BASIC SCIENCE: GYNECOLOGY

Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women

Melinda B. Nye, PhD; Jane R. Schwebke, MD; Barbara A. Body, PhD

OBJECTIVE: We evaluated the performance characteristics of APTIMA *Trichomonas vaginalis* (ATV) transcription-mediated amplification (TMA) for diagnosis of *T vaginalis* (TV) infection from female vaginal swab, endocervical swab, and urine specimens and from male urethral swab and urine specimens. Performance of ATV TMA was compared with wet mount microscopy, culture, and polymerase chain reaction (PCR).

STUDY DESIGN: In all, 296 female and 298 male subjects who attended the Jefferson County Health Department sexually transmitted diseases clinic were enrolled in the study and provided specimens for each test. Results were analyzed using 3 interpretative algorithms.

RESULTS: For women, vaginal swab ATV TMA was significantly more sensitive than wet mount or culture. In male subjects, urethral swab ATV TMA was significantly more sensitive than culture or PCR.

CONCLUSION: ATV TMA provides a sensitive, commercially available nucleic acid amplification test for improved diagnosis of TV in male and female patients.

Key words: sensitivity, specificity, transcription-mediated amplification, *Trichomonas vaginalis*, trichomoniasis

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Trichomoniasis is a sexually transmitted infection (STI) caused by the protozoan *Trichomonas vaginalis* (TV). Although TV infection is not a reportable disease in the United States, an estimated 7.4 million new infections occur each year,[1] or approximately double the number of *Chlamydia* and gonorrhea infections estimated to be acquired annually. TV infection may present as vaginitis in women and urethritis in men; however, it is frequently asymptomatic. TV infection may cause adverse health consequences, including preterm labor and pelvic inflammatory disease in women, as well as infertility and increased incidence of human immunodeficiency virus transmission in women and men.[2-7]

Both the lack of testing and the absence of an ideal diagnostic test hamper TV diagnosis. No guidelines for TV testing have been published. Wet mount microscopy, which is performed in the clinic setting on vaginal fluid, has high specificity but low sensitivity. Despite its imperfect performance, the wet mount is most frequently used to diagnose TV in women. Wet mount microscopy is not used for TV diagnosis in male subjects because of poor sensitivity. Culture is considered the traditional gold standard laboratory test for TV diagnosis in men and women but requires 5 days for completion. Two Food and Drug Administration (FDA)-approved point-of-care tests, Affirm VP III (BD Diagnostic Systems, Sparks, MD) and OSOM *Trichomonas* rapid test (Genzyme Diagnostics, Cambridge, MA), have been shown to be more sensitive than vaginal wet mount microscopy for TV diagnosis[8-10] but are limited to vaginal specimens.

Nucleic acid amplification tests (NAATs) have been developed for detection of TV infections in both men and women and combine excellent performance characteristics with a more rapid turnaround time compared with culture. Laboratory-developed polymerase chain reaction (PCR) tests have been shown to be superior to culture for diagnosis of TV in male subjects using first-fraction urine and urethral swab specimens.[11-13] PCR with female genital specimens has also detected more positive patients than culture or wet mount. In these studies, PCR was consistently more sensitive than either culture or wet mount microscopy using vaginal specimens.[13-16] Cervical specimens and female first-fraction

From Laboratory Corporation of America, Burlington, NC (Drs Nye and Body); and the Department of Medicine, University of Alabama at Birmingham School of Medicine, Birmingham, AL (Dr Schwebke).

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Reprints: Melinda B. Nye, PhD, Laboratory Corporation of America, 1447 York Ct., Burlington, NC 27215. Nyem@labcorp.com.

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TABLE 1
Subject demographics

Demographic	Female (n = 296)	Male (n = 298)
Race/ethnicity		
African American, not Hispanic/Latino	266	279
White, not Hispanic/Latino	24	15
Other	6	4
Age range (y)		
< 20	30	18
20-24	100	94
25-29	83	83
30-39	66	59
≥ 40	16	44
Not collected	1	0

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urine have also identified more positive patients than culture or wet mount.[13] Currently, there is no commercially available, FDA-cleared NAAT for TV.

APTIMA (Gen-Probe Inc, San Diego, CA) assays use target capture and transcription-mediated amplification (TMA) to selectively purify, amplify, and detect species-specific 16S ribosomal RNA. The APTIMA technology is a common methodology for *C trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) NAAT, with 28% of respondents on a recent national laboratory proficiency survey reporting the use of APTIMA for CT/NG NAAT.[17] APTIMA CT/NG testing has high analytic sensitivity, has low inhibition rates, can use a variety of specimen types, and can be performed on an automated platform. APTIMA TV (ATV) analyte-specific reagents (ASRs) provide a method for developing a qualitative NAAT for TV. The purpose of this study was to evaluate the performance characteristics of ATV TMA NAAT for diagnosis of TV from female vaginal swab, endocervical swab, and urine specimens and from male urethral swab and urine specimens. Performance of ATV TMA was compared with wet mount, culture, and PCR. Test results for each specimen type were analyzed using infected patient status algorithms, with and without PCR, and a molecular-resolved status algorithm.

MATERIALS AND METHODS

Study population

All adult women and men aged 18 years or older who attended the Jefferson County Health Department sexually transmitted diseases (STD) clinic (Birmingham, AL) were invited to participate in this prospective study. Subjects were excluded from the study if they had taken an antibiotic within the last 14 days, had urinated within 1 hour prior to the collection of the urine specimen, or could not provide all required specimens. The study protocol was approved by the University of Alabama, Birmingham Institutional Review Board and Western International Review Board (Olympia, WA). All subjects provided informed consent. Women were enrolled from January to August 2006; men were enrolled from March to November 2006. Patient demographics are described in Table 1.

Specimen collection and processing

Each female participant had 2 vaginal swabs, 1 endocervical swab, and 1 (25-mL) first-fraction voided urine collected for study purposes. One vaginal swab was also collected per routine care for wet mount microscopy. For male participants, 2 urethral swabs and 1 (25-mL) first-fraction urine specimen were collected for study purposes. Specimen collection order was randomized weekly to diminish bias introduced by collection order and clinician.

One vaginal swab was placed into a Gen-Probe Inc APTIMA vaginal swab transport media tube and the endocervical swab was placed into the Gen-Probe Inc APTIMA unisex swab transport media tube for NAAT analysis. A male urethral swab was added to a Gen-Probe Inc APTIMA unisex swab transport media for NAAT analysis. For both male and female subjects, a 2-mL aliquot of first-void urine was added to the Gen-Probe Inc APTIMA urine transport media for NAAT analysis. Specimens for NAAT were sent to the reference laboratory for testing by TMA and PCR.

Clinical data

Enrolled male and female subjects characterized themselves as symptomatic or asymptomatic. Male subjects who reported sexual contact with a partner diagnosed with TV were noted. The clinician recorded the presence of cervical discharge, vaginal discharge, and vaginal itch/irritation, if observed, for female subjects and signs of urethritis in male subjects, if present.

Specimen testing

Wet mount microscopy was performed, as previously described, using a vaginal swab at the STD clinic per standard of care.[18] Culture was performed at the STD clinic on vaginal swab or combined male urethral swab/urine sediment specimens using the InPouch TV culture test (Biomed Diagnostics, White City, OR) per the manufacturer's instructions. Microscopic examination for motile trichomonads was performed daily, for up to 5 days.

Specimens were tested by ATV TMA using APTIMA general-purpose reagents, including target capture, amplification, enzyme, probe, and selection reagents, combined with ATV ASR oligonucleotides. The reconstituted amplification reagent was supplemented with 50 μL of ATV ASR oligonucleotides 1 and 2 and 50 μL of a 1/10,000 dilution of oligonucleotide 3. The ATV assay was performed on the semiautomated direct tube sampling (DTS) platform, as described in the APTIMA Combo 2 (Gen-Probe Inc) test package insert. An arbitrary cutoff of 100,000 relative light U (RLU) was used to determine the reactivity of specimens tested with ATV TMA. Specimens with a test result < 100,000 RLU were considered negative. Specimens with a test result ≥ 100,000 RLU were considered positive.

All APTIMA swab and urine specimens were also tested using a LabCorp-developed real-time PCR assay targeting the TV β-tubulin gene using the LightCycler (Roche Applied Science, Indianapolis, IN).[19] PCR-negative specimens