Comparison between the Gen-Probe TMA *Trichomonas vaginalis* Research Assay and a Real-time PCR for *Trichomonas vaginalis* using the Roche Lightcycler Instrument in Female Self-obtained Vaginal Swabs and Male Urine Samples

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Results from this analysis were presented in part at the International Society for Sexually Transmitted Diseases Research, Amsterdam, The Netherlands, July 10-13, 2005. Funding was in part from Gen-Probe, Inc., San Diego, CA.
Abstract

This study compared two assays for *Trichomonas vaginalis* detection, Gen-Probe’s TMA-TV and BTUB FRET PCR in self-obtained clinical samples from 611 patients. Infection status was defined as 2 positives by 2 different tests. Initial TMA-TV sensitivity was 96.7%; specificity was 97.5%. The TMA-TV assay was comparable to BTUB FRET PCR.

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*Trichomonas vaginalis* (TV) is the most common parasitic sexually-transmitted infection in the world and is estimated to cause 3 million infections in the US annually year (1, 21). TV can cause vaginitis, cervicitis, preterm labor, urethritis and prostatitis (3,32,33). TV is cytopathic to vaginal cells and is associated with other STDs infections, including transmission of HIV (2, 7, 26, 29).

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

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

We report a comparison of a research transcription-mediated amplification assay for TV (TMA-TV), now available as an analyte specific reagent (ASR) (Gen-Probe Inc., San Diego, CA) with BTUB FRET PCR (BTUB), a real-time research PCR (9).
We screened 615 people attending two STD clinics; 611 had male urine samples (N=290) or duplicate female self-obtained vaginal swabs (SOVS) (N= 321) collected. Institutional Review Board approval was obtained, and the study was funded in part by Gen-Probe.

**BTUB PCR.** For females, two SOVS for BTUB and TMA-TV 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, of which 200 ul was used for DNA robotic extraction. Similarly 200ul 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 FRET® probe chemistry (Roche Diagnostics). Use of positive and negative controls, thermocycling protocol, data analysis, and specific sequences of primers and probes were previously published using the Roche LightCycler Instrument (9).

**TMA-TV.** The SOVS were placed in Specimen Transport Media (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 (HPA) in a manner similar to the other Gen-Probe APTIMA® family of assays (Gen-Probe® APTIMA COMBO® 2 Package insert, IN0037-04 Rev A, Gen-Probe Incorporated, San Diego, CA). Primers, probes and target capture oligomers were designed for TV based on the 16S rRNA gene target. The cut-off for positive reactions was set by the investigators at 60,000 relative light units.

Samples that tested positive by both the BTUB and TMA-TV were considered true positives. Discordant samples were adjudicated by another research PCR with
another primer set, TVK3 and TVK4, utilizing gel electrophoresis for end-point analysis (14). Results for vaginal wet preparation for motile trichomonads obtained during the clinic visit for females were also recorded for samples; no culture for trichomonas 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 analysis.

Overall, 59 samples were positive by BTUB and TMA-TV; 535 were negative by both; 15 were TMA-TV positive and BTUB negative; and 2 were TMA-TV negative and BTUB positive. Initially, compared only to BTUB results, TMA-TV sensitivity, specificity, PPV and NPV were 96.7%, 97.3%, 79.7%, and 99.6%, respectively (Table 1). After further testing of the 15 TMA-TV positive/BTUB negative samples, 10 were resolved as true positives; of 2 TMA-TV negative/BTUB positive samples, 1 female resolved as a true positive. Final TMA-TV sensitivity was 98.6% (69/70) and specificity was 99.1% (536/541) (Table 1).

For males, final sensitivity and specificity was 100%. All male TMA-TV positive/BTUB negative samples were TVK3/4 positive (Table 2). Final prevalence in males was 4.5%. For females, final sensitivity and specificity were 98.2% (56/57) and 98.1% (259/264), respectively (Table 3). Of the 12 female TMA-TV positive/BTUB negative samples, 7/12 were TVK3/4 positive, 2/12 were TVK3/4 negative, 2/12 were of quantity not sufficient (QNS) for TVK3/4 analysis. They were graded 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 results...
Overall prevalence in women was 17.8%. The prevalence by vaginal wet preparation in the clinic was 12.1%; sensitivity was 68.4% (39/57).

Kappa statistic analysis for comparison between TMA-TV and BTUB indicated an overall kappa value of 0.858. For men, 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% of *T. vaginalis* in our study population. Females had a higher prevalence (17.8%) for TV in our population than men (4.5%). This data is similar to our STD clinic population and Miller et al. (18, 30, 31).

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

Culture, the gold standard, is inherently limited because it relies on viable organism for detection. Wet preparation and culture are subject to interpretation by the microscopist, whereas nucleic acid amplification assays offer may offer more accurate and objective results (16, 30). The Gen-Probe TMA-TV assay is commercially available as an ASR (analyte specific reagent) and offers laboratories a highly sensitive and
specific assay for use clinically; hopefully it will become available as a fully FDA cleared test.

Reference List


Ann Epidemiol 5:325-332.
Table 1. Comparison of TMA-TV results to BTUB FRET PCR results for *Trichomonas vaginalis* from 611 patients; 321 women and 290 men.

<table>
<thead>
<tr>
<th>Data type</th>
<th>PCR</th>
<th>Sensitivity</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>TMA-TV</td>
<td>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</td>
<td>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</td>
<td>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-TV</td>
<td>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</td>
<td>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</td>
<td>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-TV compared to BTUB FRET PCR  
*b* Sensitivity and specificity where infection status is determined by two positive amplified results from TMA-TV, BTUB FRET PCR or alternate PCR using primer set TVK3 and TVK4 or wet preparation result

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Table 2. Additional testing of Male discordant samples

<table>
<thead>
<tr>
<th>3 Male discordant samples: all initially TMA-TV Pos / BTUB FRET PCR Neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeat TMA-TV</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
</tbody>
</table>

1 Male Discordant sample: initially TMA-TV Neg / BTUB Pos

| 1.             | —           | +               | -              | True neg     |
Table 3. Additional testing of female discordant samples for 12 Female Discordant samples: all initially TMA-TV Pos / BTUB Neg (QNS = quantity not sufficient)

<table>
<thead>
<tr>
<th></th>
<th>Repeat TMA</th>
<th>Rpt. BTUB</th>
<th>TVK 3/4</th>
<th>Wet Prep. Results</th>
<th>True Pos or Neg</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>True pos</td>
</tr>
<tr>
<td>2.</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>True pos</td>
</tr>
<tr>
<td>3.</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>True pos</td>
</tr>
<tr>
<td>4.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>True pos</td>
</tr>
<tr>
<td>5.</td>
<td>Not done</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>True pos</td>
</tr>
<tr>
<td>6.</td>
<td>Not done</td>
<td>-</td>
<td>+</td>
<td>Not done</td>
<td>True pos</td>
</tr>
<tr>
<td>7.</td>
<td>Not done</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>True pos</td>
</tr>
<tr>
<td>8.</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>True neg</td>
</tr>
<tr>
<td>9.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>True neg</td>
</tr>
<tr>
<td>10.</td>
<td>-</td>
<td>-</td>
<td>Not done</td>
<td>-</td>
<td>True neg</td>
</tr>
<tr>
<td>11.</td>
<td>-</td>
<td>-</td>
<td>Not done</td>
<td>-</td>
<td>True neg</td>
</tr>
<tr>
<td>12.</td>
<td>+</td>
<td>QNS</td>
<td>QNS</td>
<td>-</td>
<td>True neg</td>
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</table>

Additional testing of female discordant samples
1 Female Discordant sample: initially TMA-TV Neg / BTUB Pos

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>+</th>
<th>+</th>
<th>-</th>
<th>True pos</th>
</tr>
</thead>
</table>