Comparison between the Gen-Probe Transcription-Mediated Amplification Trichomonas vaginalis Research Assay and Real-Time PCR for Trichomonas vaginalis Detection Using a Roche LightCycler Instrument with Female Self-Obtained Vaginal Swab Samples and Male Urine Samples

Andrew Hardick, Justin Hardick, Billie Jo Wood, and Charlotte Gaydos*

Division of Infectious Diseases, Johns Hopkins University, School of Medicine, Baltimore, Maryland

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Trichomonas vaginalis is the cause of the most common parasitic sexually transmitted infection in the world and is estimated to affect 3 million infections in the United States annually (1, 21). T. vaginalis can cause vaginitis, cervicitis, preterm labor, urethritis, and prostatitis (3, 32, 33). T. vaginalis is cytopathic to vaginal cells and is associated with other sexually transmitted diseases (STDs), including transmission of human immunodeficiency virus (2, 7, 26, 29).

Conventional methods for diagnosing T. vaginalis are microscopic examination of wet-mount preparations and culture-based systems. Both methods rely on the collection of viable organisms and suffer from poor sensitivity (25). More sensitive research-based PCRs for the diagnosis of T. vaginalis have been described (9, 11–16, 22, 24).

The development of a commercially available amplification assay for T. vaginalis using self-collected samples would be highly desirable from patient, clinical, and public-health perspectives (6). Other commercially available FDA-cleared STD tests, such as those for chlamydia and gonorrhea, could also be performed on the self-obtained samples (4, 5, 27, 28).

We report a comparison of a research transcription-mediated amplification (TMA) assay for Trichomonas vaginalis and BTUB FRET PCR, using self-obtained clinical samples from 611 patients. Infection status was defined as two positive results by two different tests. The initial TMA assay sensitivity was 96.7%; specificity was 97.5%. The TMA assay was comparable to BTUB FRET PCR.

**BTUB PCR.** For females, two SOVS for BTUB and TMA testing were collected in random order. SOVS for BTUB testing were transported in a dry state and were rehydrated in 1 ml of Tris-EDTA buffer, 200 μl of which was used for DNA robotic extraction. Similarly, 200 μl of male urine was subjected to this automated DNA extraction (MagNA Pure LC instrument; Roche Diagnostics, Indianapolis, IN). The BTUB PCR assay design was based on fluorescent resonance energy transfer (FRET) probe chemistry (Roche Diagnostics). The use of positive and negative controls, the thermocycling protocol, data analysis, and specific sequences of primers and probes using the Roche LightCycler Instrument were previously published (9).

**TMA for T. vaginalis.** The SOVS were placed in specimen transport medium (Gen-Probe), and male urine was pipetted into urine specimen transport tubes for testing. The male urine and female SOVS were tested using target capture TMA and detection by the hybridization protection assay in a manner similar to the other members of the Gen-Probe APTIMA family of assays (Gen-Probe APTIMA COMBO 2 Package insert, IN0037-04 Rev A; Gen-Probe Incorporated, San Diego, CA). The primers, probes, and target capture oligomers were designed for T. vaginalis based on the 16S rRNA gene target. The cutoff for positive reactions was set by the investigators at 60,000 relative light units.

Samples that tested positive by both the BTUB and TMA assays were considered true positives. Discordant samples were adjudicated by another research PCR with another primer set, TVK3 and TVK4, utilizing gel electrophoresis for endpoint analysis (14). Results for vaginal wet preparations for motile trichomonads obtained from females during the clinic visit were also recorded for samples; no culture for trichomonads was performed. Samples positive by two of the three amplified tests or by one amplified test and a positive wet-preparation result were also considered to be true positives for final sensitivity and specificity analyses.

Overall, 59 samples were positive by the BTUB and TMA assays, 535 were negative by both, 15 were TMA positive and...
BTUB negative, and 2 were TMA negative and BTUB positive. Initially, compared only to the BTUB results, the TMA sensitivity, specificity, positive predictive value, and negative predictive value were 96.7%, 97.3%, 79.7%, and 99.6%, respectively (Table 1).

After further testing of the 15 TMA-positive/BTUB-negative samples, 10 were resolved as true positives; of 2 TMA-negative/BTUB-positive samples, 1 sample from a female resolved as a true positive. The final TMA sensitivity was 98.6% (69/70), and the specificity was 99.1% (536/541) (Table 1).

For males, the final sensitivity and specificity were 100%. All TMA-positive/BTUB-negative samples from males were TVK3 and -4 positive (Table 2). The final prevalence in males was 4.5%. For females, the final sensitivity and specificity were 98.2% (56/57) and 98.1% (259/264), respectively (Table 3). Of the 12 TMA-positive/BTUB-negative samples from females, 7/12 were TVK3 and -4 positive, 2/12 were TVK3 and -4 negative, and 2/12 were “quantity not sufficient” (QNS) for TVK3 and -4 analysis. They were graded as true negatives based on wet-preparation negative results. The remaining discordant sample (1/12) was QNS for further resolution. It was considered negative based on wet preparation and TVK3 or TVK4 or wet-preparation result.

Kappa statistic analysis for comparison between the TMA and BTUB assays indicated an overall kappa value of 0.858. For men, the kappa value was 0.826; for women, it was 0.858. Kappa analysis indicated “almost-perfect” agreement.

There was a high prevalence of 11.5% for T. vaginalis in our study population. Females had a higher prevalence (17.8%) of T. vaginalis in our population than men (4.5%). These data are similar to those for our STD clinic population and those of Miller et al. and Wendel et al. (18, 30, 31).

Male urine and SOVS have been shown to be acceptable for the detection of T. vaginalis (9, 10, 16, 20, 23, 31). In our study, these self-obtained samples demonstrated a very high initial sensitivity and specificity for TMA of 96.7% and 97.3%, respectively, and the TMA assay had excellent resolved sensitivity and specificity at 98.6% and 99.1%. Kappa statistic analysis between the two assays indicated “almost-perfect” agreement. There were very few discordant samples, although the TMA assay identified more positives, indicating that the TMA assay may be more sensitive. Additional testing was not performed on all of the samples due to economic constraints. We recognize that this potentially introduced some bias into the results (8, 17, 19).

Culture, the gold standard, is inherently limited because it relies on viable organisms for detection. Wet preparation and culture are subject to interpretation by the microbiologist, whereas nucleic acid amplification assays may offer more accurate and objective results (16, 30). The Gen-Probe TMA assay is commercially available as an analyte-specific reagent.

### Table 1. Comparison of TMA results to BTUB FRET PCR results for T. vaginalis from 611 patients, 321 women and 290 men

<table>
<thead>
<tr>
<th>Data type</th>
<th>PCR Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>TMA 96.7 (59/61)</td>
<td>97.3 (535/550)</td>
<td>79.7 (59/74)</td>
<td>99.6 (535/537)</td>
</tr>
<tr>
<td></td>
<td>Female 98.0 (49/50)</td>
<td>95.6 (259/271)</td>
<td>80.3 (49/61)</td>
<td>99.6 (259/260)</td>
</tr>
<tr>
<td></td>
<td>Male 90.1 (10/11)</td>
<td>98.9 (276/279)</td>
<td>76.9 (10/13)</td>
<td>99.6 (276/277)</td>
</tr>
<tr>
<td>Resolved</td>
<td>TMA 98.6 (69/70)</td>
<td>99.1 (536/541)</td>
<td>93.2 (69/74)</td>
<td>99.8 (536/537)</td>
</tr>
<tr>
<td></td>
<td>Female 98.2 (56/57)</td>
<td>98.1 (259/264)</td>
<td>91.8 (56/61)</td>
<td>99.6 (259/260)</td>
</tr>
<tr>
<td></td>
<td>Male 100 (13/13)</td>
<td>100 (277/277)</td>
<td>100 (13/13)</td>
<td>100 (277/277)</td>
</tr>
</tbody>
</table>

* a Sensitivity and specificity of TMA compared to those of BTUB FRET PCR.
* b Sensitivity and specificity, where infection status is determined by two positive amplified results from TMA, BTUB FRET PCR, or alternate PCR using primer set TVK3 and TVK4 in wet-preparation result.
* c PPV, positive predictive value.
* d NPV, negative predictive value.

### Table 2. Additional testing of male discordant samples

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Repeat TMA</th>
<th>Repeat BTUB</th>
<th>Primers TVK3 and -4</th>
<th>True positive/negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>True positive</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>True positive</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>True positive</td>
</tr>
<tr>
<td>1c</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>True negative</td>
</tr>
</tbody>
</table>

* a Three male discordant samples, all initially TMA positive/BTUB FRET PCR negative.
* b +, positive; -, negative; NA, not applicable.
* c One male discordant sample, initially TMA negative/BTUB positive.

### Table 3. Additional testing of female discordant samples

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Repeat TMA</th>
<th>Repeat BTUB</th>
<th>TVK3 and -4</th>
<th>Wet preparation</th>
<th>True positive or negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>True positive</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>True positive</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>True positive</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>True positive</td>
</tr>
<tr>
<td>6</td>
<td>Not done</td>
<td>+</td>
<td>+</td>
<td>Not done</td>
<td>True positive</td>
</tr>
<tr>
<td>7</td>
<td>Not done</td>
<td>-</td>
<td>+</td>
<td>Not done</td>
<td>True positive</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>True negative</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>True negative</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not done</td>
<td>True negative</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>Not done</td>
<td>-</td>
<td>Not done</td>
<td>True negative</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>QNS</td>
<td>QNS</td>
<td>-</td>
<td>True negative</td>
</tr>
<tr>
<td>1c</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>True positive</td>
</tr>
</tbody>
</table>

* a Twelve female discordant samples, all initially TMA positive/BTUB negative.
* b +, positive; -, negative.
* c One female discordant sample, initially TMA negative/BTUB positive.
and offers laboratories a highly sensitive and specific assay for use clinically; hopefully, it will become available as a fully FDA-cleared test.

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REFERENCES