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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 women and men (10–12). Consistent with developments in the diagnosis of other STIs, culture and antigen-based tests 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). 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 secretions 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 tenax* 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 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. tenax* and *P. hominis* were negative.

All 8 primer pairs in single or nested PCR combinations gen-
TABLE 1  T. vaginalis PCR primers and their sensitivities

<table>
<thead>
<tr>
<th>Gene target</th>
<th>Primer</th>
<th>Primer sequence</th>
<th>Size (bp)</th>
<th>Sensitivity</th>
<th>PCR</th>
<th>Nested PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>KENGOE (L23861)</td>
<td>TVK3F</td>
<td>5'-ATT GTC GAA CAT TGG TCT TAC CCT C-3'</td>
<td>262</td>
<td>1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVK7R</td>
<td>5'-TCT GTG CGG TCT TCA AGT ATG C-3'</td>
<td>213</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC1F</td>
<td>5'-TCA GTC GAA AAG GCC GGT CCT-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC2R</td>
<td>5'-GTA CTT AGC CTT GGA GAC GAC ATG A-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MURESU (X83109)</td>
<td>TVC3F</td>
<td>5'-GAT GCC ATG AAC GGA AAT GTT G-3'</td>
<td>299</td>
<td>1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC4R</td>
<td>5'-TCT GGA GAA TAT TGG ATG CGC-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC11F</td>
<td>5'-GGA ATG GAA ATT CTA CTA C-3'</td>
<td>237</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC12R</td>
<td>5'-CAA CCT TCC TTC TGT GAC AAC ATG-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PACES (M86482)</td>
<td>TVC5F</td>
<td>5'-AAT TCC CGG ATA ATT GAA ACG GA-3'</td>
<td>190</td>
<td>0.1</td>
<td>0.01</td>
<td></td>
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<tr>
<td></td>
<td>TVC6R</td>
<td>5'-GAT GTG GAC GAT GTT TTA CTG G-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC8R</td>
<td>5'-GAT AAA GAA AAT GTG TTT AAG ATG AGT GA-3'</td>
<td>148</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC10R</td>
<td>5'-TCT TCG TAC ACC ACT GGT TCG AAT TT-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PACES (M86482)</td>
<td>TVOP1F</td>
<td>5'-GTG AAA ATT AAC TCA TTG GGG TAT TAA CTT-3'</td>
<td>580</td>
<td>0.01</td>
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<td>TVOP2R</td>
<td>5'-GTT TTA TTT ATC ACT GGA AAA TAA CGC TT-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>TVC9F</td>
<td>5'-AGA ATA CAA AAC ATC GCC AAC ATC TT-3'</td>
<td>358</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For each gene target, the top 2 primers are for 1st PCR and the bottom 2 primers for 2nd PCR.

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

c T. vaginalis organisms per PCR.

The discrepancy might be due to detection in the wet-mount preparations of the specimens, or with performing the tests, it is also possible that the discrepancy might be due to detection in the wet-mount preparations of a different T. vaginalis-related species, such as T. tenex and P. hominis, which cannot be differentiated by routine microscopic examination. Although, T. tenex 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. tenex, 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.

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

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>
</tr>
</thead>
<tbody>
<tr>
<td>TF1</td>
<td>5'-TCC TAC CGG ATG TGG TCA GAC GAC-3'</td>
</tr>
<tr>
<td>TF2</td>
<td>5'-AGG AAG AGA AGT CGT AAC AGG-3'</td>
</tr>
<tr>
<td>TF3</td>
<td>5'-GTA GGT GAC GAA ATC GCC GGT TCA G-3'</td>
</tr>
<tr>
<td>TR1</td>
<td>5'-GGA GGA GCC AAG ACA TCC ATT G-3'</td>
</tr>
<tr>
<td>TR2</td>
<td>5'-ATG CAA CGT TGT TCA TCA TCC T-3'</td>
</tr>
<tr>
<td>TR3</td>
<td>5'-GCG CAA TCA GTA TCA TCA AAG-3'</td>
</tr>
<tr>
<td>TR4</td>
<td>5'-GAG ATG TGT CAC GGT TAC GAC GGA-3'</td>
</tr>
<tr>
<td>TR5</td>
<td>5'-CTT TTC CCG TAT CTA TTG GAC TG-3'</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