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Clinical Validation of a Test for the Diagnosis of Vaginitis

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Abstract

OBJECTIVE—Vaginitis may be diagnosed as bacterial vaginosis, vulvovaginal candidiasis, trichomoniasis, or coinfection. A new molecular test assays the vaginal microbiome and organisms that cause three common infections. The objective of the trial was to evaluate the clinical accuracy of the investigational test for vaginal swabs collected by patients (self) or clinicians. The primary and secondary outcomes were to compare the investigational test with reference methods for the three most common causes of vaginitis and compare clinician-collected with self-collected swabs.

METHODS—We conducted a cross-sectional study in which women with symptoms of vaginitis were recruited at ten clinical centers and consented to the investigation between May and September 2015. The woman collected a vaginal swab, sheathed, and then handed it to the clinician. These swabs were to evaluate how self-collected swabs compared with clinician-collected swabs. The clinician collected an investigational test swab and reference test swabs. From 1,740 symptomatic patients, clinician-collected and self-collected vaginal swabs were

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evaluated by the molecular test and six tests. The reference methods for bacterial vaginosis were Nugent's score and Amsel's criteria for intermediate Nugent results. The reference methods for *Candida* infection were isolation of any potential *Candida* microorganisms from inoculation of two culture media: chromogenic and Sabouraud agar and sequencing. The reference methods for trichomoniasis were wet mount and culture.

RESULTS—For clinician-collected swabs, by reference methods, bacterial vaginosis was diagnosed in 56.5%, vaginal candidiasis in 32.8%, trichomoniasis in 8%, and none of the three infections in 24% with a coinfection rate of 20%. The investigational test sensitivity was 90.5% (95% confidence interval [CI] 88.3–92.2%) and specificity was 85.8% (95% CI 83.0–88.3%) for bacterial vaginosis. The investigational test sensitivity was 90.9% (95% CI 88.1–93.1%) and specificity was 94.1% (95% CI 92.6–95.4%) for the *Candida* group. Sensitivity for *Candida glabrata* was 75.9% (95% CI 57.9–87.8%) and specificity was 99.7% (95% CI 99.3–99.9%). Investigational test sensitivity was 93.1% (95% CI 87.4–96.3%) and specificity was 99.3% (95% CI 98.7–99.6%) for trichomoniasis. Results from self-collected swabs were similar to clinician-collected swabs.

CONCLUSION—A molecular-based test using vaginal swabs collected by clinicians or patients can accurately diagnose most common bacterial, fungal, and protozoan causes of vaginitis. Women and their clinicians seeking accurate diagnosis and appropriate selection of efficacious treatment for symptoms of vaginitis might benefit from this molecular test.

Vaginitis is a common problem for women associated with discomfort. Symptomatic vaginitis accounts for millions of clinical visits annually.[1] The main diagnoses are bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis.[2] In a review of symptomatic women, bacterial vaginosis was diagnosed in 22–50%, vulvovaginal candidiasis in 17–39%, and trichomoniasis in 4–35%.[3]

Lactobacilli have predominant roles in the commensal vaginal flora by producing lactic acid.[4,5] *Lactobacillus crispatus* and *Lactobacillus jensenii* are common lactobacilli in the vaginas of healthy women.[6]

In bacterial vaginosis, lactobacilli are decreased, with increases in microorganisms such as *Atopobium vaginae*, *Gardnerella vaginalis*, *Mobiluncus mulieris*, *Prevotella* species, BVAB-2, and *Megasphaera* species.[7–9] *Candida albicans* is responsible for 65–90% of vaginal *Candida* species infections, and non-albicans *Candida* species are responsible for up to 30%.[10–12]

Trichomoniasis is the most common curable sexually transmitted infection in the world.[1] The Centers for Disease Control and Prevention recommends use of molecular tests for diagnosis.[13]

Diagnosis of vaginitis is usually made by clinical findings, wet mount, Amsel criteria, or laboratory tests.[7,13] Vaginal swab Gram stain with estimates of numbers of microbial flora is an alternative method for the diagnosis of bacterial vaginosis.[14,15]

Inaccurate test results are problematic for treatment efficacy (Carr FL, Thabault P, Levenson S, Friedman RH. Vaginitis in a community based practice [abstract]. Clin Res

1992;40:554A.).[16,17] A single molecular test with high sensitivity and specificity might provide a clinical benefit.[18] The study objectives were to compare the investigational molecular test with three reference methods for the diagnosis of vaginitis and compare clinician-collected swabs with self-collected swabs. Comparisons for self-collection were made to demonstrate accuracy could be maintained if self-collection was used for busy clinicians to save time.

MATERIALS AND METHODS

The study design was a diagnostic accuracy cross-sectional study (Fig. 1). At ten clinical centers, all eligible patients were recruited consecutively from clinics identified as sexually transmitted diseases, human immunodeficiency virus, family planning, and gynecology if they reported symptoms of vaginitis (at least one of the following symptoms: abnormal vaginal discharge, painful or frequent urination, vaginal itching or burning or irritation, painful or uncomfortable intercourse, vaginal odor) and enrolled if they provided informed consent and met the minimum age required by their institutional review board (18 at nine sites, and 14 at one site). Exclusion criterion was if they were previously enrolled in this study. The clinical centers were either academic medical center clinics or community clinics. Before giving informed consent, each patient reviewed the study procedures, risks, and benefits. After written informed consent to clinical research, vaginal discharge specimens were collected between May and September 2015. Eligible specimens meeting study inclusion criteria were included in the sensitivity, specificity, and prevalence calculations (Appendix 1, available online at http://links.lww.com/AOG/A954).

A single investigational test swab was used by the patient to self-collect a vaginal specimen in the clinic and then placed in a sheath and handed to the clinician (first collected swab at nine clinical sites and last collected swab at one site). Vaginal swabs collected by the clinician were investigational test swab, two cotton swabs, one BD Liquid Amies Elution Swab Collection and Transport System in random order, and lastly one APTIMA swab. The investigational test swab was placed in an investigational test buffer tube to transport the specimen to the laboratory. Three of the ten collection sites were used as laboratory sites for the investigational test and two additional reference laboratories were used for investigational and reference tests.

The investigational tests were performed with the BD MAX System using the investigational test swabs. The investigational test provided positive or negative results for three diagnoses as follows: 1) bacterial vaginosis, by an algorithmic analysis of molecular DNA detection of lactobacilli (*Lactobacillus* species *[L crispatus* and *L jensenii*]) and also with bacteria associated with bacterial vaginosis*, G vaginalis*, *A vaginae*, *Megasphaera*-1, BVAB-2; 2) vaginal candidiasis by molecular DNA detection of a *Candida* group (*C albicans*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida dubliniensis*) or *Candida glabrata* or *Candida krusei*; and 3) trichomoniasis by molecular DNA detection of trichomonad protozoa. The molecular test was performed on the investigational test device; an automated sample-in and answer-out instrument that combines sample extraction, polymerase chain reaction setup, and real-time polymerase chain reaction on a walkaway platform.

There were three reference methods. Appendix 2, available online at http://links.lww.com/AOG/A954, summarizes the reference methods for each of the three diagnoses. The bacterial vaginosis reference method was comprised of two tests: Amsel's criteria[7] from a wet mount prepared from one cotton swab and Nugent[14] scoring from a split elution swab sample performed at a single reference laboratory. A 50-microliter aliquot from each elution swab sample was placed on a glass microscope slide and air-dried. Each slide was Gram-stained and used for a scored microscopic interpretation. A semiquantitative evaluation of the three bacterial morphotypes (lactobacilli, *G vaginalis*, and *Mobiluncus* spp.) and a numeric value were assigned for each type, the sum of which was then totaled: a score of 0–3 interpretation "normal flora," a score of 4–6 interpretation "intermediate flora," and 7–10 interpretation "bacterial vaginosis flora." Each Gram-stained slide was read by two different readers blinded from the result of the other reader. A third reading by an arbiter was required for discordant results, or a score of 4–6 by both readers, or slide "not readable" by both readers. The score was final if the same result was reported by at least two readers. Disagreement across all three readers was resolved by a panel review of the slide for an adjudicated score. If the final Nugent score was 4–6, this indeterminate result was nondiagnostic and therefore Amsel's criteria were used. A modified Amsel-positive result in this study was the presence of at least two of the three following criteria: vaginal pH greater than 4.5, clue cells seen during microscopic examination of the wet mount by expert clinicians, and "whiff test"-positive. The pH was determined from chromatic paper touched to vaginal discharge on the cotton swab that was used for the wet mount or directly. Whiff test was determined after adding potassium hydroxide to the vaginal discharge and detecting a "fishy" amine odor. The discharge criterion of Amsel's was not considered as a result of its poor sensitivity.

The candidiasis reference method was comprised of two tests: a yeast culture from the split elution swab sample performed at a single reference laboratory by inoculating two different culture media: chromogenic medium BD BBL CHROMagar *Candida* plate that was read after 36–48 hours of incubation at 33–37°C and the BD BBL Sabouraud Dextrose Agar, Emmons plate that was read after 36–48 hours and until 80 hours of incubation at 25–30°C. The growth level was estimated on both media in an increasing manner: 1 colony, 1+, 2+, 3+, 4+ (where n+ represented the number of quadrants showing *Candida* spp. growth). Identification of the isolated yeast was then performed by bidirectional sequencing of the *its2* gene.[19]

The trichomoniasis reference method was comprised of two tests: microscopic examination of the wet mount with visualization of motile trichomonads[20] and InPouch TV Culture System inoculated with one cotton swab. Incubation at 35–37°C was started within 48 hours after inoculation and readings were performed daily over a 5- to 7-day period. If either reference test was positive, the patient's status was established as infected. Both reference tests had to be negative to establish a noninfected patient status. The APTIMA Trichomonas vaginalis Assay performed from the last swab collected was used in case of discordance.

A panel of 50 *C glabrata* and 50 *C krusei* strains spiked at varying concentrations and 50 negative (defined "contrived") samples was prepared in a unique (not pooled) negative natural vaginal matrix. These were masked, randomly intermixed, and tested at three

reference laboratories to supplement the specimens enrolled in the event prevalence of *C glabrata* and *C krusei* was low within the enrolled population.

The population demographics were tabulated by geographic area, clinical type, race or ethnicity, age, and education level. Prevalence rate, sensitivity, specificity, positive predictive value, and negative predictive value were calculated according to standard equations. Sensitivity and specificity analyses were stratified by collection method (self- and clinician-collected) and specimen type (prospective and contrived). The confidence intervals for sensitivity and specificity were calculated using the score method.[21] The confidence intervals for positive predictive value and negative predictive value were calculated using the exact method. Logistic modeling was performed to determine if sensitivity and specificity are statistically different between collection methods or other subgroups. A significant *P* value, set at ≤.05, indicates that there is a statistically significant difference.

Institutional review board approvals were obtained locally by all clinical centers. The protocol and ethical consent were reviewed by each center participating in the study. The study was conducted in accordance with the ethical principles derived from the Declaration of Helsinki and Belmont Report and in compliance with U.S. Food and Drug Administration (FDA) and Good Clinical Practice Guidelines set forth by the International Conference on Harmonization (ICH-E6).

RESULTS

A total of 1,763 women were enrolled for investigational test testing, of whom 23 were excluded. Reasons for exclusion of participants were patient withdrawn (13), informed consent process incorrect (7), asymptomatic patient enrolled (2), and more than one specimen obtained for the same patient (1). Of the 1,740 evaluable participants, the sensitivity and specificity were calculated for clinician- and self-collected swabs for bacterial vaginosis (n=1,559; 1,582), *Candida* species (n=1,618; 1,628), and *Trichomonas vaginalis* (1,600; 1,610) (Appendix 1, http://links.lww.com/AOG/A954). Demographic data are shown for the 1,686 participants who had a reportable reference method and reportable investigational test results for at least one target for clinician-collected swabs (Table 1). The prevalence of the different targets is presented (Table 2) for the 1,471 and 1,494 specimens that yielded reportable reference method and reportable investigational test results for all the targets for clinician- and self-collected swabs, respectively.

Table 1 provides detailed demographics of the study population stratified by geographic area, clinic type, race and ethnicity, age, educational level, number of sexual partners in the past year, human immunodeficiency virus status, types and number of symptoms, use of antibiotics, and antifungals. The majority of specimens (greater than 70%) was collected from the eastern and south–central U.S. regions from family planning centers (66.7%) and in the 18- to 29-year age group (63.3%). No patients younger than 18 years old were enrolled in the study. Black or African American patients made up the largest racial group (53.3%) followed by Caucasian patients (24.9%). The education level of the majority of participants was high school or above (1,586 [94.1%]). Most of the participants (53.3%) had a single sexual partner or none in the past year, and a small minority of participants self-reported

human immunodeficiency virus status as positive (17 [1%]). The most frequently experienced symptoms were abnormal vaginal discharge (1,256 [74.5%]) and vaginal itching, burning, or irritation (872 [51.7%]) with more than two thirds of participants experiencing two or more symptoms (1,143 [67.8%]).

Fewer than 25% of participants were treated with either oral or vaginal antibiotics in the 30-day period before the study or with vaginal antifungals in the 14-day period before the study. For bacterial vaginosis, sensitivity for clinician- and self-collected swabs with the use of oral antibiotics was 82.3% and 84.4%, respectively. With the use of antifungals, bacterial vaginosis sensitivity was 80.4% and 80.0%, respectively. The use of antibiotics or antifungals was not shown to have an effect on the sensitivity of the diagnostic test for *Candida* species (*P* value range .25–.82). Fewer than 20 women who tested positive for *T vaginalis* used antibiotics or antifungals. Table 2 illustrates the number of single and multiple infections by bacterial vaginosis organisms, *Candida* group, *C glabrata*, *C krusei*, and *T vaginalis*. Overall disease prevalence as identified by the reference method and the investigational test was similar (Table 2). For clinician-collected swabs, by reference methods, bacterial vaginosis was diagnosed in 56.5%, *Candida* group in 31.9%, *C glabrata* in 1.7%, *C krusei* in 0%, trichomoniasis in 8%, and none of the three infections in 24% with a coinfection rate of 20%. Total vaginal candidiasis was diagnosed in 32.8%. Self-collected swabs showed very similar prevalence distributions (Table 2).

The clinical performance of the investigational test for the detection of each organism or group of organisms is shown in Table 3, which describes the performance of both clinician- and self-collected swabs as well as the contrived specimen results for *C glabrata* and *C krusei*. Sensitivity and specificity for bacterial vaginosis, *Candida* group, *C glabrata* and *T vaginalis* against reference method samples was 90.5% and 85.8%, 90.9% and 94.1%, 75.9% and 99.7%, and 93.1% and 99.3%, respectively. For the self-collected swabs, the sensitivity and specificity for bacterial vaginosis, *Candida* group, *C glabrata,* and *T vaginalis* was 90.7% and 84.5%, 92.2% and 91.9%, 86.7% and 99.6%, and 93.2% and 99.3%, respectively.

DISCUSSION

The investigational test is an FDA-approved nucleic acid amplification test for detection of the major causes of vaginitis. The test performed with high sensitivity and specificity in 1,740 evaluable symptomatic patients from which clinician- and self-collected specimens were obtained and tested. For bacterial vaginosis, the sensitivity of the investigational test compared with the reference methods was 90.5% and the specificity was 85.8%. For vaginal candidiasis, the investigational test sensitivity and specificity were 90.9% and 94.1%, respectively, for the *Candida* group; for *C glabrata*, sensitivity was 75.9% and the specificity was 99.7%. For the detection of *T vaginalis*, the investigational test demonstrated a sensitivity and specificity of 93.1% and 99.3%, respectively, indicating performance similar to other FDA nucleic acid amplification test–cleared assays (Van Der Pol B, Williams JA, Eddleman L, Fuller D, Taylor S, Schwebke J, et al. P5.093 evaluation of a new amplified DNA assay on the becton dickinson viper system in extracted mode for the detection of

trichomonas vaginalis from vaginal specimens [abstract]. Sex Transm Infect 2013;89:A364.).[22,23]

The investigational test provided a single vaginal swab, single molecular multiplex assay capable of assisting in the diagnosis of the three types of vaginitis. The investigational test performance was comparable with other research molecular assays for these infections.[9,22,24–26] The performance of self-collected swabs could potentially expand available models of care for busy clinicians, saving them time with patients collecting their own specimens and for recurrent cases of vaginitis. Additionally, this assay potentially eliminates time to perform whiff tests and microscopic examinations for clue cells, trichomonas, and yeast.

For *T vaginalis* analysis, an FDA-cleared amplification assay used for specimens with discordant results between the investigational test and wet preparation revealed that seven of nine investigational test "false-negative" specimens were also negative. This suggested that some samples were perhaps incorrectly read as *T vaginalis*–positive on wet mount.

Although traditional methods that diagnose bacterial vaginosis have relied on methods such as the microscopic assessment of bacterial morphotypes (Nugent) or some combination of patient examination and vaginal discharge (Amsel), recent research by Fredricks et al has indicated more complex processes implicating the role of bacterial pathogens in the etiology of bacterial vaginosis.[9,24,25] Many of these organisms, such as *A vaginae, Prevotella* species, and others, can be detected only by amplification tests, because they are not easily grown or are uncultivable. These discoveries have created an opportunity to advance the molecular diagnosis of bacterial vaginosis. Additionally, certain Lactobacillus species (*L crispatus* and *L jensenii* but not *Lactobacillus iners*[9,25]) are important contributors to the maintenance of the normal vaginal flora and are decreased or lost in bacterial vaginosis. These dynamic bacterial population changes are complex.[5,9,18,24] A molecular assay based on the presence of lactobacilli and the absence of deleterious organisms represents an opportunity to improve the diagnosis of bacterial vaginosis.[5,9,18,24]

Although this new assay performed well, a fuller understanding of its true performance is constrained by the known limitations of the reference methods.[27] The investigational test performance for bacterial vaginosis could be attributed to specimens with an intermediate Nugent, for which Amsel was used to determine the final reference method result. Based on published data, the Amsel demonstrated only 81.0% positive percent agreement and 86.0% negative percent agreement compared with positive (7–10) and negative (0–3) Nugent (Beqaj SS, Lebed J, Smith B, Farrell M, Schwebke JR, Rivers CA, et al. P142 Comparison of conventional and modified Amsel's criteria with nugent score and impact on PCR-based bacterial vaginosis infection status evaluation [abstract]. Int J STD AIDS 2015;26:96.).

The investigational test provided results for a third common cause of vaginitis, *Candida* species. Although microscopic wet prep examination may be limited by poor sensitivity and culture requires prolonged time, an amplified molecular technique has the opportunity to offer improvements. The ability to identify *C glabrata* and *C krusei*, which have fluconazole resistance, represents an advantage not possible with wet prep and culture.[26] Numbers of

women who tested positive for *C glabrata* were small, and misses occurred where culture load was low. Contrived *C glabrata* and *C krusei* samples showed positive and negative percent agreements of 100%.

This study has limitations that may preclude its generalizability to other populations of women. The majority of women were African American and the majority came from family planning clinics. Study limitations also included the use of traditional reference methods of bacterial vaginosis, which are becoming recognized as less than accurate compared with research demonstrating the importance of bacterial vaginosis-associated organisms that are difficult to grow or are uncultivable.[8,9,24]

This study had strengths and was unique because it used one vaginal swab, which performed accurately for the simultaneous detection of the three causes of vaginitis. Research will be required to demonstrate performance and outcomes in various populations such as pregnant women, hypoestrogenic women, and asymptomatic women. In summary, the investigational test appears to be a promising molecular assay for detection of vaginitis using molecular amplification of vaginal microbiome organisms, indicating a one-assay platform could potentially aid clinicians in diagnosing vaginitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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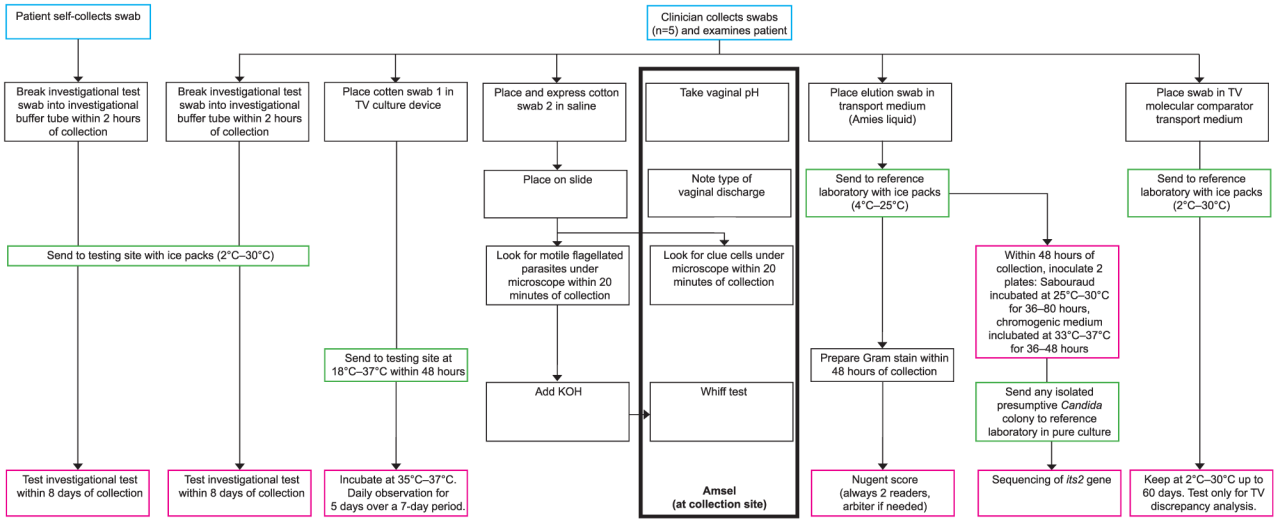

Fig. 1.

Testing algorithm. *Blue boxes* indicate patient- or clinician-collected swabs, *green boxes* refer to specimen transport, and *pink boxes* refer to testing of the different methods. TV, *trichomonas vaginalis*; KOH, potassium hydroxide.

Gaydos. Molecular Diagnosis of Vaginitis. Obstet Gynecol 2017.

Table 1

Demographic Data of the Study Population (n=1,686)

Demographic	Value
Geographic area	
U.S. east	632 (37.5)
U.S. center south	629 (37.3)
U.S. center north	165 (9.8)
U.S. west	260 (15.4)
Clinic type	
STD or HIV	309 (18.3)
Family planning	1,124 (66.7)
Obstetrics and gynecology	253 (15.0)
Race or ethnicity	
Native American or Alaskan Native	7 (0.4)
Asian	61 (3.6)
Black or African American	898 (53.3)
Caucasian (not Hispanic or Latina)	419 (24.9)
Hispanic or Latina	149 (8.8)
Native Hawaiian or other Pacific Islander	2 (0.1)
Mixed race or ethnicity	126 (7.5)
Unknown or DTA	24 (1.4)
Age (y)	27.0 (18–81) 29.2±9.4
18–29	1,067 (63.3)
30–39	377 (22.4)
40–49	171 (10.1)
50 and older	71 (4.2)
Educational level	
Less than high school	57 (3.4)
High school or high school equivalency certificate	509 (30.2)
Greater than high school	1,077 (63.9)
Unknown or DTA	43 (2.6)
Sexual partners in the past year	
1 or fewer	899 (53.3)
2–3	595 (35.3)
4–5	94 (5.6)
6 or greater	60 (3.6)
Unknown or DTA	38 (2.3)
HIV status	
Seropositive	17 (1.0)
Seronegative	1,409 (83.6)
Unknown or DTA	258 (15.3)
NA	2 (0.1)

Demographic	Value
Type of symptom	
Abnormal vaginal discharge	1,256 (74.5)
Painful or frequent urination	198 (11.7)
Vaginal itching or burning or irritation	872 (51.7)
Painful or uncomfortable intercourse	169 (10.0)
Vaginal odor	813 (48.2)
No. of symptoms	
1	543 (32.2)
2	757 (44.9)
3	304 (18.0)
4	71 (4.2)
5	11 (0.7)
Use of oral antibiotics *	
Yes	196 (11.6)
Use of vaginal antibiotics *	
Yes	51 (3.0)
Use of antifungals *	
Yes	150 (8.9)

STD, sexually transmitted disease; HIV, human immunodeficiency virus; DTA, declined to answer; NA, not available.

Data are n (%), median (minimum–maximum), or mean±standard deviation.

* Use of antibiotics and antifungals was from medical record abstraction or self-report.

Table 2

Overall* Infection Prevalence by Reference Method and Investigational Test

	Clinician-Collected (n=1,471)		Self-Collected (n=1,494)	
Infection Pattern	**RM**	**Investigational**	**RM**	**Investigational**
BV only	549 (37.3) (34.9, 39.8)	531 (36.1) (33.7, 38.6)	550 (36.8) (34.4, 39.3)	522 (34.9) (32.6, 37.4)
All negative	353 (24.0) (21.9, 26.2)	350 (23.8) (21.7, 26.0)	364 (24.4) (22.3, 26.6)	347 (23.2) (21.2, 25.4)
Cgrp only	243 (16.5) (14.7, 18.5)	238 (16.2) (14.4, 18.1)	247 (16.5) (14.7, 18.5)	245 (16.4) (14.6, 18.4)
BV, Cgrp	186 (12.6) (11.0, 14.4)	205 (13.9) (12.3, 15.8)	189 (12.7) (11.1, 14.4)	229 (15.3) (13.6, 17.2)
BV, TV	64 (4.4) (3.4, 5.5)	72 (4.9) (3.9, 6.1)	64 (4.3) (3.4, 5.4)	68 (4.6) (3.6, 5.7)
BV, Cgrp, TV	23 (1.6) (1.0, 2.3)	21 (1.4) (0.9, 2.2)	24 (1.6) (1.1, 2.4)	23 (1.5) (1.0, 2.3)
TV only	22 (1.5) (1.0, 2.3)	23 (1.6) (1.0, 2.3)	22 (1.5) (1.0, 2.2)	27 (1.8) (1.2, 2.6)
Cgla only	11 (0.7) (0.4, 1.3)	10 (0.7) (0.4, 1.2)	11 (0.7) (0.4, 1.3)	5 (0.3) (0.1, 0.8)
BV, Cgrp, Cgla	6 (0.4) (0.2, 0.9)	7 (0.5) (0.2, 1.0)	6 (0.4) (0.2, 0.9)	9 (0.6) (0.3, 1.1)
Cgrp, TV	6 (0.4) (0.2, 0.9)	4 (0.3) (0.1, 0.7)	7 (0.5) (0.2, 1.0)	5 (0.3) (0.1, 0.8)
Cgrp, Cgla	4 (0.3) (0.1, 0.7)	3 (0.2) (0.1, 0.6)	5 (0.3) (0.1, 0.8)	6 (0.4) (0.2, 0.9)
BV, Cgla	3 (0.2) (0.1, 0.6)	3 (0.2) (0.1, 0.6)	3 (0.2) (0.1, 0.6)	7 (0.5) (0.2, 1.0)
Cgrp, Cgla, TV	1 (0.1) (0.0, 0.4)	0 (0.0) (0.0, 0.3)	1 (0.1) (0.0, 0.4)	0 (0.0) (0.0, 0.3)
BV, Cgrp, Ckru	0 (0.0) (0.0, 0.3)	2 (0.1) (0.0, 0.5)	0 (0.0) (0.0, 0.3)	0 (0.0) (0.0, 0.3)
Ckru only	0 (0.0) (0.0, 0.3)	2 (0.1) (0.0, 0.5)	0 (0.0) (0.0, 0.3)	0 (0.0) (0.0, 0.3)
BV, Cgla, TV	0 (0.0) (0.0, 0.3)	0 (0.0) (0.0, 0.3)	1 (0.1) (0.0, 0.4)	1 (0.1) (0.0, 0.4)

RM, reference methods; BV, bacterial vaginosis; Cgrp, *Candida* group (*Candida albicans, Candida tropicalis, Candida parapsilosis*, or *Candida dubliniensis*); TV, *Trichomonas vaginalis*; Cgla, *Candida glabrata;* Ckru, *Candida krusei*.

Data are n (%) (upper and lower confidence interval).

*Prevalence was calculated with the same denominator for the reference method and the investigational test.

Table 3

Overall Investigational Test Performance

Identification	Prevalence	Specimen Type	Sensitivity	Specificity	PPV (95% CI)	NPV (95% CI)
BV	55.8 (899/1,610)	Clinician (n=1,559)	90.5 (797/881) (88.3–92.2)	85.8 (582/678) (83.0–88.3)	89.0 (87.1–90.7)	87.7 (85.4–89.8)
		Self (n=1,582)	90.7 (803/885) (88.6–92.5)	84.5 (589/697) (81.6–87.0)	88.1 (86.2–89.9)	87.8 (85.5–90.0)
Cgroup[*]	31.6 (523/1,656)	Clinician (n=1,618)	90.9[†] (462/508) (88.1–93.1)	94.1[‡] (1,045/1,110) (92.6–95.4)	87.8 (85.1–90.2)	95.7 (94.5–96.8)
		Self (n=1,628)	92.2 (470/510) (89.5–94.2)	91.9 (1,028/1,118) (90.2–93.4)	84.1 (81.3–86.7)	96.2 (95.0–97.2)
C glabrata	1.8 (30/1,656)	Clinician (n=1,618)	75.9[§] (22/29) (57.9–87.8)	99.7[//] (1,584/1,589) (99.3–99.9)	81.6 (65.3–93.0)	99.6 (99.2–99.8)
		Self (n=1,628)	86.7 (26/30) (70.3–94.7)	99.6 (1,592/1,598) (99.2–99.8)	81.0 (66.7–91.6)	99.8 (99.4–99.9)
	NA	Contrived (n=100)	100[¶] (50/50) (92.9–100)	100[#] (50/50) (92.9–100)	NA	NA
C krusei	0.0 (0/1,656)	Clinician (n=1,618)	ND	99.8 (1,614/1,618) (99.4–99.9)	NA	NA
		Self (n=1,628)	ND	100.0 (1,628/1,628) (99.8–100.0)	NA	NA
	NA	Contrived (n=100)	100[¶] (50/50) (92.9–100)	100[#] (50/50) (92.9–100)	NA	NA
TV	8.2 (135/1,638)	Clinician (n=1,600)	93.1[**] (121/130) (87.4–96.3)	99.3[††] (1,459/1,470) (98.7–99.6)	91.8 (86.4–95.6)	99.4 (98.9–99.7)
		Self (n=1,610)	93.2 (124/133) (87.6–96.4)	99.3 (1,466/1,477) (98.7–99.6)	91.8 (86.4–95.6)	99.4 (98.9–99.7)

PPV, positive predictive value; NPV, negative predictive value; BV, bacterial vaginosis; NA, not applicable; ND, no data, TV, *Trichomonas vaginalis*; “Clinician,” clinician-collected specimens, “Self,” self-collected specimens; “Contrived,” samples spiked with isolated strains.

Data are % (n/N), % (n/N) (95% confidence interval), or % (95% confidence interval).

[*] *Cgroup=Candida albicans, Candida dubliniensis, Candida parapsilosis,* and *Candida tropicalis*.

[†] *Candida* group sensitivity is 80.0% (140/175) (95% CI 73.5–85.3%) and 96.7% (322/333) (95% CI 94.2–98.2%) when *Candida* group load was less than 2+ and 2+ or greater after vaginal specimen culture, respectively, for clinician-collected swabs. All *Candida* group strains isolated from culture and frozen were successfully detected by the investigational test when retesting the isolated strain directly.

[‡] Forty-three of 65 false-positive investigational test specimens were confirmed positive for *Candida* group by a molecular amplification method.

[§] *Candida glabrata* sensitivity is 40.0% (2/5) (95% CI 11.8–76.9%) and 83.3% (20/24) (95% CI 64.1–93.3%) when *C glabrata* load was less than 2+ and 2+ or greater after vaginal specimen culture, respectively, for clinician-collected swabs.

[//] All five false-positive investigational test specimens yielded low *C glabrata* load by a molecular amplification method.

[¶] Positive percent agreement.

[#] Negative percent agreement.

[**] Seven of nine false-negative investigational test specimens were confirmed negative with the TV molecular comparator and two were positive with the TV molecular comparator. The latter two specimens were positive with the TV culture device test after 4 days of incubation.

††Ten of 11 false-positive investigational test specimens were confirmed positive with the TV molecular comparator.