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CLINICAL STUDY

Real-time PCR improves detection of Trichomonas vaginalis infection compared with culture using self-collected vaginal swabs

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Abstract
Objective. To compare a real-time polymerase chain reaction (PCR) assay with broth culture for the detection of Trichomonas vaginalis using self-collected vaginal swabs.
Methods. Self-collected vaginal swabs were obtained from adolescent and young adult African-American women participating in HIV-1 prevention programs. T. vaginalis culture was performed using the InPouch TV System. Samples for the real-time PCR assay were collected using the BDProbeTec ET Culturette Direct Dry Swab system and tested in a laboratory-developed assay which targeted a repeated sequence of the genome. Discrepant samples that were culture negative and positive in the real-time PCR assay were tested in a confirmatory PCR which targeted a different region of the T. vaginalis genome, the 18S ribosomal DNA gene.
Results. Of the 524 specimens tested by both culture and real-time PCR, 36 were culture positive and 54 were positive in the real-time PCR assay; 16 of the 18 discrepant specimens were also positive in the confirmatory PCR assay. Using a modified gold standard of positive by culture or positive in both PCR assays, the sensitivity of the real-time PCR assay was 100% and the specificity was 99.6%, whereas culture had a sensitivity of 69.2% and a specificity of 100%.
Conclusions. The real-time PCR assay was sensitive and specific for the detection of T. vaginalis DNA from self-collected vaginal swab specimens. The ability to use the BDProbeTec dry swab system for the real-time PCR testing allowed for the detection of Chlamydia trachomatis, Neisseria gonorrhoeae, and T. vaginalis from a single specimen.

Keywords: Trichomoniiasis, DNA, adolescent women

Introduction
Trichomonas vaginalis is one of the most common sexually transmitted diseases, with an estimated 5 million cases per year in the United States [1] and as many as 200 million cases annually worldwide. In women, common symptoms of T. vaginalis infection include vaginal discharge and vulval irritation. Complications of T. vaginalis infection can occur in untreated women and include endometritis, infertility and cervical erosion [2,3]. In pregnant women, T. vaginalis infections can lead to severe complications including premature rupture of membranes [4], preterm deliveries and low-birth-weight infants [4]. In men, T. vaginalis infection is a cause of nongonococcal urethritis, [5] and if untreated may lead to complications including chronic prostatitis, urethral strictures, epididymitis and/or infertility [2,3]. T. vaginalis infection is also associated with an increased risk of acquiring human immunodeficiency virus type 1 (HIV-1) infection [6–9], which is related to local inflammation within the genital tract that occurs with T. vaginalis infections. It has been estimated that between 10% and 50% of T. vaginalis infections are asymptomatic [10], supporting the need for more intensive screening for T. vaginalis infections in sexually active men and women as well as pregnant women.
Methods used for the diagnosis of *T. vaginalis* infection include microscopic examination of wet-mount preparations, culture and, more recently, polymerase chain reaction (PCR). Wet mount examination of genital discharge material is rapid and inexpensive, but the sensitivity is only 40% to 70% [11]. This poor sensitivity may be due in part to the rapid loss of the characteristic protozoan motility once the organism has been removed from an environment at 37°C. Currently, broth culture is considered to be the gold standard for detecting *T. vaginalis* [12]. Successful growth in culture can be achieved with as few as 300 to 500 TV organisms per milliliter of vaginal fluid, but requires 2 to 7 days of incubation and daily microscopic examination for optimal performance. However, the sensitivity of culture is also low, ranging from 50% to 80%. In addition, culture requires a specialized medium such as Diamond’s broth or Tricosal medium. These limitations of wet-mount examination and culture has led to interest in using molecular assays for the detection of *T. vaginalis* DNA from clinical specimens. PCR assays have been developed that are specific and show an improved sensitivity compared with both culture and wet-mount examination [13–17].

The majority of sexually transmitted diseases occur in adolescents and, although screening for sexually transmitted disease has been shown to be cost effective, compliance with testing, particularly among teenagers, remains an important challenge [18]. Self-collected vaginal swabs offer a method of testing adolescent women that could improve compliance with testing, and a recent study has shown that this approach can be easily implemented in a high-school setting, with high acceptability [13]. Thus, self-collection of vaginal swabs improves the diagnosis and treatment of sexually transmitted diseases in adolescents. In this study, we compared the sensitivity of a real-time PCR assay with culture for the diagnosis of *T. vaginalis* infections using self-collected vaginal swabs. Specimens were collected using the BDProbeTec ET Culturette Direct Dry Swab system, allowing for simultaneous testing for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *T. vaginalis* from a single specimen.

Subjects and methods

Patients and samples

Samples were collected from African-American adolescent and young adult females enrolled in one of two HIV-1 prevention programs. In the first study, females aged 15 to 21 years were recruited from a county clinic for sexually transmitted diseases. The second study included women between the ages of 18 to 29 years, who were enrolled at Kaiser Permanente in Atlanta, Georgia, USA. As part of the study, the participants provided self-collected vaginal swab samples to be tested for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*. Both studies were approved by the Emory University Institutional Review Board (#327–99, #534–2002). Participants collected two vaginal swabs, one for *T. vaginalis* culture and the other for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* nucleic acid testing. The results of the *C. trachomatis* and *N. gonorrhoeae* testing were not included in this study.

For *T. vaginalis* culture, the specimens were collected using Dacron tip swabs. They were inoculated into the InPouch TV System (BioMed Diagnostics, San Jose, CA, USA) onsite, and were transported to the clinical laboratory where they were immediately incubated at 37°C. For *T. vaginalis* PCR testing, self-collected vaginal swabs were obtained using the BDProbeTec ET Culturette Direct Dry Swab system (Becton Dickinson, Sparks, MD, USA) and transported to the laboratory on icepacks. They were refrigerated until processed, which was carried out within 6 days of collection.

*Trichomonas vaginalis* culture

*T. vaginalis* cultures were performed in the Clinical Microbiology Laboratory at Emory University Hospital following standard procedures. The specimens were incubated at 37°C and examined at 72 hours for the presence of *T. vaginalis* trophozoites.

*T. vaginalis* real-time PCR assay

The vaginal swabs collected in the BDProbeTec ET Culturette Direct Dry Swab system were processed as outlined by the manufacturer for cervical swabs. Briefly, the swabs were placed in a vial of BDProbeTec ET diluent (2 ml); the swab was swirled in the diluent for 5 to 10 seconds and then removed. The tubes were then incubated at 112 °C to 116°C in a dry bath for 30 minutes. An aliquot of the sample was used for *T. vaginalis* PCR testing. The target for the real-time PCR assay was a 67-base pair region of a repeated sequence of the *T. vaginalis* genome [19] (Gene Bank Accession Number L23861). The primers and probes were designed using Primer Express (Applied Biosystems, Foster City, CA, USA). The PCR reaction contained 25 μl of 2X TaqMan® Universal Master Mix (Applied Biosystems, manufactured by Roche, Branchburg, NJ, USA), 5 μl each (final concentration 900 nM) of primer TV forward (5’ CAT TGA CCA CAC GGA CAA AAA G 3’) and primer TV reverse (5’ CGA AGT GCT CGA ATG CGA 3’), 5 μl (final concentration 225 nM) of probe (5’FAM - TCA
TTT CGG ATG GTC AAG CAG CCA - TAMRA 3’ and 5 μl of sample DNA. The samples were amplified in an ABI Prism 7000 Sequence Detector (Applied Biosystems, Foster City, CA, USA) using the following thermal cycling conditions: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and at 60°C for 1 minute. Each run included a positive and negative control. A positive T. vaginalis result was defined as a cycle threshold (Ct) less than 40; samples with a Ct of > 38 and < 40 were repeated to confirm the result. All samples with a Ct > 38 and ≤40 on the initial run were confirmed on repeat testing.

Culturing T. vaginalis in the InPouch TV System and then counting the organisms using a hemocytometer determined the limit of detection of the assay. The T. vaginalis organism was obtained from BioMed Diagnostics (San Jose, CA, USA). The limit of detection was 0.2 organisms per reaction. The specificity of the assay was assessed by testing a variety of organisms commonly isolated from the female genital tract, including Escherichia coli, Candida albicans, Enterococcus faecalis, Gardnerella vaginalis, Staphylococcus aureus and Lactobacillus acidophilus, and genital pathogens including cytomegalovirus, herpes simplex virus, Chlamydia trachomatis and Neisseria gonorrhoeae. In addition, cultures of normal vaginal flora grown on sheep blood agar obtained from the Clinical Microbiology Laboratory were also tested in the assay. All of these specimens tested negative for T. vaginalis DNA in the real-time PCR assay.

Confirmatory T. vaginalis PCR assay

Discrepant specimens that were culture negative and positive by the real-time PCR assay were also tested in a confirmatory PCR assay that targets a different region of the T. vaginalis genome. For this testing an aliquot of the specimen, which had been stored at −70°C, was sent to a second laboratory for confirmatory PCR testing. The confirmatory PCR assay amplified a 312-base pair region of the 18S ribosomal DNA gene [20]. One μl of the extracted sample was added to a 50 μl volume of master mix comprising 1X Qiagen Hotstar buffer (Qiagen Inc, Valencia, CA, USA), 0.4 μM of each primer (TV1: 5’TAA TGG CAG AAT CTT TGG AG 3’, TV2: GAA CTT TAA CCG AAG GAC GAC TTC 3’), 200 μM dNTPs (with UTP), 1.25 U Qiagen Hotstar Taq and 0.5 U AmpErase (Applied Biosystems, Foster City, CA, USA). The reaction was amplified in a Gene Amp 9600 (Applied Biosystems, Foster City, CA, USA) using the following parameters: 10 minutes at room temperature, 15 minutes at 95°C, then 40 cycles of 30 seconds at 95°C, 1 minute at 57°C and 30 seconds at 72°C. The product was visualized on a 3% agarose gel (Nusieve GTG). Positive and negative controls were included in each run. A visible band in the gel at the appropriate molecular weight was considered a positive result.

Statistical analysis

Differences in Ct values between the culture-positive/real-time PCR-positive specimens and the culture-negative/real-time PCR-positive/confirmatory PCR positive specimens were analyzed using Student’s t-test.

Results

The real-time PCR assay had a limit of detection of < 0.2 organism per reaction, which was equivalent to approximately 40 copies/ml of specimen. A representative standard curve is shown in Figure 1. The assay was specific for T. vaginalis; there was no cross-reactivity with other genital pathogens or organisms found in normal vaginal flora (see Methods for a list of the organisms tested).

A total of 524 pairs of specimens were tested by culture and real-time PCR: 36 specimens were positive for T. vaginalis by culture and 54 were positive for T. vaginalis DNA by PCR (Table I). Using culture as the gold standard, the real-time PCR assay had a sensitivity of 100% and a specificity of 96.3%. The 18 specimens that were positive by real-time PCR and culture negative, as well as 36 specimens that were negative by real-time PCR and culture, were tested in a confirmatory PCR assay, which targeted a different region of the T. vaginalis genome. All 36 of the specimens negative by culture and the real-time PCR assay were also negative by the confirmatory PCR assay; 16 of the 18 specimens tested negative for T. vaginalis DNA in the real-time PCR assay.

![Figure 1. A representative standard curve for the real-time Trichomonas vaginalis PCR assay. The log10 concentration of T. vaginalis DNA (organisms/ml of specimen) is plotted against the Ct value.](image-url)
that were culture negative, and positive in the real-time PCR assay, were positive in the confirmatory PCR assay (Table II). These 16 specimens were considered true positive results, whereas the 2 specimens that were not positive in the confirmatory PCR assay were considered false positive results. The Ct values on the real-time PCR assay for the false-positive specimens were 35.1 and 39.4. Using a modified gold standard of positive by culture or positive in both PCR assays, the sensitivity of the real-time PCR assay was 100% and the specificity was 99.6%, whereas culture had a sensitivity of 69.2% and a specificity of 100%. Using the modified gold standard, the prevalence of T. vaginalis infection in our population was 10% (52 of 524) and the positive predictive value of the real-time PCR assay was 96.3% with a negative predictive value of 100%.

Discussion

We developed a real-time PCR assay for the detection of T. vaginalis that was both sensitive and specific. This study confirms the results of others [14–17,21] in showing that PCR testing was more sensitive than culture for the detection of T. vaginalis; in this study, the real-time PCR assay detected about 40% more positive samples than culture. This increase in sensitivity was obtained with very little loss of specificity, i.e., 99.6% for the PCR assay compared with 100% for culture. Although the real-time PCR assay was not designed to quantify the amount of T. vaginalis DNA in the specimen, the Ct-value was inversely related to the concentration of DNA in the specimen: samples with a low organism burden have a higher Ct value. The comparison of Ct values between the culture-positive, real-time PCR-positive (Cx+/PCR+), culture-negative, real-time and confirmatory PCR-positive (Cx-/PCR+ confirmed) and culture-negative, real-time PCR-positive, confirmatory PCR-negative (Cx-/PCR+ unconfirmed) samples. The line represents the mean Ct value.

Table I. Comparison of culture and real-time PCR for the detection of T. vaginalis.

<table>
<thead>
<tr>
<th>Real-time PCR</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>470</td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction; real-time PCR sensitivity = 100%, specificity = 96.3%.

Table II. Comparison of culture and real-time PCR for the detection of T. vaginalis.

<table>
<thead>
<tr>
<th>Culture/confirmatory PCR</th>
<th>Real-time PCR</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>52</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>470</td>
<td></td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction; real-time PCR sensitivity = 100%, specificity = 99.6%.

the culture-positive REAL-TIME PCR-positive specimens was > 2 log_{10} higher than that observed in the culture-negative/real-time and confirmatory PCR-positive specimens.
Neisseria gonorrhoeae tests are FDA cleared for the BDProbeTec ET Culturette Direct Dry Swab system for clinical laboratories. In this study we successfully cultured and urine specimens and are widely used in the detection of these pathogens in urethral, endocervical and urine specimens and are widely used in clinical laboratories. In this study we successfully used the BDProbeTec ET Culturette Direct Dry Swab system for specimen collection. This real-time PCR assay as designed provides a simplified approach to improving the detection of N. gonorrhoeae and T. vaginalis infection, improving compliance in high-risk groups. Such an improvement, according to what has been observed for C. trachomatis infection, would be predicted to reduce the incidence of T. vaginalis infection, possibly to reduce HIV-1 transmission and even to reduce poor pregnancy outcomes.

References
