Rapid Antigen Testing Compares Favorably with Transcription-Mediated Amplification Assay for the Detection of *Trichomonas vaginalis* in Young Women

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**Background.** Diagnosis of *Trichomonas vaginalis* (TV) infection is limited by imperfect testing methods. Newer tests, such as rapid antigen and nucleic acid amplification tests, are often compared with culture, which is not widely used but is more sensitive than wet mount. We assessed the sensitivity and specificity of 4 tests for the identification of TV using 3 statistical approaches.

**Methods.** Sexually active adolescent women aged 14–21 years (*n* = 330) were recruited from a teen health center and emergency department. Vaginal swabs were tested for TV using wet mount, culture (InPouch TV; Biomed Diagnostics), rapid antigen testing (OSOM TV; Genzyme Diagnostics), and transcription-mediated amplification testing (TMA; APTIMA TV analyte specific reagents; Gen-Probe).

**Results.** TV was detected in 61 participants (18.5%). Compared with a composite reference standard (i.e., any TV test with positive results), the sensitivities of wet mount, culture, rapid antigen testing, and TMA were 50.8%, 75.4%, 82%, and 98.4%, respectively. Using latent class analysis, the sensitivity of wet mount (56%) was significantly lower than that of other tests, and the sensitivities of culture and rapid antigen testing were similar (83% and 90%, respectively); specificity was 100% for each of these 3 methods. TMA had a sensitivity of 98.2% and a specificity of 98%. Tests performed equally well regardless of whether the participant had bleeding or other infections. The sensitivities of the rapid antigen test and TMA were comparable (92.5% and 97.5%, respectively) in women who had vaginal symptoms.

**Conclusions.** Wet mount alone is insufficient for the reliable diagnosis of TV infection in women. TMA and rapid antigen tests are highly sensitive and specific, and both are superior to wet mount. Rapid antigen testing is equivalent to culture, and it compares favorably with the sensitivity of TMA for the detection of TV.

Although epidemiologic data are sparse, among adolescent women, infection with *Trichomonas vaginalis* (TV) appears to be more common than infection with *Neisseria gonorrhoea* (GC) and is nearly as prevalent as infection with *Chlamydia trachomatis* (CT) [1, 2]. However, this infection is often overlooked, perhaps because of a lack of understanding of the clinical consequences of infection. For example, there are no published recommendations for TV testing, whereas they exist for CT and GC [3, 4]. In addition, the development of diagnostic methods for TV infection has lagged behind methods for CT and GC infection. Because there are both behavioral and biological risks associated with all sexually transmitted infections (STIs) for adolescent women, and because this population is often excluded from initial trials of new diagnostic testing methods, they are an important group in which to study diagnostic methods for determining TV infection.

Wet mount, the most commonly used diagnostic method for detecting TV infection, is relatively insensitive (36%–70%), compared with culture [5–7]. How-
ever, culture is not widely used; although it is assumed to have perfect specificity, sensitivity can be as low as 70%–85% [8–10]. Recently, an objective rapid antigen detection test for TV was developed (OSOM TV; Genzyme Diagnostics) [11, 12]. In addition, several nucleic acid amplification tests perform quite well in research settings [2, 13, 14]. One nucleic acid amplification test that uses transcription-mediated amplification (TMA; APTIMA TV analyte–specific reagents; Gen-Probe) is commercially available but has not been cleared by the US Food and Drug Administration. Despite the limited sensitivities of wet mount and culture, a combination of these methods is often used as the comparator for new tests, resulting in biased specificity estimates for the new tests. In addition, the effects of clinical factors on test characteristics are not known.

In this study, we assessed the sensitivity and specificity of 4 diagnostic methods for the detection of TV using vaginal swabs obtained from adolescent women and 3 statistical approaches. In addition, we examined whether test sensitivity was affected by vaginal symptoms, bleeding, or other STIs.

SUBJECTS, MATERIALS, AND METHODS

We recruited a convenience sample of female adolescent patients from a teen health center and emergency department between July 2004 and June 2006. Young women aged 14–21 years who presented with genitourinary symptoms (vaginal itching or discharge, dysuria, abnormal vaginal bleeding, or lower abdominal or pelvic pain) or risk of STI (sexual intercourse without a condom, ≥2 sexual partners in the past 3 months, or contact with an individual with an STI) and who had a history of heterosexual, vaginal intercourse in the past 6 months were eligible. Subjects who had used metronidazole within the 2 weeks prior to presentation were excluded. The study was approved by the hospital’s Institutional Review Board. The use of a waiver of parental permission for subjects aged <18 years was instituted in May 2005.

Participants were interviewed confidentially regarding demographic characteristics, specific symptoms, history of STIs, condom use, number of sexual partners, and number of new sexual partners in the past 3 months. During the pelvic examination, a speculum was inserted, and 4 swabs from the vaginal fornices and any other clinically indicated endocervical swabs were obtained. Interview data and clinical findings were recorded on a data collection instrument.

Laboratory assessment. Endocervical swabs were tested for CT using the BD ProbeTec ET strand displacement assay (Becton Dickinson). Endocervical swabs were tested for GC using either Thayer-Martin culture or the BD ProbeTec ET assay at the clinician’s discretion. Vaginal swabs were collected in random order for TV testing, as described below.

Wet mount was performed as a part of routine clinical care. In the teen center, a clinician performed the wet mount analysis in an in-office laboratory. All clinicians are required to take a yearly wet mount proficiency test. In the emergency department, wet mounts were transported to the clinical laboratory and examined by medical technologists. Wet mounts were considered to be positive for TV if motile trichomonads were observed. Because wet mounts were a part of routine clinical care, the time interval between specimen collection and microscopy was not recorded.

A second vaginal swab was tested using the OSOM TV rapid antigen test according to the manufacturer’s instructions. A blue test line and a red control line indicated positive results. Tests that displayed only a red control line were considered to have negative results. Rapid antigen tests and wet mounts were performed independently.

A third vaginal swab was used to inoculate InPouchTV culture media. Cultures were examined daily by a trained microscopist up to 5 days after inoculation or until a positive result (i.e., observation of motile trichomonads) was obtained.

The fourth vaginal swab was placed in a dry test tube and maintained at −80°C until recruitment was complete. Swabs were transferred to buffer in APTIMA vaginal swab specimen collection tubes and were stored at −80°C until testing. Specimens were tested in a Gen-Probe DTS 400 instrumentation system using TV analyte–specific reagents with APTIMA General Purpose Reagents. TV analyte–specific reagents include target capture, TMA, and hybridization protection, using primers and probes that specifically target TV rRNA. Reagents were reconstituted as described in the GPR product insert. Fifty μL of each oligonucleotide (1 target capture oligonucleotide, 3 amplification oligonucleotides, and 1 probe oligonucleotide) was added to the appropriate general purpose reagent component, except for TV analyte–specific reagent amplification oligonucleotide 3, which was diluted to 1:10,000 before 50 μL of the solution was added to the reconstituted amplification reagent. Purified TV nucleic acids and sterile water were used as positive and negative controls, respectively. TMA testing was performed using 400 μL of processed vaginal swab specimens, as described in the APTIMA Combo 2 kit product insert (Gen-Probe). Gen-Probe Data Acquisition Software returns assay result values in relative light units (RLUs) emitted by the hybridized labeled probe and detected by the Leader HC+ luminometer. We retested all samples with RLU values of >10,000. Samples with at least 1 TMA result of >30,000 RLUs were considered to be positive.

Statistical analysis. Data were analyzed using statistical software (Stata software, version 8; Statacorp). Differences in subject characteristics between those with and those without TV were compared using the χ² test for dichotomous variables and Student’s t test for continuous variables.

To assess sensitivity and specificity, we used the following 2 reference standards to define trichomoniasis: (1) the traditional
standard, which is defined as a positive culture or wet mount result, assumes that both tests are 100% sensitive and specific; and (2) the composite reference standard, which is positive if any test for TV had positive results and negative if all tests (wet mount, culture, rapid antigen, and TMA) had negative results, assumes 100% specificity for each test and allows for the comparison of relative sensitivities. In addition, we examined test performance of all 4 tests simultaneously using latent class analysis [15]. Latent class analysis uses maximum likelihood estimation to determine the combination of sensitivity and specificity for each test that is most likely to yield the observed test results, assuming conditional independence.

For TMA, we compared reliability between initial and repeat RLU values using Spearman’s correlation coefficient. We performed a nonparametric receiver-operating characteristics analysis to examine sensitivity and specificity at different RLU cutoffs, compared with any positive non-TMA test result.

**RESULTS**

**Patient population.** We recruited 376 young women; 330 had complete laboratory data. The mean age of participants was 17.7 years, 84% were black, and 63% reported experiencing at least 1 vaginal symptom (table 1). In 28 patients (8.5%), all 4 tests had results positive for TV infection; in 269 patients (81.5%), all test results were negative. TV was detected by wet tests had results positive for TV infection; in 269 patients least 1 vaginal symptom (table 1). In 28 patients (8.5%), all 4

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 330)</th>
<th>TV present† (n = 61)</th>
<th>TV absent (n = 269)</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years ± SD</td>
<td>17.7 ± 1.6</td>
<td>17.7 ± 1.7</td>
<td>17.8 ± 1.6</td>
<td>.672</td>
</tr>
<tr>
<td>Age ≥18 years</td>
<td>142 (43.0)</td>
<td>25 (41.0)</td>
<td>117 (43.5)</td>
<td>.721</td>
</tr>
<tr>
<td>Black race</td>
<td>271 (82.1)</td>
<td>57 (93.4)</td>
<td>214 (79.6)</td>
<td>.011</td>
</tr>
<tr>
<td>Recruited from ED</td>
<td>187 (56.7)</td>
<td>41 (67.2)</td>
<td>146 (54.3)</td>
<td>.066</td>
</tr>
<tr>
<td>Vaginal symptomsd</td>
<td>210 (63.6)</td>
<td>40 (65.6)</td>
<td>170 (63.2)</td>
<td>.728</td>
</tr>
<tr>
<td>Any symptomsg</td>
<td>294 (89.1)</td>
<td>58 (95.1)</td>
<td>236 (87.7)</td>
<td>.096</td>
</tr>
<tr>
<td>New sexual partner in the past 3 months</td>
<td>100 (30.3)</td>
<td>23 (37.7)</td>
<td>77 (28.6)</td>
<td>.164</td>
</tr>
<tr>
<td>&gt;1 Sexual partner in the past 3 months</td>
<td>86 (26.1)</td>
<td>21 (34.4)</td>
<td>65 (24.2)</td>
<td>.099</td>
</tr>
<tr>
<td>History of prior STI</td>
<td>205 (62.1)</td>
<td>41 (67.2)</td>
<td>164 (61.0)</td>
<td>.364</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em> infection †</td>
<td>79 (24.5)</td>
<td>25 (43.4)</td>
<td>54 (20.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><em>Neisseria gonor rhoea</em> infectiong</td>
<td>35 (10.8)</td>
<td>14 (23.7)</td>
<td>21 (7.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Either <em>C. trachomatis</em> or <em>N. gonorrhoea</em> infection†</td>
<td>97 (30.1)</td>
<td>30 (50.8)</td>
<td>67 (25.5)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. ED, emergency department; STI, self-reported sexually transmitted infection including chlamydia, gonorrhea, trichomoniasis, genital herpes, or genital warts.

† TV present by composite reference standard (i.e., positive results with any TV test).

‡ Determined using the y2 test, except where indicated.

§ Determined using Student’s t test.

d Including vaginal discharge, itching, or pain.

g Including vaginal symptoms, dysuria, abnormal bleeding, or lower abdominal or pelvic pain.

h Results were missing for 7 subjects, 2 of whom had TV infection and 5 of whom did not have TV infection.

i Results were missing for 5 subjects, 2 of whom had TV infection and 3 of whom did not have TV infection.

j Results were missing for 8 subjects , 2 of whom had TV infection and 6 of whom did not have TV infection.

...
The positivity of TMA results.

SUMMARY

Our goal was to examine the characteristics of various tests to detect TV infection in adolescent women. Not surprisingly, culture, rapid antigen testing, and TMA were all more sensitive than wet mount, which is the most widely used test for the detection of TV. Sensitivity estimates for wet mount were significantly lower than that of culture and TMA remained stable or improved slightly (table 3). TMA was significantly more sensitive than rapid antigen testing in women without vaginal symptoms.

DISCUSSION

The rapid antigen test performed as well as culture and was equivalent to TMA. In earlier studies comparing the rapid test with a traditional reference standard of wet mount and culture, apparent false-positive results were problematic [11, 12]. The high specificity demonstrated in our study should reassure clinicians who use the rapid antigen test in populations with a lower prevalence of TV infection. The rapid antigen test is an objective, inexpensive, point-of-care test that allows for immediate treatment of and counseling for STIs. Thus, the rapid antigen test may be especially important for use in adolescents and patients who are seen in emergency departments, both of whom present challenges to follow-up.

In women without vaginal symptoms, TMA appears to be the most sensitive test to detect TV. Asymptomatic patients may be infected with fewer organisms, and accurate detection may require biological amplification in culture or nucleic acid amplification. However, our objective was not to evaluate test performance for screening low-risk women. Even when TMA or other nucleic acid amplification tests are available, cost and time constraints may limit their use.

Most reports of nucleic acid amplification tests used to detect TV infection describe in-house PCR assays [2, 13, 14]. We demonstrated that TMA has a high test-retest reliability, and test performance calculated by latent class analysis (98.2% sensitivity and 98.0% specificity) is comparable with figures reported for TMA compared with real time PCR (96.7% sensitivity and 97.5% specificity) [18]. On the basis of the results of the receiver-operating characteristics analysis, a reasonable method for interpretation of TMA results is to classify samples with values of <10,000 RLUs as negative, those with values of >30,000 RLUs as positive, and those having values of 10,000–30,000 RLUs as equivocal, to be retested and classified as positive if the repeat result is >10,000 RLUs.

Latent class analysis is a reasonable approach to assess test performance; however, it assumes conditional independence of tests. The results of 4 TV detection tests using samples from the same subject will be affected by host characteristics, specimen factors, and handling and, thus, are not completely independent. However, 4 separate swabs were obtained, each test was performed by an individual who was unaware of other test
Table 3. Differences in test sensitivity stratified by the presence or absence of vaginal symptoms.

<table>
<thead>
<tr>
<th>Test method</th>
<th>Sensitivity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients (n = 330)</td>
</tr>
<tr>
<td>Wet mount</td>
<td>50.8 (37.7–63.9)</td>
</tr>
<tr>
<td>Culture</td>
<td>75.4 (62.7–85.5)</td>
</tr>
<tr>
<td>Rapid test</td>
<td>82.0 (70.0–90.6)</td>
</tr>
<tr>
<td>TMA</td>
<td>98.4 (91.2–99.9)</td>
</tr>
</tbody>
</table>

NOTE. The comparator was any test result positive for *Trichomonas vaginalis* infection. TMA, transcription-mediated amplification.

results, and the tests have different technological methodologies that may be affected by host and specimen factors in different ways. Thus, conditional independence is likely.

This study highlights the need for improved detection of TV infection in adolescent women, because one-half of the cases were missed by wet mount. TV infection has been implicated in the acquisition and shedding of several serious infections, including herpes simplex virus, human papillomavirus, and HIV infections [17, 19–21]. There may be other health consequences of trichomoniasis that have been obscured because older studies relied upon insensitive detection methods. Providers who care for adolescent women who are at risk for acquiring STIs are encouraged to use rapid antigen testing, culture, or nucleic acid amplification testing methods with specimens obtained from patients who had negative wet mount results for a more reliable diagnosis of TV infection.

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References