Clinical validation of the Aptima Bacterial Vaginosis and Aptima Candida/Trichomonas Vaginitis Assays: results from a prospective multi-center clinical study

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ABSTRACT (256 words)

Infectious vaginitis due to bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and Trichomonas vaginalis accounts for a significant proportion of all gynecologic visits in the United States. A prospective multicenter clinical study was conducted to validate the performance of two new in vitro diagnostic transcription-mediated amplification nucleic acid amplification tests (NAAT) for diagnosis of BV, VVC, and trichomoniasis. Patient- and clinician-collected vaginal swab samples obtained from women with symptoms of vaginitis were tested with the Aptima BV and Aptima CV/TV assays. Results were compared to Nugent score (plus Amsel for intermediate Nugent) for BV, Candida cultures and DNA sequencing for VVC, and a composite of a NAAT and culture for T. vaginalis. The prevalence of infection was similar for clinician- and patient-collected samples: 49% for BV, 29% for VVC due to Candida species group, 4% for VVC due to C. glabrata, and 10% for T. vaginalis. Sensitivity and specificity estimates for the investigational tests in clinician-collected samples were, respectively, 95.0% and 89.6% for BV, 91.7% and 85.8% for Candida species group, 84.7% and 99.1% for C. glabrata, and 96.5% and 98.9% for T. vaginalis. Sensitivity and specificity were similar in patient-collected samples. In a secondary analysis, clinician’s diagnosis, in-clinic assessments, and investigational assay results were compared to gold standard reference methods. Overall, the investigational assays had higher sensitivity and specificity than clinician’s diagnoses and in-clinic assessments, indicating the investigational assays were more predictive of infection than traditional diagnostic methods. These results provide clinical efficacy evidence for two IVD NAATs that can detect the main causes of vaginitis.
INTRODUCTION

Vaginitis is responsible for as much as 50% of all gynecologic visits in the United States (US) and represents a major contributor to healthcare expenses (1). Infectious vaginitis due to bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis accounts for up to 90% of these cases (2). Unlike trichomoniasis, both BV and VVC are attributable to several pathogens.

For VVC, overgrowth of C. albicans is predominant, although other Candida species including C. glabrata may contribute as well (3). BV is harder to diagnose because the pathogenesis involves decreased levels of Lactobacillus bacteria concomitant with increased concentrations of BV-associated bacteria such as G. vaginalis, Mobiluncus spp., and A. vaginae (4, 5).

Various diagnostic methods are available to identify the underlying cause of vaginitis. In the clinician’s office, a combination of pH, a potassium hydroxide (KOH) test, and microscopic examination of fresh samples of vaginal discharge are routinely used, despite their relatively poor performance (1, 6). For BV, diagnosis often relies on the use of either clinical Amsel criteria or Gram stain and Nugent score (considered the gold standard laboratory method for diagnosis of BV). Examination of wet mount with KOH preparation and/or vaginal cultures for Candida are the most common diagnostic tools for VVC. Highly sensitive and specific nucleic acid amplification tests (NAATs) are recommended for detecting T. vaginalis, but examination of wet mount preparations is still commonly used in clinical practice (6). However, several barriers are associated with the use of non-molecular methods, including lack of equipment in the clinic, subjectivity of clinical endpoints used and inconsistent employment between practitioners, lack of proper training in microscopy, and overall poor sensitivity of the tests (7-9). Diagnosis of the underlying cause of vaginitis is further complicated by the common
symptomatology reported for BV, VVC, and trichomoniasis (2, 6), the incidence of mixed or co-
infections (10-12), and the recurrence of vaginal symptoms (13-16).

Taken together, these barriers result in many women being misdiagnosed based on non-specific
observations, leading to incorrect, misguided, or prolonged treatment (7, 9). This article reports
the results from the clinical validation of two newly developed US Food and Drug
Administration (FDA) -cleared NAATs for the detection of infectious vaginitis. The study also
compares the investigational assay results, in-clinic testing results, and clinician’s diagnosis to
reference methods for BV, Candida spp. group, C. glabrata, and T. vaginalis.

MATERIAL AND METHODS

Study design and ethics approval. A multi-center, cross-sectional diagnostic accuracy study
was conducted to evaluate the clinical performance of two NAATs, the Aptima BV and Aptima
CV/TV assays. The studies were conducted in accordance with the ethical principles derived
from the Declaration of Helsinki and Belmont Report and in compliance with the FDA and Good
Clinical Practice Guidelines set forth by the International Conference on Harmonization (ICH-
E6). The study protocols were approved by the local institutional review board at every site.

Written informed consent was obtained from each subject at the time of enrollment, prior to
specimen collection. Participants were compensated for study participation.

Study Population. Subjects at least 14 years of age with symptoms of vaginitis (eg, abnormal
vaginal discharge, vaginal odor, genital itching or irritation, pain/discomfort during sexual
intercourse or urination, edema, or erythema) were eligible for enrollment. Subjects were
enrolled at 21 US sites (clinical research centers and emergency medicine, family planning,
public health, sexually transmitted infection (STI), family medicine/obstetric-gynecologic clinics) between June and October 2018. Exclusion criteria included use of douches, vaginal deodorants or intravaginal products within 48 hours of enrollment, or prior enrollment in the study.

For each subject, the collection site provided subject demographic and clinical data, including the following: clinician’s diagnosis, and subject-reported date of birth, sex, ethnicity, race, and symptoms of STIs, pregnancy status, menstrual status, recent (ie, within 24 hours) unprotected sexual intercourse, HIV diagnosis, history of recurrent symptoms of vaginitis within 12 months, and use of feminine products within 4 weeks.

**Sample Collection.** Six vaginal swab samples were collected in the clinic from each patient during routine clinical visits (Figure 1) using a predetermined rotating order: two investigational assay swabs (one patient-collected and one clinician-collected; Aptima Multitest swab; Hologic, Inc.; San Diego, CA), one patient-collected swab for *T. vaginalis* NAAT testing (Xpert Vaginal/Endocervical swab; Cepheid; Sunnyvale, CA), one clinician-collected swab for *Candida* spp. culture (BD BBL CultureSwab EZ; Becton, Dickinson and Company; Sparks, MD), and one clinician-collected cotton swab each for Nugent score/Amsel criteria and *T. vaginalis* culture. All swab samples were collected according to package insert directions and instructions for use for each collection device.

Each investigational swab was tested with both the Aptima BV and Aptima CV/TV assays on the Panther system (Hologic, Inc.; San Diego, CA). Paired investigational swab samples were split evenly among three U.S. laboratories. The Aptima BV assay reported positive or negative results for BV based on a mathematical algorithm analysis of ribosomal RNA detection of *Lactobacillus*
species, *G. vaginalis*, and *A. vaginae*. The Aptima CV/TV assay reported positive or negative results based on the detection of RNAs for: 1) *Candida* species group (*C. albicans, C.
*dubliniensis, C. parapsilosis, C. tropicalis*), 2) *C. glabrata*, and 3) *T. vaginalis*.

For BV, the reference method was comprised of a consensus Nugent score, and modified Amsel criteria if necessary. Using modified Amsel criteria, the presence of ≥20% clue cells together with either vaginal pH greater than 4.5 (from chromatic paper touched to the swab) or a positive whiff test (potassium hydroxide test on the swab) indicated BV infection. For each subject, a single vaginal swab first was smeared on a glass microscope slide to prepare the Nugent scoring slide, and then used to complete Amsel evaluation. The slide was then Gram-stained and assigned a Nugent score based on the relative combined concentration of gram-positive rods (eg, *Lactobacillus* spp.), gram-variable/negative rods and cocci (eg, *G. vaginalis, Bacteroides* spp.), and curved gram-variable rods (eg, *Mobiluncus* spp.) characteristic of bacterial vaginosis (19).

The sum of the three components was calculated for the final Nugent score and interpretation; using Nugent criteria, a sample with a total score of 7 or more was considered BV positive, a score of 3 or less was considered BV negative, and a score of 4 to 6 was considered intermediate. Each Gram stained slide was independently reviewed by three different reviewers at a single reference laboratory, blinded to each other’s interpretations. If the BV interpretations (positive, negative, intermediate) agreed for at least two reviewers, consensus was reached and the Nugent interpretation was final. Disagreement across all three reviewers was resolved via panel review of the same slide at a multi-headed microscope. If the final consensus Nugent interpretation was intermediate, modified Amsel criteria, which excluded abnormal discharge, were used to determine BV status.
For candidiasis, the reference method was comprised of yeast culture, and PCR/bidirectional sequencing for a subset of specimens. For each subject, a single vaginal swab was used to inoculate two different culture media at a single reference laboratory: Sabouraud Dextrose Agar and CHROMagar Candida (CHROMagar; Paris, France), both read after 48 hours. The growth level on both media was reported as no colony, 1+, 2+, 3+ and 4+ (n+ represented the number of quadrants showing Candida growth). For subjects with a positive culture result on either media, identification of the isolated yeast species was performed by bidirectional sequencing of the its2 gene (20) using both clinician- and patient-collected Aptima vaginal swab samples; an isolated Candida species was determined to be present if it was detected on either swab.

For T. vaginalis, the reference method was comprised of the Xpert TV assay (Cepheid; Sunnyvale, CA) and the InPouch TV culture system (Biomed Diagnostics, Inc.; White City, OR). The Xpert TV assay swab sample from each subject was tested at a single reference laboratory. For each subject, one cotton swab was used to inoculate the InPouch TV system at the clinical site; each inoculated pouch was incubated within 48 hours at a single reference laboratory, and readings performed daily for up to 7 days. If either test was positive for a subject, the subject’s status was “infected”; both reference tests had to be negative to establish a “noninfected” status. Aptima swab samples leftover after Aptima CV/TV assay testing were tested with the Aptima Trichomonas vaginalis assay in the case of discordance between the investigational assay result and the TV infection status.

**In-clinic Assessments.** For this study, the following in-clinic testing was performed and documented for vaginitis. For BV, Amsel criteria were assessed individually (pH >4.5, clue cells ≥20% of total cells, or positive Whiff test), or as a group with (Original Amsel) or without
(Modified Amsel) abnormal vaginal discharge factored in. For candidiasis, KOH test results were assessed. Results from standard in-clinic testing for *T. vaginalis*, such as wet mount microscopy, were not documented for this study; InPouch culture performed at a central laboratory was used as a surrogate for in-clinic testing.

**Clinician Diagnosis.** Diagnosis was based on the clinician’s assessment of in-clinic testing or other standard of care testing (eg, wet mount for *T. vaginalis*, detection of pseudohyphae for *Candida*), clinical signs, patient-reported symptoms, and subject history.

**Statistical Methods.** Prevalence, sensitivity, specificity, positive predictive value, and negative predictive value were calculated according to standard equations. Analyses were performed separately for clinician- and patient-collected Aptima swab samples. The confidence intervals for sensitivity and specificity were calculated using the score method (21). The confidence intervals for positive and negative predictive values were calculated based on exact confidence intervals for the ratio of two independent binomial proportions (22). Samples with inconclusive reference results and samples with invalid or missing investigational assay results were excluded from the analyses. Analyses were performed with SAS software (version 9.4; SAS Institute Inc, Cary, NC).
RESULTS

Study Design and Subject Accountability

There were 1519 subjects enrolled for participation in both studies. Of these, 17 subjects were withdrawn for various reasons, including ineligibility to participate or self-termination for participation. An additional 85 subjects were excluded from the Aptima BV analysis (Nugent not evaluable \( n=58 \) or available \( n=26 \), intermediate Nugent score could not be resolved \( n=1 \)), and 6 subjects were excluded from the Aptima CV/TV analysis (withdrawn reference samples \( n=4 \), invalid Aptima CV/TV results for both swabs \( n=1 \), no swabs collected \( n=1 \)). A total of 1501 subjects were evaluable for BV and/or CV/TV and are included in the evaluable study population; 1417 subjects were evaluable for BV and 1496 for CV/TV in at least one sample type.

Table 1 provides summarized patient-reported demographics and clinical characteristics of the evaluable study population. Most subjects were between 18 and 39 years of age (69.4%), black or African American (50.2%), from southeast or southwest clinical centers (66.0%), and enrolled at clinical research centers or obstetrics and gynecology clinics (75.3%). Most of the subjects were not pregnant (98.2%), were of reproductive age but not actively menstruating (81.5%) or had not been diagnosed with HIV (97.7%). The most frequently reported symptoms were abnormal vaginal discharge (81.9%), vaginal odor (56.8%), or genital itching, irritation or soreness (62.2%). Almost 60% of the evaluable subjects had a history of recurrent symptoms of vaginitis within 12 months. A minority of the subjects (20.3%) reported the use of feminine products (eg, douches, tampons, deodorants) within 4 weeks of collection.

Infection Rates
Single and multiple infection rates by BV-associated organisms, *Candida* species group, *C. glabrata*, and *T. vaginalis* are shown in Table 2 based on detection in samples tested by reference methods or in clinician- and patient-collected samples tested with the investigational assays. Overall, infection rates identified by the reference methods and the investigational assays were similar. Using reference methods, BV alone was detected in 31.9% of the total subjects, *Candida* species group alone in 13.9%, *C. glabrata* alone in 0.9%, trichomoniasis alone in 1.8%, and no infection in 31.1%. Infections rates were similar for the investigational assays in clinician- and patient-collected swabs. Coinfection rates by 2 or more organisms were 20% by reference testing and approximately 25% by investigational testing.

Prevalence and Clinical Performance

BV, candidiasis, and trichomoniasis prevalence and the clinical performance of the investigational assays are shown in Table 3 by target for both clinician- and patient-collected samples. The overall prevalence of infection was similar for clinician- and patient collected vaginal swabs. The prevalence was highest for BV (49%), followed by candidiasis due to *Candida* species group (29%). For clinician-collected samples, sensitivity and specificity against reference method samples were 95.0% and 89.6% for BV, 91.7% and 94.9% for *Candida* species group, 84.7% and 99.1% for *C. glabrata*, and 96.5% and 95.1% for *T. vaginalis*. For patient-collected samples, sensitivity and specificity against reference method samples were 97.3% and 85.8% for BV, 92.9% and 91.0% for *Candida* species group, 86.2% and 98.7% for *C. glabrata*, and 97.1% and 98.9% for *T. vaginalis*. 
Clinical sensitivity and specificity of clinician’s diagnosis, in-clinic assessments, and the investigational assays was assessed compared to gold standard reference methods: Nugent score for BV, culture for VVC, and NAAT testing for trichomoniasis. Paired clinical sensitivity and specificity estimates for each target are shown in Figure 2. Overall, the investigational assays had higher sensitivity and specificity than clinician’s diagnosis and in-clinic assessments. For BV, sensitivity and specificity were ≥96.2% and ≥92.4%, respectively for the investigational assay samples, compared to 83.4% and 85.5% for clinician’s diagnosis, 75.9% and 94.4% for original Amsel criteria, 81.1% and 90.1% for modified Amsel criteria, and ≤82.8% and ≤91.1% for any of the individual Amsel criteria components (vaginal pH, clue cells, whiff test). For VVC due to Candida species group or C. glabrata, sensitivity and specificity were ≥91.2% and ≥98.9%, respectively for the investigational assay samples, compared to ≤27.9% and ≤56.4% for potassium hydroxide testing and ≤54.9% and ≤85.5% for clinician’s diagnosis. For trichomoniasis, sensitivity was ≥96.4% for the investigational assay samples compared to 78.8% for culture and 38.1% for clinician’s diagnosis; specificity estimates were greater than 95% for all trichomoniasis detection methods. Supplemental Table 1 shows the positive and negative predictive values for the same comparisons; in general, the investigational assays were more predictive of infection than clinical assessments and clinician’s diagnosis.

DISCUSSION

Vaginitis affects millions of women annually and is the primary reason women in the US visit their doctor (1). Women suffering from vaginitis are often underserved by the current paradigm of inaccurate or incomplete diagnosis guiding inadequate or inappropriate treatment. The resultant prolonged or recurrent vaginal dysbiosis impacts quality of life and has implications for
preterm birth, acquisition/transmission of STIs and HIV, increased risk of pelvic inflammatory disease, and neonatal infections (23-31). Further, correct diagnosis of trichomoniasis is imperative in that sexual partners require treatment for control of this STI (6, 32-33).

The investigational assays described here are FDA-cleared for the differential diagnosis of BV, VVC, and trichomoniasis in symptomatic women; the tests are approved for use with both clinician-collected and patient-collected vaginal swabs. The results of these studies showed comparable sensitivity and specificity between clinician- and patient-collected samples for BV, VVC, VVC attributed to C. glabrata, and T. vaginalis. The investigational tests allow differential diagnosis of the 3 primary causes of vaginitis from a single vaginal swab rather than requiring the collection of multiple specimens to support microscopy, culture, KOH, and other methods. Specimen handling and contamination risk is minimized, with the swab placed directly into a sealed collection tube for testing on the automated instrument. Specimen integrity and stability for long term storage is maintained by the stabilizing buffer within the collection tube.

Current in-clinic methods for diagnosing BV, VVC, and trichomoniasis present challenges that impact diagnostic accuracy and treatment. For BV, accurate diagnosis involves identifying the shift from a protective lactobacillus-dominated vaginal environment to pathogenic anaerobe bacterial growth (34). Microbes indicated as causative agents in BV such as Gardnerella, Atopobium, Prevotella, and others are present in both women with and without the current definition of BV, so mere detection will not provide adequate specificity. The subjectivity of Nugent scoring of a Gram stain is apparent by the need of consensus scoring to improve accuracy, something not practical in clinical practice. Microscopy also presents challenges in terms of training, expertise and equipment and many clinicians have defaulted to empiric
diagnosis and treatment leading to incorrect management of infectious vaginitis. Multiplexed
amplified molecular methods offer a viable alternative, where multiple microbes can be
accurately quantitated at very high numbers (10^6 CFU/mL or more), allowing the retraction of
lactobacilli and overgrowth of Gardnerella, Atopobium, and other microbes in BV to be
objectively measured, analyzed and assessed. For diagnosis of candidiasis, culture is routinely
performed but is time consuming, labor intensive and often lacks the sensitivity and species level
identification of molecular methods (35, 36). Accurate identification of azole-resistant species
such as C. glabrata is also important in guiding appropriate treatment. In the case of TV, wet
mount must be performed within 10 minutes of sample collection while T. vaginalis is alive and
motile; a positive wet mount result requires organisms to be motile, not merely present.

Molecular methods are capable of overcoming many of these challenges, providing objective and
reproducible results. Multiplex capabilities allow for differential diagnoses for BV, VVC, and
trichomoniasis, unaffected by coinfection status. Concordance between the clinician- and patient-
collected swabs for the investigational tests described here also indicate these tests are robust
against sample collection, preparation and interpretation artifacts which can confound diagnoses.
The patient-collected swab also allows the potential for patient comfort and convenience while
streamlining clinical workflow.

Strengths of the study include the broad geographic US distribution of enrolled subjects,
ensuring a robust evaluation of the investigational tests in these populations. The use of multiple
reference methods for all indications (BV: Nugent + Amsel to adjudicate Nugent intermediates;
VVC: 2 cultures + PCR/Bidirectional sequencing of culture positives; *T. vaginalis*: Composite of culture + NAAT) improves accuracy and reduces potential bias towards any single metric.

Limitations include limited representation by certain ethnic groups, primarily Asians and Pacific Islanders (which were grouped with ‘Other’). Molecular methods are also highly specific, potentially impacting the sensitivity to disease attributed to minor species (eg, *Prevotella, C. krusei*) not included in the assay design.

The availability of highly sensitive and specific NAAT for vaginitis that can be performed on automated analyzers are a welcome addition to previously available tests. The investigational molecular assays offer sensitive and specific detection of vaginitis and are capable of providing differential diagnoses of multiple etiologies from a single vaginal swab. In addition, the differentiation of *C. glabrata* from other species causing VVC may be helpful in guiding therapy for this infection which is often azole resistant. These types of tests can provide objective tools for the clinician to accurately diagnose and treat their patients.

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Figure 1. Sample workflow and testing algorithm. Red boxes denote sample preparation or handling. Shaded boxes denote testing for each swab.

Table 1. Demographic and clinical characteristics for evaluable subjects.

Table 2. Single and multiple infection rates determined by clinical reference method and investigational NAAT.

Table 3. Overall investigational assay performance

Figure 2. Comparison of clinical performance versus reference standards. Paired clinical sensitivity and specificity estimates (with corresponding 95% CI values) for Aptima results, clinician’s diagnoses, and clinical assessments compared to laboratory method diagnostic reference standards. (A) Detection of bacterial vaginosis compared to Nugent score. (B) Detection of *Candida spp. group* infection compared to culture. (C) Detection of *C. glabrata* infection compared to culture. (D) Detection of *T. vaginalis* compared to NAAT (Xpert TV assay). Note: Some Specificity error bars are too small to be visible.

Original Amsel = Positive if at least 3 of the following: 1) clinician-reported signs of abnormal vaginal discharge that is thin and white; 2) pH >4.5; 3) clue cells ≥20% total cells' 4) positive Whiff test. Modified Amsel = Positive if clue cells ≥20% total cells and either pH >4.5 or positive Whiff test.

CVS = clinician-collected swab, PVS = patient-collected swab
Six (6) vaginal swab samples were collected from each eligible symptomatic subject during the study visit.

- **Aptima:** Patient-collected swab
- **Aptima:** Clinician-collected swab
- **Candida Culture:** Clinician-collected swab
- **TV NAAT:** Patient-collected swab
- **TV Culture:** Clinician-collected swab
- **Nugent/Amidol:** Clinician-collected swab

**Steps in central laboratory:**
- Sort and send to Panther testing sites
- Complete Aptima BV and Aptima CV/TV testing
- Use for additional testing (if needed):
  - Sequence if Candida culture positive or if discordant
  - Second TV NAAT testing if discordant

**Steps in reference laboratory:**
- Incubate agar within 48 hours of collection
- Complete Candida culture (Sabouraud Dextrose agar, Chromogenic agar)
- Daily observation for up to 5 days over 7-day period
- Nugent score: 3 readers (panel if needed)

Complete (in the following order): pH, clue cell, and whiff test evaluations (at collection site).

Prepare Gram stain

Prepare Nugent slide
Table 1. Demographics and clinical characteristics for Evaluable Subjects

| Category                        | Value |
|---------------------------------|-------|
| Age (years)                     |       |
| Median (min-max)                | 33.0  |
| Mean ± SD                       | 35.3 ± 11.74 |
| 14-17                           | 5 (0.3%) |
| 18-29                           | 554 (36.9%) |
| 30-39                           | 485 (32.3%) |
| 40-49                           | 247 (16.5%) |
| ≥50                             | 210 (14.0%) |
| Race/Ethnicity                  |       |
| Asian                           | 74 (4.9%) |
| Black / African American        | 753 (50.2%) |
| White (Hispanic/Latino)         | 269 (17.9%) |
| White (Not Hispanic/Latino)     | 341 (22.7%) |
| Other                           | 64 (4.3%) |
| Geographic Region               |       |
| Mid-Atlantic                    | 244 (16.3%) |
| Northeast                       | 220 (14.7%) |
| Northwest                       | 49 (3.3%) |
| Southeast                       | 571 (38.0%) |
| Southwest                       | 417 (27.8%) |
| Site Type                       |       |
| Clinical research center        | 659 (43.9%) |
| Family planning clinic          | 282 (18.8%) |
| Hospital system high-risk STI clinic | 18 (1.2%) |
| Obstetrics & gynecology clinic  | 467 (31.1%) |
| Public health clinic            | 75 (5.0%) |
| Subject-reported Symptoms<sup>a</sup> |       |
| Abnormal vaginal discharge      | 1230 (81.9%) |
| Vaginal odor                    | 853 (56.8%) |
| Genital itching/irritation/burning/soreness | 933 (62.2%) |
| Pain during sex and/or urination| 371 (24.7%) |
| Edema                           | 127 (8.5%) |
| Erythema                        | 138 (9.2%) |
| Other                           | 30 (2.0%) |
| Pregnant                        |       |
| Yes                             | 21 (1.4%) |
| No                              | 1474 (98.2%) |
| Unspecified                     | 6 (0.4%) |
| Menstrual Status                |       |
| With menses                     | 120 (8.0%) |
| Without menses                  | 1224 (81.5%) |
| Post-menopausal                 | 157 (10.5%) |
| Diagnosed with HIV              |       |
| Yes                             | 14 (0.9%) |
| No                              | 1467 (97.7%) |
| Unspecified                     | 20 (1.3%) |
| History of Recurrent Symptoms Within 12 Months |
|----------------------------------------------|
| **No** 623 (41.5%)                          |
| **Yes** 874 (58.2%)                          |

| 1-2 occurrences | 396 (45.9%) |
| 3-4 occurrences | 242 (28.1%) |
| >4 occurrences  | 224 (26.0%) |

| Use of Feminine Products Within 4 Weeks |
|----------------------------------------|
| **Yes** 305 (20.3%)                    |
| **N/A = not applicable**               |
| **2** Includes patient-reported other, mixed, and unknown races. |
| **3** May report multiple responses.    |

| 423 (41.5%) | 62 (8.2%) |

2

on March 22, 2020 by guest
Table 2. Single and multiple infection rates determined by clinical reference method and investigational NAAT.

|                  | Reference Method (N=1365) | Investigational NAAT                  |
|------------------|---------------------------|---------------------------------------|
|                  |                           | Clinician-collected (N=1491) | Patient-collected (N=1480) |
|                  | n (%)                     |                           |                           |
| All Negative     | 425 (31.1)               | 430 (28.8)                 | 399 (27.0)                 |
| BV, Cspp, Cglab, TV | 3 (0.2)            | 2 (0.1)                     | 4 (0.3)                     |
| BV only          | 435 (31.9)               | 427 (28.6)                 | 456 (30.8)                 |
| BV, Cspp         | 135 (9.9)                | 166 (11.1)                 | 202 (13.6)                 |
| BV, Cglab        | 4 (0.3)                  | 7 (0.5)                     | 12 (0.8)                    |
| BV, TV           | 71 (5.2)                 | 108 (7.2)                   | 80 (5.4)                    |
| BV, Cspp, Cglab  | 8 (0.6)                  | 8 (0.5)                     | 8 (0.5)                     |
| BV, Cspp, TV     | 19 (1.4)                 | 25 (1.7)                    | 29 (2.0)                    |
| BV, Cglab, TV    | 1 (0.1)                  | 2 (0.1)                     | 1 (0.1)                     |
| Cspp only        | 190 (13.9)               | 212 (14.2)                  | 212 (14.3)                  |
| Cspp, Cglab      | 22 (1.6)                 | 13 (0.9)                    | 15 (1.0)                    |
| Cspp, TV         | 12 (0.9)                 | 15 (1.0)                    | 16 (1.1)                    |
| Cspp, Cglab, TV  | 3 (0.2)                  | 2 (0.1)                     | 1 (0.1)                     |
| Cglab only       | 12 (0.9)                 | 28 (1.9)                    | 27 (1.8)                    |
| Cglab, TV        | 0 (0.0)                  | 1 (0.1)                     | 0 (0.0)                     |
| TV only          | 25 (1.8)                 | 45 (3.0)                    | 18 (1.2)                    |

BV = bacterial vaginosis, Cspp = Candida species group, Cglab = C. glabrata, TV = T. vaginalis

* The summary in each column includes only subjects with valid conclusive results for all four analytes.
Table 3. Overall investigational assay performance

| Target                  | Prevalence | Specimen Type (n) | Sensitivity % (95% CI) | Specificity % (95% CI) |
|-------------------------|------------|-------------------|------------------------|------------------------|
| BV                      | 49.2%      | Clinician-collected (1413) | 95.0 (93.1-96.4) | 660/695 | 89.6 (87.1-91.6) | 643/718 |
|                         | 49.3%      | Patient-collected (1405) | 97.3 (95.8-98.2) | 673/692 | 85.8 (83.1-88.2) | 612/713 |
| Candida spp. group      | 28.6%      | Clinician-collected (1485) | 91.7 (88.7-94.0) | 389/424 | 94.9 (93.4-96.1) | 1007/1061 |
|                         | 28.6%      | Patient-collected (1477) | 92.9 (90.0-95.0) | 392/422 | 91.0 (89.1-92.6) | 960/1055 |
| C. glabrata             | 4.0%       | Clinician-collected (1483) | 84.7 (73.5-91.8) | 50/59 | 99.1 (98.4-99.5) | 1411/1424 |
|                         | 3.9%       | Patient-collected (1475) | 86.2 (75.1-92.8) | 50/58 | 98.7 (98.0-99.2) | 1399/1417 |
| T. vaginalis            | 9.9%       | Clinician-collected (1438) | 96.5 (92.0-98.5) | 137/142 | 95.1 (93.8-96.2) | 1233/1296 |
|                         | 9.8%       | Patient-collected (1433) | 97.1 (92.9-98.9) | 136/140 | 98.9 (98.2-99.4) | 1279/1293 |

Candida species group = C. albicans, C. dublieniensis, C. parapsilosis, and C. tropicalis.

* Of the 35 false negative results, 10 were Nugent intermediates and had BV infection status determined by modified Amsel criteria, and 15 were negative by modified Amsel criteria.

* Of the 75 false positive results, 46 subjects were Nugent intermediates and had BV infection status determined by modified Amsel criteria, and 6 were positive by modified Amsel criteria.

* Of the 19 false negative results, 6 subjects were Nugent intermediates and had BV infection status determined by modified Amsel criteria, and 7 were negative by modified Amsel criteria.

* Of the 101 false positive results, 55 subjects were Nugent intermediates and had BV infection status determined by modified Amsel criteria, and 9 were positive by modified Amsel criteria.

* All 9 samples with false negative results showed no growth of C. glabrata on chromogenic agar.

* Of the 13 samples with false positive results, 2 showed high (4+) growth, 2 showed low (≤2+) growth, and 9 showed no growth of C. glabrata on chromogenic agar.

* Of the 8 samples with false negative results, 7 showed no growth and 1 showed high (4+) growth of C. glabrata on chromogenic agar.

* Of the 18 samples with false positive results, 2 showed high (4+) growth, 2 showed low (≤2+) growth, and 14 showed no growth of C. glabrata on chromogenic agar.

* Of the 5 samples with false negative results, 3 were confirmed negative with the Aptima Trichomonas vaginalis assay.

* Of the 63 samples with false positive results, 56 were confirmed positive with the Aptima Trichomonas vaginalis assay.

* Of the 4 samples with false negative results, 3 were confirmed negative with the Aptima Trichomonas vaginalis assay.

* Of the 14 samples with false positive results, 8 were confirmed positive with the Aptima Trichomonas vaginalis assay.