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ORIGINAL ARTICLE

Accuracy of the BD MAX™ vaginal panel in the diagnosis of infectious vaginitis

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Abstract

The aim of this study was to evaluate the BD MAX™ vaginal panel in the diagnosis of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis by comparing it with conventional methods: (i) combination of Hay criteria and presence of clue cells with predominant growth of *Gardnerella vaginalis*, (ii) yeast culture, and (iii) combination of culture, wet mount microscopic examination, and an alternative molecular assay. One thousand vaginal samples of women ≥ 14 years were analyzed; 5% of the samples belonged to pregnant women. 19.3% were classified as BV, in 33.6% yeasts were recovered and in 1.5% TV was detected. For BV, sensitivity and specificity were of 89.8% and 96.5%, respectively; for VVC, sensitivity and specificity were of 97.4% and 96.8%, respectively, and for *T. vaginalis*, the sensitivity and specificity were of 100%. The BD MAX™ vaginal panel is highly sensitive and specific and simplifies the identification of infectious vaginitis.

Keywords Bacterial vaginosis · *Gardnerella vaginalis* · Vulvovaginal candidiasis · *Trichomonas vaginalis* · Vaginitis · BD MAX vaginal panel

Introduction

Vaginitis is an inflammation of the vagina that can result in abnormal discharge, odor, irritation, itching or burning, and pain. The most common causes of vaginitis are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis. Other non-infectious causes such as skin disorders, estrogen deficiency, or allergic reactions may produce vaginitis but are less frequent [1]. Due to its association with direct and indirect economic costs, sexually transmitted infections (STI), and ascending genital tract infection, vaginitis is currently considered an important public health problem and thus, its accurate diagnosis is crucial for appropriate treatment.

BV, the most common cause of abnormal vaginal discharge among women in reproductive age group, is an ecological imbalance of the vaginal microbiota characterized by a reduction in lactobacilli that are part of the normal flora and its replacement by a mixed flora consisting of facultative and strict anaerobes [2].

The etiology of BV is complex; bacteria associated include *Gardnerella vaginalis*, *Prevotella* species, *Atopobium vaginae*, *Megasphaera* type I, *Sneathia sanguinegens*, and BV-associated bacteria 1–3 type [BVAB-1]. Moreover, certain lactobacilli (*L. crispatus*, *L. jensenii*, *L. gasseri*, and *L. iners*) are important contributors to the maintenance of the normal vaginal flora and are decreased or lost in BV.

Diagnosis of BV remains challenging; clinical diagnosis is based on Amsel et al.'s criteria [3] whereas microbiological diagnosis is based on the Gram stain and culture; however, many of the bacteria related to BV such as *A. vaginae* or *Prevotella* species are not easily grown or are even uncultivable. The grading of the microbial flora observed in the Gram stain smear of vaginal secretions based on the quantification of *Lactobacillus* morphotypes and pathogenic bacteria was first described by Spiegel et al. [4] and later modified by Nugent et al. [5]. A simplification of the score by defining three categories (normal, intermediate, and suggestive of BV depending on the relative amount of *Lactobacillus* morphotypes compared to the *Gardnerella vaginalis* and other bacterial morphotypes) was defined by Ison and Hay [6] and has shown comparable results with the classic scores [7]. Other authors have stated that the presence of clue cells

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(squamous epithelial cells coated with Gram-negative to Gram-variable coccobacilli) is the key pathognomonic feature of BV [8].

VVC is the second most common cause of vaginitis after BV [1]. Risk factors include pregnancy, immunosuppression, and recent antibiotic use. Although *Candida albicans* is the most common cause of VVC (accounts for 85 to 90% of the cases), the involvement of other species such as *C. glabrata*, *C. parapsilosis*, and *C. krusei* is emerging [9]. It should be noted that asymptomatic colonization with *Candida* species appears in 17–33% of women [10]; therefore, the isolation of *Candida* species of vaginal swabs is not necessarily related to symptoms.

Trichomonas vaginalis is a sexually transmitted protozoan parasite that causes vaginitis and cervicitis and is a risk factor for HIV transmission but studies suggest that nearly 85% of women infected can be asymptomatic [11, 12]. Trichomoniasis is the most common curable STI in the world. Although extremely insensitive, microscopic examination of a wet mount preparation of vaginal secretions is the traditional diagnostic method for this parasite and remains widely used. Recent advances in molecular diagnostic test have improved the sensitivity but in some settings are still not available [13].

Recently developed molecular techniques that target bacterial vaginosis, *Candida* spp., and *T. vaginalis* may both increase the diagnostic accuracy and simplify the current diagnostic procedure [14–16]. The recently launched into the market BD MAX™ vaginal panel (Becton Dickinson, MA) is a multiplex assay which uses an algorithm based on quantitative assessment of different species of lactobacilli (*L. crispatus* and *L. jensenii*), *G. vaginalis*, *A. vaginae*, *Megasphaera-1*, and BVAB-2 to diagnose BV. This molecular test also detects *Candida* group (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. dubliniensis*), *C. glabrata*, *C. krusei*, and *T. vaginalis.*

The objective of this study is to evaluate the accuracy of the BD MAX™ vaginal panel in the diagnosis of BV, VVC, and trichomoniasis by comparing it to conventional methods: (i) combination of Hay criteria and presence of clue cells with predominant growth of *G. vaginalis*, (ii) yeast culture, and (iii) combination of wet mount microscopic examination and an alternative molecular assay.

Materials and methods

Sample collection

Over a period of 2 months and until completing a panel of 1000 samples, vaginal swabs obtained by insertion of a speculum of woman ≥ 14 years old presenting with or without symptoms received in the Microbiology Laboratory of the University Hospital of Álava (Vitoria-Gasteiz, Spain) were analyzed. Only one sample per patient was considered.

A single swab per patient was collected in ESwab™ (Copan Diagnostics Inc., Murrieta, CA). Samples were immediately processed after the reception.

The median age of participants was 39 years (range, 14 to 98 years). Of the analyzed samples, 50 (5%) belonged to pregnant women.

Conventional diagnostic assessment

Columbia colistin-nalidixic acid agar supplemented with 5% sheep blood (CNA), chocolate agar, candida chromogenic agar CHROMagar™ (Becton Dickinson, MA), and Roiron enrichment broth (bioMérieux, Madrid, Spain) were inoculated with each swab immediately after its reception in the laboratory.

CNA and chocolate agar plates were incubated with 5% CO_2 at 35 °C for up to 48 h, whereas candida chromogenic agar plates and Roiron broth media were incubated at 37 °C for up to 48 h and 5 days, respectively. Species identification for both bacteria and yeasts was performed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) Microflex LT (Bruker Daltonics, Germany).

Gram stains were examined under oil immersion objective and scored following Hay's criteria: grade I (predominantly *Lactobacillus* morphotypes), grade II (mixed *Lactobacillus* and other morphotypes), grade III (few or absent *Lactobacillus* morphotypes, but greatly increased number of morphotypes compatible with *G. vaginalis* and other bacterial morphotypes) [6]. Other relevant information such as the presence of clue cells and leucocytes was also considered.

A clinical microbiologist initially examined all the smears which were later re-examined by another clinical microbiologist. In case of disagreement, a third clinical microbiologist evaluated the smear.

Wet mount microscopic examination combined with culture in Roiron medium and an alternative multiplex polymerase chain reaction (M-PCR) (Anyplex™ II STI-7, Seegene, South Korea) which targets for *T. vaginalis* as well as for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *M. hominis*, *Ureaplasma urealyticum*, and *U. parvum* was considered the reference method in the diagnosis of trichomoniasis.

BD MAX™ vaginal panel

The BD MAX™ vaginal panel (Becton Dickinson, MA) uses real-time PCR for the amplification of specific DNA targets, followed by fluorogenic, target-specific probes to differentially detect infectious vaginitis: (i) algorithmic analysis of lactobacilli (*L. crispatus* and *L. jensenii*) and bacteria involved in BV (*G. vaginalis*, *A. vaginae*, *Megasphaera-1*, and BVAB-2); (ii) algorithmic analysis of *Candida* group (*C. albicans*,

C. tropicalis, *C. parapsilosis*, and *C. dubliniensis*), *C. glabrata*, and *C. krusei*; and (iii) algorithmic analysis of *T. vaginalis*.

The assay was performed following the instructions provided.

Specimen turnaround time depends on the number of specimens analyzed (60 to 144 min if one sample or 24 samples, respectively, are analyzed).

Results

A summary of the obtained data is presented in Table 1.

A total of 193 samples were classified as BV based on a combination of Hay's criteria, the presence of clue cells, and a predominant growth of *G. vaginalis*; median age was of 33 years and 3.6% (7/193) belonged to pregnant women. In 33.7% (65/193) of the BV-positive samples, yeasts were recovered, *C. albicans* accounting for 95.4% (62/65) of them. *T. vaginalis* was concomitantly diagnosed in 2.1% (4/193) samples.

The BD MAX™ vaginal panel was positive for BV in 199 samples; 2% (4/199) of them were initially invalidated. In 14.6% (29/199) samples, neither clue cells were observed, nor BV-related species were isolated. Conversely, in 2.7% (22/801) samples categorized as negative by the molecular panel under evaluation, clue cells were observed, Hay's score was of III, and *G. vaginalis* was predominantly recovered. The sensitivity of the test compared to the conventional method was 89.8% (95% confidence interval [CI], 85.0–93.1) and the specificity was 96.5% (95% CI, 95.1–97.6). The positive predictive value (PPV) of the investigational test was 86.9 (95% CI, 81.9–90.7), and the negative predictive value (NPV) was 97.3% (95% CI, 96.0–98.2).

With respect to VVC, in a total of 336 (33.6%) samples, yeasts were recovered; the mean age was of 35 years and 7.7% (26/336) belonged to pregnant women. A total of nine different species of *Candida* were isolated. *C. albicans* was the commonest isolate accounting for 90.8% (305/336) of the total yeast isolates. Of the non-albicans *Candida* species, *C. glabrata* was the most frequent isolate (23/336; 6.8%), followed by *C. parapsilosis* (11/336; 3.3%). Other less common species were concomitantly isolated with *C. albicans*: *C. lusitaniae*, *C. tropicalis*, and *C. orthopsilosis*, in one sample each. *C. kefyr* and *C. guilliermondii* were each isolated in the absence of other *Candida* species (Table 2).

In 344 (34.4%) samples, *Candida* species (including *C. glabrata* and *C. krusei*) were detected by the BD MAX™ vaginal panel; 3.5% (12/344) of the samples were initially invalidated and yielded a result after repetition, resulting in an overall sensitivity of 97.4% (95% CI, 95.1–98.6) and a specificity of 96.8% (95% CI, 95.2–97.9). PPV was of 93.9% (95% CI, 90.9–95.9) and NPV of 98.7 (95% CI, 97.5–99.3).

Non-*C. glabrata*, non-*C. krusei* positive *Candida* species (including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. dubliniensis*) was detected in 324 samples; *C. albicans* was isolated in 91.0% (295/324) and *C. parapsilosis* in 2.8% (9/324). In the remaining 6.8% (22/324) *Candida* sp.-positive samples, no fungi were recovered after a 5-day incubation period. Conversely, in five and two samples initially categorized as negative by the BD MAX™ vaginal panel, *C. albicans* and *C. parapsilosis*, respectively, were recovered after 48 h of incubation. Therefore, for *Candida* species (targeted by the panel), the sensitivity was of 97.2 (95% CI, 94.8–98.5) and the specificity of 96.8% (95% CI, 95.3–97.9). PPV was of 93.5% (95% CI, 90.3–95.6) and NPV of 98.7% (95% CI, 97.5–99.3).

C. glabrata was detected in 23 samples (2.3%) whereas *C. krusei* was detected in five (0.1%). Both species were recovered after 48 h of incubation. However, in one sample categorized as negative, *C. krusei* was afterwards recovered. For *C. glabrata*, sensitivity and specificity were of 100% (95% CI, 85.7–100) and of 100% (95% CI, 99.6–100), respectively, and PPV and NPV were of 100% (95% CI, 85.7–100) and of 100% (95% CI, 99.6–100), respectively, whereas for *C. krusei*, the sensitivity was of 83.3 (95% CI, 43.6–97.0) and the specificity of 100% (95% CI, 99.6–100). PPV was of 100% (95% CI, 56.6–100) and NPV was of 99.9% (95% CI, 99.4–100).

Table 1 Overall prevalence and investigational test performance

Condition	Prevalence (%)	BD MAX™	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
BV	19.3 (193/1000)	199/1000	89.8 (85.0–93.1)	96.5 (95.1–97.6)	86.9 (81.9–90.7)	97.3 (96.0–98.2)
Candida sp. (including *C. glabrata*, *C. krusei*)	33.6 (336/1000)	344/1000	97.4 (95.1–98.6)	96.8 (95.2–97.9)	93.9 (90.9–95.9)	98.7 (97.5–99.3)
Cgroup	31.4 (314/1000)	324/1000	97.2 (94.8–98.5)	96.8 (95.3–97.9)	93.5 (90.3–95.6)	98.7 (97.5–99.3)
C. glabrata	2.3 (23/1000)	23/1000	100 (85.7–100)	100 (99.6–100)	100 (85.7–100)	100 (99.6–100)
C. krusei	0.5 (5/1000)	4/1000	83.3 (43.6–97.0)	100 (99.6–100)	100 (56.6–100)	99.9 (99.4–100)
TV	1.5 (15/1000)	15/1000	100 (79.6–100)	100 (99.6–100)	100 (79.6–100)	100 (99.6–100)

BV, bacterial vaginosis; *Cgroup*, *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. dubliniensis*; *TV*, *Trichomonas vaginalis*; *PPV*, positive predictive value; *NPV*, negative predictive value

Table 2 Prevalence of *Candida* spp.

Species	No. (%)
C. albicans	305 (90.8)
C. glabrata	23 (6.8)
C. guilliermondii	1 (0.3)
C. kefyr	1 (0.3)
C. krusei	5 (1.5)
C. parapsilosis	11 (3.3)
C. orthopsilosis	1 (0.3)
C. tropicalis	1 (0.3)

T. vaginalis was recovered in 15 (1.5%) samples and the alternative multiplex PCR assay (Anyplex™ II STI-7, Seegene, South Korea) yielded a TV-positive result in all of them. However, the parasite was microscopically observed in 26.7% (4/15). The Gram stain revealed a high number of leucocytes in 40% (6/15) of the smears positive to TV. The mean age was of 36 and none of the TV-positive samples were detected in pregnant women. The BD MAX™ vaginal panel detected TV in all the 15 positive samples and the parasite was not detected in any of the 985 samples that were TV negative by either culture or the alternative PCR assay, resulting in a sensitivity of 100% (95% CI, 79.6–100) and a specificity of 100% (95% CI, 99.6–100).

Of the 50 samples of pregnant women analyzed, 56% (26 /50) yielded a positive *C. albicans* culture, and in 14% (7/50), BV was diagnosed following traditional criteria. The BD MAX™ vaginal panel initially invalidated 18% (9/50) of the samples belonging to pregnant women but results were available after repetition in 77.8% (7/9).

Forty-three (4.3%) of the 1000 samples analyzed were initially invalidated; after repetition, 30.2% (13/43) remained invalidated whereas 69.8% (30/43) provided a valid result.

Discussion

Overall, the BD MAX™ vaginal panel performed with high sensitivity and specificity.

The population prevalence of BV in this study was of 19.3 and was comparable to the one obtained in other studies [2, 17]. Traditional BV microbiological diagnosis is based on a combination of Gram stain and *G. vaginalis* culture since this microorganism is stated to play a significant role in the etiology of BV [18–20]. Gram staining is time consuming and depends on the experience of the observer and plates should be examined after 48 h of incubation to ensure sufficient growth of *G. vaginalis*. Therefore, molecular tests that detect both lactobacilli and BV-associated bacteria are a promising tool to rapidly and objectively diagnose BV. However, as the assay does not differentiate non-pathogenic growth from the pathogenic one, it can result in overdiagnosis of BV. Thus, 14.9% (29/199) of the samples were incorrectly categorized as BV, whereas 2.7% (22/801) of the samples categorized as negative by the panel yielded positive results with traditional methods. Therefore, we discourage leaving aside traditional techniques such as Gram stain for the correct diagnosis of BV.

Regarding VVC, the population prevalence of *Candida* spp. in this study was 33.6%, which is in line with other studies [14]. Although *C. albicans* is responsible for the clear majority of VVC, the frequency of other *Candida* species such as *C. glabrata*, *C. parapsilosis*, and *C. krusei* is increasing [9]. Moreover, other species such as *C. guilliermondii* and *C. kefyr* are infrequently isolated. The molecular panel under evaluation detects the six most frequent *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, and *C. dubliniensis*); hence, the presence of other species is interpreted as negative. Therefore, in areas with high prevalence of species different of the ones targeted by the panel or when those are suspected, yeast culture is mandatory. In our case, *C. guilliermondii* and *C. kefyr* were recovered from specimens classified as negative by the BD MAX™ vaginal panel. In any case, recovering yeasts from culture is important specially in those cases in which an antifungigram is expected to be performed, either after therapeutic failure or if resistances are suspected.

The high sensitivity of molecular tests is probably responsible for the 6.4% false positive VVC results. Clinical uncertainties may appear whenever the PCR detects yeasts which are not afterwards recovered; in these cases, we recommend adding a comment to the final report explaining the high sensitivity of the molecular test.

The prevalence of trichomoniasis in this study was 1.5%, similar to other studies performed in Spain [21]. For *T. vaginalis*, microscopic examination of wet mount preparations of vaginal secretions is the traditional diagnostic procedure. This method is highly specific; in fact, the observation of the characteristic tumbling motility is considered 100% specific. Besides, the test is inexpensive and easy to perform and remains widely used, above all in laboratories with limited resources. Nevertheless, specimens need to be examined immediately after collection and the sensitivity is very poor, ranging from 44 to 68%, depending on the expertise of the reader. Sensitivity dramatically increases with molecular techniques. In our case, in only 26.7% (4/15) of the 15 TV-positive samples, *T. vaginalis* was observed and so, the sensitivity of the microscopic examination compared to the molecular assay was of 57.7%.

Invalid results were obtained in 4.3% (43/1000) of the samples analyzed; after repetition, in 30.2% (13/43) of these samples, the result remained invalid. As specified in the manufacturer's instructions, the PCR amplification is highly sensitive to inhibition by vaginal lubricants and creams which are widely used, especially among women who have some type of

vaginal symptomatology. Although the percentage of samples that did not yield a valid result was low, in the daily practice, it would imply a delay in the report of results and an increase in the cost of the test that should be considered.

As in Spain prevention of early-onset group B streptococcal (GBS) disease is carried out by a screening-based strategy in which a vaginal and rectal swab is obtained at 35–37 weeks of gestation, pregnant women whose samples have been included in this study had vaginal discomfort or symptoms. The prevalence of BV among pregnant women was of 14% (7/50), similar to other studies carried out in pregnant women [22]. However, the VVC prevalence (56% (26/50)) is somewhat higher than the one obtained in other studies [23, 24]; this could be due to the fact that only symptomatic women were evaluated.

Although infectious vaginitis generates significant costs and morbidities, it is frequently underdiagnosed and even trivialized. BV has been linked to pelvic inflammatory disease; increased risk of both acquisition and transmission of STIs, including immunodeficiency virus (HIV); and adverse pregnancy outcomes, such as preterm labor and delivery, preterm premature rupture of membranes, chorioamnionitis, spontaneous preterm birth, and postpartum endometritis. Furthermore, colonization with *Candida* species in early pregnancy has been associated with preterm delivery and low birthweight, and so, some authors recommend routine screening of pregnant women [25]. Similarly, trichomoniasis is an important source of reproductive morbidity. Therefore, a reliable and rapid diagnosis of vaginitis is essential for proper management.

In our experience, the BD MAX™ vaginal panel had some limitations: (i) it does not quantify, and growth curves are not available; (ii) the high sensitivity of the test can act as a double-edged sword confusing the clinician and leading to unnecessary prescription of antimicrobials; and (iii) taking into account that the majority of the *Candida* species isolated are *C. albicans*, a specific target for this species could speed up the diagnosis. Therefore, we consider that the panel under evaluation should be used as a supplementary tool to support the final diagnosis. Gram stain provides additional valuable information in the diagnosis of vaginitis. On the one hand, grading of lactobacilli gives an idea about the status of the vaginal flora; on the other, the presence of leucocytes and/or predominant morphotypes of bacteria provides further information which can guide to the final microbiological diagnosis. As stated before, the evaluated assay can result in overdiagnosis of BV, and so, we recommend carrying out Gram stains or wet mount exams in all samples. On the other hand, yeast cultures allow the performance of antifungigrams when requested or needed.

Molecular diagnostic assays are objective and can detect fastidious bacteria and results are available within the same day of sample reception. The BD MAX™ vaginal panel is highly sensitive and specific and increases the accuracy in the diagnosis of women presenting a vaginitis syndrome. The results of the present study demonstrate that the BD MAX™ vaginal panel accurately diagnoses most common bacterial, fungal, and protozoan causes of vaginitis and improves and simplifies the identification of infectious vaginitis.

This study has some limitations. Firstly, vaginitis, especially VVC, is frequently clinically diagnosed without the support of laboratory diagnosis. Thus, samples are only microbiologically analyzed whenever the patient experiences no recovery. Therefore, the relative contribution of other species to the burden of VVC is difficult to measure and could be overestimated. Secondly, information regarding antibiotic or antifungal treatment before sample collection was lacking. At this point, it should be noted that in Spain vaginal antifungals can be acquired in pharmacies without prescription and patients frequently make use of these products without a clinical diagnosis. Thirdly, clinical outcomes for patients with discordant results were not evaluated.

Compliance with ethical standards

Conflict of interest A. Canut-Blasco reports personal fees from Pfizer, Merck, Roche, and Werfen unrelated to the current study. All other authors declare that they have no conflict of interest.

Ethical approval For this type of study, formal consent is not required.

Transparency declaration The authors of this manuscript affirm that it is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been eliminated; and that any discrepancies from the study as planned have been explained.

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