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

Nancy Fajman, Emory University
Claudiu Bandea, Center for Disease Control and Prevention
Kahaliah Joseph, Center for Disease Control and Prevention
Evan W. Secor, Center for Disease Control and Prevention
Laurie A. Jones, Center for Disease Control and Prevention
Joseph U. Igietseme, Center for Disease Control and Prevention
Robert L. Sautter, Carolinas Pathology Group
Margaret R. Hammerschlag, SUNY Downstate Medical Center
Rebecca G. Girardet, University of Texas - Houston Medical School
Carolyn M. Black, Center for Disease Control and Prevention

Journal Title: Journal of Clinical Microbiology
Volume: Volume 51, Number 4
Publisher: American Society for Microbiology | 2013-04-01, Pages 1298-1300
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/JCM.03101-12
Permanent URL: https://pid.emory.edu/ark:/25593/rxbjv

Final published version: http://dx.doi.org/10.1128/JCM.03101-12

Copyright information:
© 2013, American Society for Microbiology. All Rights Reserved.
This is an Open Access work distributed under the terms of the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/licenses/by/3.0/).

Accessed October 26, 2019 6:35 AM EDT
Development of PCR Assays for Detection of *Trichomonas vaginalis* in Urine Specimens

Claudiu I. Bandea,a Kahaliah Joseph,a Evan W. Secor,a Laurie A. Jones,a Joseph U. Igietseme,a Robert L. Sautter,b Margaret R. Hammerschlag,a Nancy N. Fajman,a Rebecca G. Girardet,a Carolyn M. Blacka Centers for Disease Control and Prevention, Atlanta, Georgia, USAa; Carolinas Pathology Group, Charlotte, North Carolina, USAa; SUNY Downstate Medical Center, Brooklyn, New York, USAa; Emory University School of Medicine, Atlanta, Georgia, USAa; The University of Texas—Houston Medical School, Houston, Texas, USAa

*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 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 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 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/TV6R and TV7F/TV8R) were positive for all 4 specimens.

We selected the nested primer sets TVC3F/TVC4R and TVC11F/TVC12R to screen urine specimens from the CDC multilocus strain typing (MLST) panel of culture isolates and the 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 macroscopic 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 transmitted 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.

From the 406 girls, ages 0 to 13 years, only 85 met the clinical criteria for performing a wet-mount test for detection of T. vaginalis (16). Five (5.9%) of these girls were positive for T. vaginalis by wet mount (16). Our PCR assay detected 8 (9.4%) T. vaginalis-positive patients in this group but only confirmed 4 of the 5 patients reported positive for T. vaginalis by wet mount (16). Although there are multiple potential explanations for this discrepancy, such as errors associated with collecting or managing the specimens, or with performing the tests, it is also possible that the discrepancy might be due to detection in the wet-mount prep-

---

### 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 CCG 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 GTT GCC AAA GGC AGT CCT C-3’</td>
<td>299</td>
<td>1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC2R</td>
<td>5’-GTA AGT CTT GSG AAG GAC ATG A-3’</td>
<td>237</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MURESU (X83109)</td>
<td>TVC3F</td>
<td>5’-GAT GCC ATG AAC GGA AAT GTT C-3’</td>
<td>190</td>
<td>0.1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC4R</td>
<td>5’-TCT GGA GAA TAT TGG ATC CGC C-3’</td>
<td>148</td>
<td>1</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>580</td>
<td>1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC12R</td>
<td>5’-CAA CCT TCT TGG TCA GAC AAC TGG-3’</td>
<td>358</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PACES (M86482)</td>
<td>TVC5F</td>
<td>5’-AAT TCC CGG ATG CTA GCA AGC GA-3’</td>
<td>262</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC8R</td>
<td>5’-GAT GTG GGT GAT GTT TTG TAT TCT G-3’</td>
<td>299</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC7F</td>
<td>5’-GAT AAA GAA AAT GTG TTT AAG ATG TCG AGA-3’</td>
<td>190</td>
<td>0.1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC8R</td>
<td>5’-TTG TAT TCG GAC AGT GGT TCC AAT TT-3’</td>
<td>148</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PACES (M86482)</td>
<td>TVOP1F</td>
<td>5’-GTA AAA ATC TCA TTG GGG TAT TAA CTT-3’</td>
<td>580</td>
<td>1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVOP2R</td>
<td>5’-GTT TTA TTT ATC ACT GGA AAA TAA CGC TT-3’</td>
<td>358</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC9F</td>
<td>5’-AGA ATC GAA ACG ATC CCG AAC ATC TT-3’</td>
<td>262</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TVC10R</td>
<td>5’-GCG ATT CTT TTA GAC CCT TCA GAT T-3’</td>
<td>299</td>
<td>1</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

---

**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 CGA TTG GAT GAC TCG TCG-3’</td>
</tr>
<tr>
<td>TF2</td>
<td>5’-GGA AGG AGA AGT CGT AAC AGG-3’</td>
</tr>
<tr>
<td>TF3</td>
<td>5’-GTA GGT GAA GAC GCC GATG ATT-3’</td>
</tr>
<tr>
<td>TR1</td>
<td>5’-TGA GGA GCC AGC ACA TCC ATT G-3’</td>
</tr>
<tr>
<td>TR2</td>
<td>5’-ATG CAA CGT TCT TGA TCG-3’</td>
</tr>
<tr>
<td>TR3</td>
<td>5’-GCG CAA TTT GCA TTC AAA GAT-3’</td>
</tr>
<tr>
<td>TR4</td>
<td>5’-GAG ATG CCT CAT TCG AGC AGG GGG-3’</td>
</tr>
<tr>
<td>TR5</td>
<td>5’-CTT TTC CTC CGC TTA TTG ATG-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