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Molecular Testing for *Trichomonas vaginalis* in Women: Results from a Prospective U.S. Clinical Trial▽†

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Trichomoniasis is a common sexually transmitted disease associated with preterm birth, low birth weight, and increased susceptibility to infection with other pathogenic sexually transmitted microorganisms. Nucleic acid amplification tests for *Trichomonas vaginalis* have improved sensitivity for detecting infected individuals compared to existing culture-based methods. This prospective, multicenter U.S. clinical trial evaluated the performance of the automated Aptima *T. vaginalis* assay for detecting *T. vaginalis* in 1,025 asymptomatic and symptomatic women. Vaginal swab, endocervical swab, ThinPrep PreservCyt, and urine specimens were collected. Subject infection status was determined by wet-mount microscopy and culture. Aptima *T. vaginalis* assay performance was determined for each specimen type by comparison to subject infection status. Of 933 subjects analyzed, 59.9% were symptomatic. Aptima *T. vaginalis* clinical sensitivity and specificity were, respectively, 100% and 99.0% for vaginal swabs, 100% and 99.4% for endocervical swabs, 100% and 99.6% in ThinPrep samples, and 95.2% and 98.9% in urine specimens. Aptima *T. vaginalis* performance levels were similar in asymptomatic and symptomatic subjects. This study validates the clinical performance of the Aptima *T. vaginalis* assay for screening asymptomatic and symptomatic women for *T. vaginalis* infection.

Trichomoniasis, a common sexually transmitted disease (STD) caused by the protozoan *Trichomonas vaginalis*, affects approximately 180 million persons per year worldwide, making it the most common nonviral STD agent in the world. An estimated 7.4 million new cases occur annually in the United States (1), and the disease has an overall prevalence of 3.1% (24). Both women and men can be infected, although symptoms are more common in women. Symptomatic women have a diffuse, malodorous, yellow-green vaginal discharge with vulvar irritation which may be confused with bacterial vaginosis. Infected men may temporarily have urethral irritation, mild discharge, or slight burning after urination or ejaculation (5). Many infections do not produce symptoms and when left untreated may lead to preterm birth, low birth weight, and pelvic inflammatory disease (5). *T. vaginalis* infection also increases susceptibility to infection with HIV (14, 22, 23). Effective and inexpensive antibiotic therapy for *T. vaginalis* infection is readily available, and detection and treatment of *T. vaginalis* in symptomatic or asymptomatic women with a high risk of STD are important to prevent disease acquisition, transmission, and associated complications.

Currently, the gold standard for the diagnosis of *T. vaginalis* infection is culture; however, the sensitivity of commercially available culture has been reported to be 75% to 89% compared to amplified methods (13, 20). Tests with improved sensitivity are needed to diagnose this prevalent STD. The Aptima *Trichomonas vaginalis* assay, an FDA-cleared, fully automated nucleic acid amplification test, has demonstrated high sensitivity and specificity compared to culture (20). Herein, we report the results of a large prospective multicenter trial designed to determine the sensitivity and specificity of this new assay in multiple specimen types from women.

MATERIALS AND METHODS

Subjects and study conduct. This clinical trial enrolled 1,025 women attending participating U.S. obstetrics and gynecology (OB/GYN), family planning, or STD clinics (collection sites). Subjects 14 years of age or older were enrolled in the study if they demonstrated symptoms consistent with a suspected STD, such as vaginal odor, vaginal discharge, vaginal/vulvar itching, pain/discomfort during sexual intercourse or urination, and/or lower abdominal discomfort; were asymptomatic and had sexual contacts with persons with confirmed or suspected STD(s); were asymptomatic and undergoing screening evaluation for possible STDs; or were undergoing routine Papanicolaou (Pap) screening. Subjects were excluded if they took antibiotic medications within 14 days of enrollment. Subjects signed Institutional Review Board (IRB)-approved informed consent before being enrolled in the study. The protocol of this study was approved by IRBs from all participating sites.

Specimen collection and processing. At each of the 9 collection sites, 6 specimens were collected from each subject as follows (in order of collection): 1 first-catch urine, 3 vaginal swabs, 1 endocervical swab, and 1 ThinPrep PreservCyt liquid cytology cervical specimen (collected with a broom-like device or an endocervical brush and spatula). Per the study protocol, a 20- to 30-ml urine specimen was self-collected by each subject; all other specimens were clinician collected. One vaginal swab specimen was tested using wet-mount microscopic examination, and another vaginal swab specimen was cultured with the InPouch *T. vaginalis* test (BioMed Diagnostics, White City, OR) at the collection site's laboratory or a designated laboratory (if the site did not routinely perform culture with the InPouch *T. vaginalis* test) to determine infection status. The

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order of the vaginal sample collected for use in the Aptima *T. vaginalis* assay, wet mount, or culture was randomized for each patient.

One first-catch urine, 1 vaginal swab, 1 endocervical swab, and 1 ThinPrep specimen were processed for Aptima *T. vaginalis* assay testing by the collection site in accordance with the appropriate package insert instructions as follows. An aliquot of the urine specimen was transferred into the Aptima urine specimen transport tube and stored at 2°C to 30°C. The vaginal swab specimen was placed into an Aptima vaginal swab specimen collection kit tube and stored at 2°C to 30°C. The endocervical swab was placed into an Aptima unisex swab specimen collection kit tube and stored at 2°C to 30°C. The ThinPrep endocervical specimen was processed by immersing and rinsing the collection device (broom-like device or endocervical brush and spatula) in PreservCyt solution in the ThinPrep Pap test vial; an aliquot was transferred into an Aptima specimen transfer kit tube, and the processed ThinPrep sample was stored at 2°C to 8°C. Approximately one-third of all processed samples were then shipped to 1 of 3 designated testing sites.

Sample evaluation. (i) Wet-mount microscopic examination and InPouch *T. vaginalis* test culture. Collection site laboratories performed wet-mount microscopic examination using a vaginal swab specimen. In general, the specimen on the swab was treated with a saline solution and examined under a microscope for motile trichomonads. The collection site's laboratory performed culture with the InPouch *T. vaginalis* test for all except three sites, for which the InPouch *T. vaginalis* test was performed at an outside laboratory. The pouch was examined daily for the presence of motile trichomonads in accordance with the package insert instructions (2) or for up to 5 days. Technicians performing these procedures were blind to molecular test results.

(ii) Aptima *T. vaginalis* assay. The Aptima *T. vaginalis* assay is a nucleic acid amplification test that utilizes target capture, transcription-mediated amplification (TMA), chemiluminescent probe hybridization, and the automated Tigris DTS system to detect *T. vaginalis* 18s rRNA. Processed samples were tested in accordance with the investigational Aptima *T. vaginalis* assay package insert instructions (9). Each Aptima *T. vaginalis* assay testing site tested approximately one-third of the study samples and used 3 lots of Aptima *T. vaginalis* assay reagent kits over the course of the study. Operators performing the Aptima *T. vaginalis* assay were blind to the subjects' clinical data and wet-mount microscopic examination and culture results. Aptima *T. vaginalis* assay results were not used for determining treatment or patient care.

(iii) Discordant analysis. Supplemental testing of discordant specimens was conducted using (i) a characterized real-time *T. vaginalis* PCR assay for the detection of *T. vaginalis* DNA (10), and (ii) an alternate TMA (Alt-TMA) *T. vaginalis* assay developed at Gen-Probe which has been previously used to resolve *T. vaginalis* test discordant results (18–20). This alternate assay uses unique primer, probe, and target capture oligomers to target a different region of the *T. vaginalis* 18s rRNA (20). A cutoff for *T. vaginalis* positivity of 100,000 RLU was used with the Alt-TMA *T. vaginalis* assay for consistency with the Aptima *T. vaginalis* assay. Both the PCR and Alt-TMA *T. vaginalis* assays exhibit analytical sensitivity of <1 organism/reaction.

Infection status. The infection status was established by testing 2 vaginal swab specimens with wet-mount microscopic examination and by culturing with the InPouch *T. vaginalis* test. The infection status was considered positive if the InPouch *T. vaginalis* test and/or the wet-mount microscopic examination result was positive. The infection status was considered negative if both InPouch *T. vaginalis* test and wet-mount microscopic examination results were negative. The infection status was considered indeterminate if either the InPouch *T. vaginalis* test or the wet-mount microscopic evaluation result was missing and the other test result was negative.

Data analyses: Aptima *T. vaginalis* assay performance evaluation. Samples that were not handled in accordance with the Aptima *T. vaginalis* assay package insert instructions or the clinical protocol and samples with invalid Aptima *T. vaginalis* assay results or indeterminate infection status were excluded from the analyses ($n = 354$); this allowed estimation of the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Aptima *T. vaginalis* assay. The score method was used for calculating the 2-sided 95% confidence intervals (CIs) for sensitivity and specificity. The exact method was used to compute the 2-sided 95% CIs for PPV and NPV based on the positive and negative likelihood ratios. Analyses were also stratified by collection site, symptom status, and age group (women 14 to 17 years of age versus women 18 years or older).

RESULTS

Subject disposition. A total of 1,025 women were enrolled at 9 sites; of these women, 26 were withdrawn because they did not meet study eligibility criteria, and 66 were excluded because the samples exceeded the recommended storage period before testing ($n = 48$) or did not have a conclusive infection status ($n = 18$). Thus, 933 of 1,025 (91.0%) subjects were included in the evaluable analysis set. These subjects produced 3,343 samples (735 urine, 875 vaginal swab, 920 endocervical swab, and 813 ThinPrep samples) with valid Aptima *T. vaginalis* assay results, which were included in the final analysis. A detailed subject disposition is presented in Fig. 1.

Subject demographic and disease characteristics. Subjects' demographic and clinical characteristics are presented in Table 1. The median age of the 933 evaluable subjects was 24.0 years (range: 14 to 67 years). Overall, 59.6% of the subjects were black/African American and 33.8% were white. The majority of subjects were symptomatic (59.9%). The most frequently reported symptom was vaginal discharge (75.1%), followed by vaginal odor (43.3%) and vaginal/vulvar itch (32.9%). Overall, 36.0% of the subjects were diagnosed with vaginosis, 15.9% with vaginitis, and 7.1% with cervicitis. The prevalence of *T. vaginalis* infection in this population ranged from 11.4% in both urine (84/735) and ThinPrep (93/813) samples to 12.7% (111/875) in vaginal swab samples (Table 2).

Aptima *T. vaginalis* assay performance in different specimen types in all women. The sensitivity, specificity, NPV, and PPV of the Aptima *T. vaginalis* assay in the different specimen types are presented in Table 2. Sensitivity values for the detection of *T. vaginalis* ranged from 95.2% using urine samples to 100% using vaginal swab, endocervical swab, or ThinPrep samples; the negative predictive values for *T. vaginalis* detection in these sample types ranged from 99.4% (urine) to 100% (vaginal swab, endocervical swab, ThinPrep). The specificity of the Aptima *T. vaginalis* assay for the detection of *T. vaginalis* in these samples was also high, ranging from 98.9% (urine) to ≥99% for vaginal swab, endocervical swab, or ThinPrep samples; the positive predictive values for these samples ranged from 92.0% to 96.9%. There was no significant difference between the sensitivity and specificity of detecting *T. vaginalis* in urine samples and those for other sample types ($P > 0.05$).

Analysis of false-negative and false-positive Aptima *T. vaginalis* assay results. Twenty-seven samples (0.8%) had discordant results in the Aptima *T. vaginalis* assay compared with the infection status; of these, 4 were false negatives (all urine) and 23 were classified as false positives. The PCR and Alt-TMA *T. vaginalis* assays were used to provide supplemental information on the 27 samples with discordant Aptima *T. vaginalis* assay results relative to the established infection status. The testing results were not used to modify the calculated performance characteristics of the Aptima *T. vaginalis* assay.

(i) Analysis of the 4 false-negative samples. Four urine specimens were negative by the Aptima *T. vaginalis* assay despite a positive infection status for these 4 subjects. All 4 subjects had positive Aptima *T. vaginalis* assay results for the other 3 specimen types. These 4 urine samples were also negative when tested with the Alt-TMA *T. vaginalis* assay and with PCR (Table 3), suggesting that these samples lacked *T. vaginalis* nucleic acids.

(ii) Analysis of false-positive samples. Twenty-three samples (7 urine, 8 vaginal swab, 5 endocervical swab, and 3 ThinPrep samples) with false-positive results were observed in 10 subjects with negative infection status. Nine of these 10 subjects

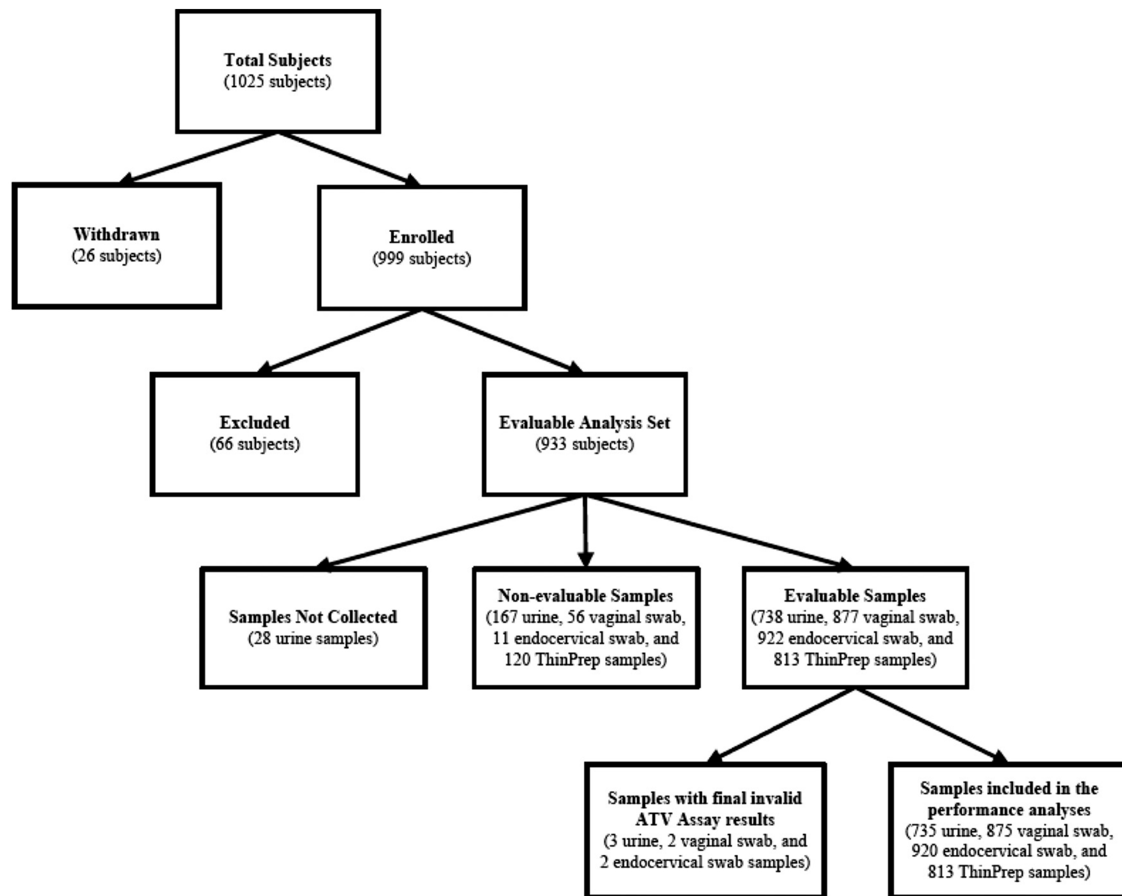

FIG. 1. Of 1,025 subjects enrolled at 9 sites, there were 933 evaluable subjects (91.0%), from which we planned to collect 3,732 samples (933 subjects with 4 samples each). Of these, 382 (10.2%) samples were either missing or considered nonevaluable (expired). Of the 3,350 evaluable samples (738 urine, 877 vaginal swab, 922 endocervical swab, and 813 ThinPrep samples), 3,343 (99.8%) had final valid results; seven (0.2%; 3 urine, 2 vaginal swab, and 2 endocervical swab) samples had final invalid results and were not included in the analyses. ATV, Aptima *T. vaginalis*.

had positive Aptima *T. vaginalis* assay results in at least 2 sample types. The *T. vaginalis* PCR assay also produced 5 positive results for the specimens considered false positive (1/7 for urine, 1/8 for vaginal swab, 2/4 for endocervical swab, and 1/3 for ThinPrep samples) (Table 3). Alt-TMA *T. vaginalis* assay test results were positive for all vaginal swab, endocervical swab, and ThinPrep samples and for 5 of the 7 urine samples with false-positive Aptima *T. vaginalis* assay results. Overall, these results indicate the presence of the *T. vaginalis* rRNA target in those specimens (Table 3).

Aptima *T. vaginalis* assay performance by symptom status. Overall, the sensitivities, specificities, and negative predictive values of the Aptima *T. vaginalis* assay were similar in asymptomatic and symptomatic subjects, despite the prevalence of *T. vaginalis* infection in symptomatic women (15.1% to 16.4%) being higher than its prevalence in asymptomatic women (6.5% to 7.0%) (Table 2). The PPV for urine and vaginal swabs was slightly lower (by approximately 6 to 10 percentage points) in asymptomatic subjects than in symptomatic subjects (Table 2). The performance of the Aptima *T. vaginalis* assay levels were very similar for samples collected from adolescent women and adult women and for samples collected from different geographic locations (data not shown).

DISCUSSION

This study was conducted to validate Aptima *T. vaginalis* assay performance characteristics for the detection of *T. vaginalis* in self-collected first-catch urine specimens and clinician-collected endocervical swab, vaginal swab, and endocervical ThinPrep samples, with standard wet-mount and culture methods used as the reference. *T. vaginalis* is widely prevalent in the U.S. population and is associated with important public health sequelae such as preterm birth and acquisition/transmission of HIV. Control of trichomoniasis has been hampered by the lack of sufficiently sensitive diagnostic tests. The most commonly used method is visualization of motile trichomonads in a saline preparation from the vaginal swab (wet-mount microscopic examination). Although this test is rapid and inexpensive, it has two important limitations: it must be performed within 10 to 20 min of collection of the specimen (or the organisms lose viability), and it has a low sensitivity (ranging from 60% to 70%) as compared to culture (5). A commercial culture

TABLE 1. Subject demographic and clinical characteristics

Characteristic	Value
Total no.	933
Age, yr	
Mean (SD)	27.0 (9.50)
Median	24.0
Min-max	14–67
Age groups, no. (%)	
14–17	81 (8.7)
18–20	177 (19.0)
21–25	284 (30.4)
26–30	136 (14.6)
31–35	88 (9.4)
36–40	76 (8.1)
>40	91 (9.8)
Ethnicity, no. (%)	
Hispanic or Latino	93 (10.0)
Not Hispanic or Latino	836 (89.6)
Unknown/refused	4 (0.4)
Race, no. (%)[a]	
White	315 (33.8)
Black or African American	556 (59.6)
Asian	8 (0.9)
American Indian/Alaska native	10 (1.1)
Unknown/refused	58 (6.2)
Symptom status, no. (%)	
Asymptomatic	374 (40.1)
Symptomatic	559 (59.9)
Reported symptom (symptomatic subjects), no. (%)[b]	
Vaginal odor	242 (43.3)
Vaginal discharge	420 (75.1)
Vaginal/vulvar itch	184 (32.9)
Pain/discomfort during sexual intercourse	33 (5.9)
Lower abdominal discomfort	99 (17.7)
Pain/discomfort during urination	54 (9.7)
Other	89 (15.9)
Clinical diagnosis, no. (%)	
Vaginosis	336 (36.0)
Vaginitis	148 (15.9)
Cervicitis	66 (7.1)
Urethritis/cystitis	12 (1.3)
Pelvic inflammatory disease	8 (0.9)
Unknown	73 (7.8)
Other[c]	446 (47.8)

[a] Subjects may have reported multiple races.
[b] Subjects may have reported multiple symptoms.
[c] Includes diagnosis of "normal."

method (InPouch *T. vaginalis* test) has a reported sensitivity of approximately 80% and a specificity of 100% compared with wet mount (21) or with Diamond's modified medium (17). Trichomonads may be visualized on the Pap smear, but this also has a limited sensitivity (approximately 60%) (15). Other tests for *T. vaginalis* include the OSOM *Trichomonas* rapid test (Genzyme Diagnostics, Cambridge, MA) and the Affirm VP III (Becton Dickenson, San Jose, CA). Both tests are performed on vaginal secretions with a sensitivity of >83% and a specificity of >97% compared with culture or wet mount (3, 4, 8, 12, 13).

In this study, the use of the highly sensitive Aptima *T. vaginalis* assay found that the prevalence of *T. vaginalis* infection ranged from 11.4% in urine and ThinPrep specimens to 12.7% in vaginal swabs. These values are higher than the national average of 3.6% (24), possibly because of the high proportion of symptomatic subjects enrolled in this study (55.1%) who presented for screening for a possible STD or who had a partner with an STD. The prevalence values reported here also agree with previously reported *T. vaginalis* molecular test prevalence rates of 11.3% to 28.7% (11, 13, 19, 20).

The Aptima *T. vaginalis* assay sensitivity (95.2% in urine samples and 100% in endocervical swab, vaginal swab, and ThinPrep samples) and specificity (~99%) values reported herein (Table 2) are similar to those reported in previous publications (11, 13, 18, 20). In the present study, the overall Aptima *T. vaginalis* assay sensitivity (≥95.2% in any specimen type) was higher than published sensitivity estimates for the wet-mount microscopic examination (60% to 70% [6]) and culture using the InPouch *T. vaginalis* test (81% and 82.4% [17, 21]). This is in agreement with studies that evaluated the analyte-specific-reagent-formatted Aptima *T. vaginalis* assay and these two reference assays in side-by-side comparisons (13, 20).

The performance levels of the Aptima *T. vaginalis* assay were nearly identical when testing endocervical ThinPrep specimens, endocervical swab samples, and vaginal swab samples. Performance of the Aptima *T. vaginalis* assay was somewhat lower in self-collected urine samples; however, the difference in assay sensitivity between vaginal or cervical (100%) and urine samples (95.2%) was not statistically significant. This finding is in agreement with results from Nye et al. (20), who reported a slightly but not significantly lower sensitivity for

TABLE 2. Performance of Aptima *T. vaginalis* assay in the different specimens by symptom status

Aptima *T. vaginalis* assay specimen	Symptom status	No. of samples[a]					% Prevalence[b]	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
		Total	TP	FP	TN	FN					
Urine	Asymptomatic	324	21	3	299	1	6.8	95.5 (78.2–99.2)	99.0 (97.1–99.7)	87.5 (71.4–96.9)	99.7 (98.4–100)
	Symptomatic	411	59	4	345	3	15.1	95.2 (86.7–98.3)	98.9 (97.1–99.6)	93.7 (85.7–98.1)	99.1 (97.7–99.8)
	All women	735	80	7	644	4	11.4	95.2 (88.4–98.1)	98.9 (97.8–99.5)	92.0 (85.1–96.4)	99.4 (98.5–99.8)
Vaginal swab	Asymptomatic	345	24	4	317	0	7.0	100 (86.2–100)	98.8 (96.8–99.5)	85.7 (70.3–95.6)	100 (98.9–100)
	Symptomatic	530	87	4	439	0	16.4	100 (95.8–100)	99.1 (97.7–99.6)	95.6 (89.5–98.8)	100 (99.2–100)
	All women	875	111	8	756	0	12.7	100 (96.7–100)	99.0 (97.9–99.5)	93.3 (87.6–97.0)	100 (99.5–100)
Endocervical swab	Asymptomatic	372	26	1	345	0	7.0	100 (87.1–100)	99.7 (98.4–99.9)	96.3 (82.4–99.9)	100 (99.0–100)
	Symptomatic	548	88	4	456	0	16.1	100 (95.8–100)	99.1 (97.8–99.7)	95.7 (89.6–98.8)	100 (99.2–100)
	All women	920	114	5	801	0	12.4	100 (96.7–100)	99.4 (98.6–99.7)	95.8 (90.7–98.6)	100 (99.6–100)
ThinPrep	Asymptomatic	353	23	0	330	0	6.5	100 (85.7–100)	100 (98.8–100)	100 (86.2–und[c])	100 (99.0–100)
	Symptomatic	460	70	3	387	0	15.2	100 (94.8–100)	99.2 (97.8–99.7)	95.9 (88.9–99.1)	100 (99.1–100)
	All women	813	93	3	717	0	11.4	100 (96.0–100)	99.6 (98.8–99.9)	96.9 (91.4–99.3)	100 (99.5–100)

[a] TP, true positive; FP, false positive; TN, true negative; FN, false negative.
[b] (TP + FN)/*n*.
[c] und, undefined; the upper boundary of the confidence interval could not be calculated.

TABLE 3. Discordant test result analysis with PCR and Alt-TMA *T. vaginalis* assays

Specimen type	Aptima *T. vaginalis* assay results	Infection status	Interpretation[a]	Total no.	No. with indicated *T. vaginalis* result by: PCR[b]		Alt-TMA	
					Positive	Negative	Positive	Negative
Urine	Positive	Negative	FP	7	1	6	5	2
	Negative	Positive	FN	4	0	4	0	4
Vaginal swab	Positive	Negative	FP	8	1	7	8	0
Endocervical swab	Positive	Negative	FP	5[b]	2	2	5	0
ThinPrep	Positive	Negative	FP	3	1	2	3	0

[a] FP, false positive; FN, false negative.
[b] One of the 5 false-positive samples was not analyzed by PCR.

urine samples (87.5%) than for vaginal and endocervical swab samples (96.6% and 89.8%, respectively) by use of the Aptima *T. vaginalis* assay in conjunction with a molecular test-resolved algorithm.

In the present study, 4 (0.43%) subjects had urine samples with negative Aptima *T. vaginalis* assay results and positive results for the other specimen types, indicating that these urine results were false negatives. The rate of urethral colonization in women with a vaginal *T. vaginalis* infection has been estimated at ~75% (16). Thus, it is possible that these 4 subjects had *T. vaginalis* infections in the vagina (they had positive vaginal samples) but were not colonized in the urethra (negative urine samples). Alternatively, it is possible that the presence of only a few trichomonad cells in the urethra or urinary meatus at the time of voiding could have resulted in very low cell concentrations in the urine sample, thus leading to sampling error upon addition of the 2-ml urine aliquot to the urine transport tube. However, in a separate study, we performed quantitative molecular testing for *T. vaginalis* in 38 urine samples obtained in this trial and found that the median trichomonad cell load was 311 cells/ml of urine, with a mean of 2,040 cells/ml of urine (data not shown). Since the analytical sensitivity of the Aptima *T. vaginalis* assay is less than 0.1 cell/ml in urine samples, we conclude that these 4 women likely were infected with *T. vaginalis* in the vagina but not in the urinary tract.

In spite of the slightly lower sensitivity of the Aptima *T. vaginalis* assay in detecting *T. vaginalis* in urine specimens, testing of urine is still of clinical benefit due to the high clinical sensitivity shown and the ease of collection of this sample type. Because first-catch urine is a noninvasive, self-collected specimen that does not require a pelvic examination, it represents a convenient specimen collection method for screening large populations, especially in STD clinics and institutional settings.

The specificity of the Aptima *T. vaginalis* assay was high, ranging from 98.9% in urine samples to 99.6% in ThinPrep samples. False-positive Aptima *T. vaginalis* assay results were observed in 10 of 933 (1.1%) subjects. However, 9 of these 10 subjects had positive Aptima *T. vaginalis* assay results in at least 2 other specimen types, suggesting that these 9 subjects were actually infected with trichomonads. Some of these specimens were also found positive by the *T. vaginalis* PCR assay and most were positive with the Alt-TMA *T. vaginalis* assay, confirming the presence of *T. vaginalis* rRNA target and indicating that these samples were likely true positives. These findings imply that the true clinical specificity of the Aptima *T. vaginalis* assay is actually higher than reported here (98.9% to 99.6%). This issue typically occurs when the performance of the evaluated assay is calculated based on reference assays with inherently lower sensitivity and has been previously reported for TMA-based assays for *Chlamydia trachomatis* detection (7).

Importantly, the performance levels were of the Aptima *T. vaginalis* assay were similar in symptomatic and asymptomatic subjects, in adolescent and adult women, and in samples obtained from different collection sites, indicating that the Aptima *T. vaginalis* assay has broad clinical utility and consistent performance for the detection of *T. vaginalis* and diagnosis of trichomoniasis in many female populations. In addition, the ability to use multiple specimen types in the Aptima *T. vaginalis* assay provides clinicians with more options for *T. vaginalis* testing, which is an important advantage over wet-mount examination or culture methods that use vaginal swabs only. Moreover, the ability to use this highly accurate molecular test on a fully automated instrumentation system represents an effective solution for large-scale screening for *T. vaginalis* infections in certain populations.

In summary, the results from this study validate the clinical performance characteristics of the Aptima *T. vaginalis* assay using self-collected first-catch urine specimens and clinician-collected endocervical swab, vaginal swab, and endocervical ThinPrep specimens from symptomatic and asymptomatic women. The superior performance of this method compared to that of the reference tests (wet-mount microscopic examination and culture) should improve the screening, diagnosis, and treatment of *T. vaginalis* infection.

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