be unresolved. After a valid repeat test, 1.3% remained unresolved. Similarly, 3.7% and 0.8% of specimens were reported as being indeterminate initially and after a valid repeat test, respectively, and 1.4% and 0.2% were reported as being incomplete initially and after a valid repeat test. The total rates of nonreportable results were 8.1% initially and 2.2% after a valid repeat test.[20] Similar rates were obtained with self-collected specimens.

TABLE 5 Nonreportable Rates[20,a]

Rate	% (No.)			
	Clinician-Collected		**Self-Collected**	
	Initial Rate *n*=1734	**Final Rate** *n*=1725	**Initial Rate** *n*=1736	**Final Rate** *n*=1733
Unresolved	3.0% (52)	1.3% (22)	2.9% (50)	0.6% (11)
Indeterminate	3.7% (64)	0.8% (13)	2.7% (47)	0.6% (10)
Incomplete	1.4% (24)	0.2% (3)	1.4% (24)	0.2% (4)
Total	8.1% (140)	2.2% (38)	7.0% (121)	1.4% (25)

[a]The final rate is calculated with valid repeats only.

DIAGNOSTIC OPTIONS TAILORED TO FIT THE NEEDS OF CLINICAL LABORATORIES AND PRACTICES

The BD MAX™ Vaginal Panel and the BD Affirm™ VPIII Microbial Identification Test provide a range of diagnostic options tailored to fit the needs of clinical laboratories and clinician practices. The BD Affirm™ test is a DNA probe test intended for use in the detection and identification of *Candida* spp., *G. vaginalis*, and *T. vaginalis* nucleic acid in vaginal fluid specimens from patients with symptoms of vaginitis/vaginosis.[17] DNA probe technology identifies target organisms in a given specimen based on their genetic fingerprints. Although the BD Affirm™ test does not identify *C. glabrata* or *C. krusei* separately, it detects them as part of a single *Candida* call. It also detects *G. vaginalis* as a marker indicative of BV; it does not provide a result for BV itself.[23] The BD Affirm™ test is well suited for practices that require faster turnaround time and results in less than 1 hour, where the number of patients is smaller and automation needs are moderate.

The BD MAX™ Vaginal Panel is a next-generation, IVD molecular solution that has the potential to improve diagnosis of vaginitis/vaginosis. As a premium performance and fully automated solution for vaginitis/vaginosis, the panel delivers objective, clear positive and negative results, removing the interpretation and subjectivity inherent in traditional diagnostic methods. Objectivity, combined with the high sensitivity and specificity of molecular methods, may provide an opportunity to reduce misdiagnosis rates and help ensure that patients receive appropriate care for their vaginal infections, while improving antimicrobial stewardship. Several key characteristics of the assay that have the potential to lead to improved performance[20]:

- The panel utilizes a BV algorithm that quantifies the ratio of flora specific to BV infections and improves the sensitivity and specificity of diagnosis, compared with methods that look for the presence or absence of specific bacteria.

- The panel provides objective positive or negative results that do not require analysis or visual interpretation, unlike traditional methods such as the NS, which also often reports an inactionable intermediate result.

- The panel has strong sensitivity and specificity for *Candida* spp. versus traditional culture without the long turnaround times typical of growing cultures.

- The panel identifies and reports *C. glabrata* and *C. krusei* separately. Because these species can be resistant to common antifungals (azoles), reporting their results separately can guide patient management and improve antifungal treatment choices.

- The panel uses nucleic acid amplification technology (NAAT) for TV, which is more accurate than traditional methods and is supported by the latest recommendations from the CDC and other guidelines.[4]

- The panel requires a single collection and test, which is simpler and more efficient than a combination of multiple manual tests. This in turn can reduce the burden on clinicians and ensures better diagnostic coverage of patient symptoms.[20]

Improving diagnosis of vaginal infections has multiple benefits. It can lead to better patient management by informing choice of prescription and over-the-counter medications. It can lead to faster infection control and improved quality of life due to symptom resolution. It can also reduce the risk of serious sequelae, such as:

- Preterm birth

- STI transmission and acquisition

- Late-term miscarriage

- PID

For the provider and broader healthcare system, consistent, accurate diagnosis can improve healthcare quality measures, reduce costs spent on ineffective treatment, and improve antimicrobial stewardship.

CONCLUSION

The BD MAX™ Vaginal Panel is an IVD molecular solution that has the potential to improve diagnosis of vaginal infections. The panel runs on the BD MAX™ System, a fully automated platform that combines real-time PCR extraction, amplification, and detection. With improved diagnosis, patients may receive appropriate treatment sooner, while avoiding the expense and burden of unnecessary care. Moreover, the BD MAX™ Vaginal Panel has the potential to improve laboratory efficiency by bringing automation, accuracy, and objectivity to vaginitis/vaginosis diagnostic tools.

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