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Development of PCR Assays for Detection of Trichomonas vaginalis in Urine Specimens

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Trichomonas vaginalis infections are usually asymptomatic or can result in nonspecific clinical symptoms, which makes laboratory-based detection of this protozoan parasite essential for diagnosis and treatment. We report the development of a battery of highly sensitive and specific PCR assays for detection of T. vaginalis in urine, a noninvasive specimen, and development of a protocol for differentiating among Trichomonas species that commonly infect humans.

Sexually transmitted infections (STIs) caused by the protozoan parasite Trichomonas vaginalis are more prevalent than those caused by Neisseria gonorrhoeae and Chlamydia trachomatis, both globally and in the United States (1–4). In women, T. vaginalis infections cause vaginitis and cervicitis and are associated with pelvic inflammatory disease and adverse pregnancy outcomes (5). In men, T. vaginalis infections cause nongonococcal urethritis and can lead to prostatitis, epididymitis, and male factor infertility (5). Additionally, T. vaginalis infections have been implicated as a significant risk factor for sexual transmission of HIV (6, 7) and possibly other bacterial and viral STIs (8), as well as for cervical cancer (9). As with other STIs, T. vaginalis infections are usually asymptomatic or can result in nonspecific clinical symptoms (5), which makes laboratory-based detection of the protozoan parasite essential for diagnosis and treatment of trichomoniasis.

The conventional diagnostic test for T. vaginalis infection in women is direct microscopic examination of vaginal fluid in wet-mount preparations. Usually performed in physician’s offices or clinics, this test is highly specific, but its sensitivity is only about 60% of that of culture, which currently is the gold standard laboratory test for T. vaginalis infection in women and men (10–12). However, several nucleic acid amplification tests (NAATs), including PCR tests, have been developed in research laboratories and shown to be more sensitive than culture and antigen-based tests (10–12). Consistent with developments in the diagnosis of other STIs, culture and the other clinical laboratory and point-of-care rapid tests for the detection of T. vaginalis are being replaced by NAATs (13, 14).

Recently, the Centers for Disease Control and Prevention (CDC) conducted a multicenter study of diagnostic tests for STIs in children under evaluation of sexual abuse (15, 16). One of the objectives of this study was to evaluate the use of NAATs for the detection of N. gonorrhoeae and C. trachomatis in noninvasive specimens for clinical and forensic purposes. Because the collection of invasive genital samples in a pediatric population can be difficult and traumatic, the use of noninvasive specimens, such as urine, is highly recommended. Owing to lack of availability of FDA-approved NAATs at the time, the use of NAATs for the detection of T. vaginalis was not part of the protocol used in this multicenter study. However, we initiated a separate study to develop and evaluate PCR assays for potential use in similar future studies and for studies validating the performance of commercial T. vaginalis NAATs (13, 14, 17, 18). In this paper, we report the performance of a series of published and novel single and nested PCR assays for the detection of T. vaginalis in laboratory-spiked urine specimens and in clinical urine specimens.

We chose three different T. vaginalis repeat genomic sequences as targets for the PCR assays (Table 1). Two of these genomic sequences, the Kengne et al. (19) and Paces et al. (20) repeats, were previously shown to be highly sensitive and specific PCR targets. The third genomic sequence, the Muresu et al. repeat (21, 22), was previously used as a target for development of dot blot and in situ hybridization tests for detection of T. vaginalis in vaginal secre- tions and discharges. The primers used in this study included 2 previously published sets of primers (10) and 6 new primer sets (Table 1). Unlike in the previous studies, the primer sets were designed to be used in both single-round and nested PCR assays. All of the PCR assays were tested using extracted DNA from urine collected from T. vaginalis-negative, healthy persons that was spiked with known numbers of T. vaginalis organisms grown in culture. To test for the specificity of the primer sets, we included samples containing Trichomonas tenex and Pentatrichomonas hominis, which are commensal species in humans inhabiting the mouth and the gastrointestinal tract, respectively.

Briefly, the DNA lysates were prepared from spiked urine specimens using a modified High Pure PCR template preparation kit (Roche Molecular Biochemicals, Branchburg, NJ). PCR and sequencing were performed by following a general procedure that we described previously for the detection and genotyping of C. trachomatis in urine specimens (23). The results, which are expressed as the lowest number of T. vaginalis organisms per PCR test that gave a positive result as detected by agarose gel electrophoresis, are presented in Table 1. All of the PCR assays performed on the extracted DNA from T. tenex and P. hominis were negative.

All 8 primer pairs in single or nested PCR combinations gen-
erated fragments of correct size and specificity as verified by DNA sequencing. The nested PCR assays were consistently more sensitive than the single-round PCR assays; however, all of the assays detected one organism, which can be explained by the fact that all of the targets were repeat sequences in the *T. vaginalis* genome. Next, we tested all of the PCR assays (i.e., 8 single-round and 4 nested PCR assays; see Table 1) on 4 clinical urine specimens that were collected from *T. vaginalis* culture-positive patients and maintained frozen at −70°C for several years. Among the 8 single-round PCR assays, only one primer pair, TVC11/TVC12, was positive for all 4 specimens, and among the 4 nested PCRs, only 2 primer sets (TVC3F/TVC4R and TVC11F/TVC; TVC5F/TVC6R and TV7F/C8R) were positive for all 4 specimens.

We selected the nested primer sets TVC3F/TVC4R and TVC11F/TVC12R to screen urine specimens from the CDC multicenter study on STIs in children being evaluated for sexual abuse (15, 16). Of the 485 female study participants enrolled in the original study (15, 16), only 406 had specimens remaining for this study. Of these specimens, 14 (3.4%) were positive for *T. vaginalis* and positive clinical specimens detected by our diagnostic PCR assay were confirmed to be *T. vaginalis*. However, after multiple attempts, the discrepant specimen remained negative for all three *Trichomonas* species.

In summary, we have developed and validated a battery of highly sensitive and specific PCR assays that detect *T. vaginalis* in urine, a noninvasive specimen. We also developed a protocol for detection of a different *T. vaginalis*-related species, such as *T. tenax* and *P. hominis*, which cannot be differentiated by routine microscopic examination. Although, *T. tenax* and *P. hominis* usually inhabit the mouth and respiratory or gastrointestinal tracts (24, 25), it is plausible that these protozoan parasites could be inadvertently transferred by cross-contamination to the urogenital tract, particularly in very young children, such as the participants in this study population. To address this possibility, we developed a *Trichomonas* species determination protocol based on the species-specific sequence variation in the 5.8S rRNA gene and the flanking internal transcribed spacer region ITS1 (26). Using a nested PCR assay (TF1/TR5 and TF2/TR2; see Table 2), we amplified, sequenced, and analyzed this genomic region from multiple *T. vaginalis*, *T. tenax*, and *P. hominis* culture isolates and the clinical specimens used in our study. All isolates were correctly identified, and positive clinical specimens detected by our diagnostic PCR assay were confirmed to be *T. vaginalis*. However, after multiple attempts, the discrepant specimen remained negative for all three *Trichomonas* species.

### Table 1: *T. vaginalis* PCR primers and their sensitivities

<table>
<thead>
<tr>
<th>Gene target&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Primer</th>
<th>Primer sequence</th>
<th>Size (bp)</th>
<th>Sensitivity&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KENGEO (L23861)</strong></td>
<td>TVK3F</td>
<td>5'-ATT GTC GAA CAT TGG TCT TAC CCT C-3'</td>
<td>262</td>
<td>1 0.01</td>
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<tr>
<td></td>
<td>TVK7R</td>
<td>5'-TCT GTG CGG TCT TCA AGT ATG C-3'</td>
<td>213</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TVC1F</td>
<td>5'-TCA GTT GGC AAA GGC AGT CCT-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC2R</td>
<td>5'-GTA CTT AGC ATG GGA GAC ATG A-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MURESU (X83109)</strong></td>
<td>TVC3F</td>
<td>5'-GAT GCC ATG AAC GGA AAT GRT-3'</td>
<td>299</td>
<td>1 0.01</td>
</tr>
<tr>
<td></td>
<td>TVC4R</td>
<td>5'-TCT GGA GAA TAC TGG ATC CG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC11F</td>
<td>5'-GGA ATG GRA TAA CGA ATG CGA C-3'</td>
<td>237</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>TVC12R</td>
<td>5'-CAA CCT TCT TTC TGA AAC ATG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PACES (M86482)</strong></td>
<td>TVC3F</td>
<td>5'-AAT TCC CGG ATA ATT GAA ACG GA-3'</td>
<td>190</td>
<td>0.1 0.01</td>
</tr>
<tr>
<td></td>
<td>TVC6R</td>
<td>5'-GAT GTT GGC GAT GTT TGT TGT AAC AC-3'</td>
<td>148</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TVC7F</td>
<td>5'-GAT AAA GAA AAT GTG TTT AAG ATG ATG GA-3'</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>TVC8R</td>
<td>5'-TGT TAC TCT GAC ACT GGT TCC AAT TT-3'</td>
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<tr>
<td><strong>PACES (M86482)</strong></td>
<td>TVOP1F</td>
<td>5'-GTT GAA ATC TCA TGG GGG TAT TAA CT-3'</td>
<td>580</td>
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<tr>
<td></td>
<td>TVOP2R</td>
<td>5'-GTT TTA TTT ATC ACT GAA AAA TAA CGC TT-3'</td>
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<td></td>
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<tr>
<td></td>
<td>TVC9F</td>
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<td>TVC10R</td>
<td>5'-GGA ATT CTT ACC TTA GAC CCT TCA GAT T-3'</td>
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</table>

<sup>a</sup> For each gene target, the top 2 primers are for 1st PCR and the bottom 2 primers for 2nd PCR.

<sup>b</sup> The gene targets are labeled with the name of the first author in the published sequence (GenBank accession number).

<sup>c</sup> *T. vaginalis* organisms per PCR.

### Table 2: Primers used for nested amplification (TF1/TR5 [1st PCR] and TF2/TR2 [2nd PCR]) and sequencing (TF3, TR1, TR3, and TR4) of the *Trichomonas* 5.8S rRNA gene and the flanking internal transcribed spacer region ITS1

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF1</td>
<td>5'-TCC TAC CGA TTG GAT GAC TCG-3'</td>
<td>213</td>
</tr>
<tr>
<td>TF2</td>
<td>5'-GAA AGG AGA AGT CGT AAC AAG-3'</td>
<td>201</td>
</tr>
<tr>
<td>TF3</td>
<td>5'-GTA GGT GAA CCA GCC TGG TGG TGA T-3'</td>
<td>213</td>
</tr>
<tr>
<td>TR1</td>
<td>5'-TGA GGA GCC AAG ACA TCC ATT G-3'</td>
<td>221</td>
</tr>
<tr>
<td>TR2</td>
<td>5'-ATG CAA CGT TCT TCA TCG TGG-3'</td>
<td>213</td>
</tr>
<tr>
<td>TR3</td>
<td>5'-GCG CAA TTT GCA TCT AAA GAT-3'</td>
<td>213</td>
</tr>
<tr>
<td>TR4</td>
<td>5'-GAG ATG CTT CAG TCG AGC GGC TT-3'</td>
<td>213</td>
</tr>
<tr>
<td>TR5</td>
<td>5'-CTT TTC CGC TTC TTA TTG AGA TG-3'</td>
<td>213</td>
</tr>
</tbody>
</table>
differentiating among *Trichomonas* species that commonly infect humans. These assays should be useful for other studies of STIs, particularly those designed for the validation of commercial *T. vaginalis* NAATs.

REFERENCES


