18S Ribosomal DNA-Based PCR for Diagnosis of Trichomonas vaginalis

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Trichomonas vaginalis remains the most common sexually transmitted parasite in the world and is considered a major risk factor in the transmission of the human immunodeficiency virus. A PCR technique using primers targeting a specific region of the 18S rRNA gene of T. vaginalis was developed. The PCR test was standardized using 15 reference strains, giving a single product of 312 bp in all strains. No amplification was observed when DNA from related organisms or human DNA was used as a target. The test was evaluated on 372 vaginal swab specimens and 361 urine samples from women attending infertility and obstetric clinics at two separate hospitals in Lima, Peru. Compared to T. vaginalis culture, the overall sensitivity and specificity of PCR of vaginal swab samples was 100% and 98%, respectively. The PCR of urine samples was 100% sensitive and 99.7% specific compared to culture of vaginal swab, but the sensitivity drops to 83.3% when compared to PCR of vaginal swabs. All culture-positive samples were found to be positive by PCR in either urine or vaginal secretion. None of the PCR-negative samples were positive by culture. The origin of the amplification was confirmed by digestion of PCR products with HaeIII. This PCR assay, which is easy to perform and has a high sensitivity and specificity, should be useful for routine diagnosis of T. vaginalis infection.

Trichomoniasis is the most common parasitic sexually transmitted disease in the world (5, 8, 33). In addition to reproductive tract symptoms, infection with Trichomonas vaginalis is increasingly being recognized as having an association with reproductive complications including premature rupture of membranes, premature birth, low birth weight, and infections occurring after abortion and caesarean delivery (6). Even more important is its role as a risk factor for the transmission of the human immunodeficiency virus (11, 27).

As with other sexually transmitted diseases, symptoms and signs of trichomoniasis are not adequately sensitive or specific for diagnosis (32). Thus, diagnostic laboratory testing is usually required to confirm the presence of the organism. Routine clinical diagnosis usually depends on microscopic observation of motile parasites in wet-mount preparations. Although rapid and inexpensive, the wet mount may not be highly sensitive, especially when a delay in examining the sample occurs, detecting only about 60% of culture positive samples (10, 29). Culture is considered the most reliable method of diagnosis but requires a special medium and frequent microscopic observation for up to 7 days (12, 14, 20). The sensitivity of culture is less than 90%, allowing for false negatives due to lack of detection of nonviable or small numbers of parasites (3, 10, 25). Cytology preparations, such as Papanicolaou’s stain test (Pap smear) (10, 19, 29), also lack both sensitivity and specificity. Other diagnostic techniques, such as monoclonal antibodies, in situ hybridization, and immunological assays, are time-consuming and expensive and lack sensitivity (1, 10, 18, 23).

Several assays for the diagnosis of trichomoniasis based on PCR have recently been developed (9, 13, 22) and evaluated (15, 29, 30), the most common of which use DNA repetitive sequences as the target. This technique, however, allows for the production of nonspecific products due to the presence of insertion segments in some strains. Thus, different strains produce bands that migrate differently. In addition, repetitive sequences or amplification of the b-tubulin gene fails to detect some strains due to strain variation (15).

Recently, a comparative analysis of the 5.8S rRNA gene and the internal transcribed spacer regions of trichomonad protozoa demonstrated a high degree of intraspecies conservation of these sequences (2). Coding regions such as the 5.8S, 18S, and 28S genes are more conserved than the internal transcribed spacer regions. Ribosomal genes are highly conserved in their primary structure. This characteristic and their highly repetitive nature in the genome of most organisms make these genes good targets for detection by PCR. We have designed primers that are based on conserved regions of the 16S-like gene of T. vaginalis. In this study we determined the sensitivity and specificity of these primers using clinical samples from infertile and pregnant women in Lima, Peru.

MATERIALS AND METHODS

Strains. Fifteen T. vaginalis strains were isolated from Peruvian patients. Strains were grown in Diamond’s modified TYM medium and axenized (14). These strains were used as reference strains to standardize the method.

Previously extracted DNA from Trichomonas tenax ATCC 30207, Trichomonas gallinae ATCC 30002, Guarula lamba ATCC SF-741 30888, Chlamydiae sulcatus ATCC 50862, Dhaisopteria fragilis ATCC 30048, Entamoeba histolytica ATCC SF-31-90015, Chlamydia trachomatis serovar E ATCC VR3488, and Netseria gonorrheae ATCC 19424 was used to assess the specificity of the PCR primers.
TABLE 1. Sample source, results of culture, PCR techniques, and kappa values for vaginal secretions tested for T. vaginalis.

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Clinica</th>
<th>No. of patients</th>
<th>No. (%) culture positive</th>
<th>No. (%) PCR positive (swabs)</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Loyza</td>
<td>Infertility</td>
<td>109</td>
<td>7 (6.4)</td>
<td>12 (11.0)</td>
<td>0.714</td>
</tr>
<tr>
<td></td>
<td>Obstetric</td>
<td>179</td>
<td>8 (4.5)</td>
<td>9 (5.0)</td>
<td>0.938</td>
</tr>
<tr>
<td>Dos de Mayo</td>
<td>Infertility</td>
<td>34</td>
<td>4 (11.8)</td>
<td>4 (11.8)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Obstetric</td>
<td>50</td>
<td>5 (10.0)</td>
<td>6 (12.0)</td>
<td>0.898</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>372</td>
<td>24 (6.5)</td>
<td>31 (8.3)</td>
<td>0.868</td>
</tr>
</tbody>
</table>

a No statistical differences were observed in incidence among patients from the different clinics (P > 0.05).

Sample collection. A total of 378 samples of vaginal secretions were obtained after informed consent from women attending the obstetric and infertility clinics of the Dos de Mayo and Arzobipco Loyza hospitals in Lima, Peru (Table 1). These two Ministry of Health public hospitals serve lower-middle-class populations of the city of Lima. The institutional ethics committees of Asociacion Bene- fica PRISMA and the Johns Hopkins University approved the protocol.

Two endocervical samples were collected from each woman using a sterile calcium alginate swab (Fisher Scientific, Pittsburgh, Pa.). One swab was placed in 3 ml of 0.15 M NaCl-0.05 M Na2HPO4-NaH2PO4 (PBS, pH 7.2) plus antibiotics (penicillin G [200,000 U/ml], gentamicin [10 μg/ml], and amphotericin B [20 μg/ml]) and used for culturing. The other swab was placed in 500 μl of 0.01 M Tris-HCl (pH 8) and used for the PCR assay.

Patients were asked to collect urine specimens, which were kept at 4°C until they reached the laboratory, where they were processed immediately.

Culture. The PBS tube containing the vaginal swab was vortexed, the swab was discarded under sterile conditions, and the remaining liquid was centrifuged at 3,000 × g for 5 min. The supernatant was discarded, and the pellet was transferred into a tube containing 8 ml of Diamond’s modified TYM (14). The tube was then incubated at 37°C for 8 days and observed microscopically every 2 days. The sample was considered negative if no motile trichomonads were observed in the culture medium after 10 days of incubation. Culture of a vaginal sample was used as the gold standard for PCR. Urine samples were not cultured.

DNA extraction of culture strains. Mid-log-phase axenic T. vaginalis cultures (1010 parasites/ml) were chilled on ice for 10 min and then pelleted by centrifugation at 3,000 × g for 5 min at 4°C. The cell pellet was washed twice with PBS. DNA was then extracted according to a previously described procedure (23). Briefly, the cell pellet was resuspended in 1 ml of lysis buffer (450 mM NaCl, 15 mM sodium citrate, 0.2% sodium dodecyl sulfate) plus 200 μg of proteinase K per ml and incubated at 65°C for 1 h. DNA was then phenol-chloroform extracted, precipitated using ethanol and ammonium acetate, resuspended in 500 μl of TE buffer (0.01 M Tris-HCl, 0.001 M EDTA [pH 8]) containing 20 μg of RNase-free RNase per ml and incubated at 37°C for 30 min. The suspension was phenol-chloroform extracted, precipitated, and finally resuspended in 200 μl of DNase- and RNase-free water (Sigma, St. Louis, Mo.). The DNA concentration was spectrophotometrically determined (24).

Human DNA was extracted from leukocytes from a healthy volunteer using the QIAamp Tissue kit (Qiagen Inc., Chatsworth, Calif.). The DNA from Toxoplasma gondii strain RH was extracted using the Chelex procedure.

DNA extraction from clinical samples. The Tris-HCl tube containing the swab was vortexed, and after removal of the swab, a 100-μl aliquot was transferred to a 1.5-ml tube and pelleted by centrifugation at 12,000 × g for 30 s. They were either used immediately or stored at −20°C. Finally, 15 μl of PCR mix was achieved. Dilutions were then prepared by the Chelex technique as described above for clinical samples. With a similar purpose, T. vaginalis purified DNA was 10-fold diluted starting from 1 organism per 25 μl of PCR mix was achieved. Dilutions were then processed using the Chelex technique as described above for clinical samples. With a similar purpose, T. vaginalis purified DNA was 10-fold diluted starting from 1 organism per 25 μl of PCR mix was achieved. Dilutions were then processed using the Chelex technique as described above for clinical samples. With a similar purpose, T. vaginalis purified DNA was 10-fold diluted starting from 1 organism per 25 μl of PCR mix was achieved. Dilutions were then prepared by the Chelex technique as described above for clinical samples. With a similar purpose, T. vaginalis purified DNA was 10-fold diluted starting from 1 organism per 25 μl of PCR mix was achieved. Dilutions were then prepared by the Chelex technique as described above for clinical samples.
inhibition. This sample first gave a negative amplification when the PCR for β-globin was performed but became positive after being diluting 1:10 or 1:100. This sample was also gave a false-negative result when tested directly for \( T. \) \( \text{vaginalis} \), but a positive result was observed when the diluted sample was tested.

**Clinical specificity.** None of the 58 dental plaque samples gave a positive amplification when PCR was performed using primer set \( T v1 \) and \( T v2 \), although wet-mount examination revealed the presence of \( T. \) \( \text{tenax} \) in eight samples and \( \text{Entamoeba} \) \( \text{gingivalis} \) in 15 samples.

**Clinical samples.** In clinical vaginal swab samples, \( T. \) \( \text{vaginalis} \) was detected by PCR in 8.3\% (31 of 372) of the samples, compared to 6.5\% (24 of 372) detected by culture. All of the 24 isolates were detected by PCR in both vaginal secretions and urine (Table 1). When urine samples were tested for PCR, 6.9\% (25 of 361) of the samples gave a positive amplification for \( T. \) \( \text{vaginalis} \). One of the PCR-positive, culture-negative patients did not have her urine sample tested by PCR. One woman with a vaginal sample positive for \( T. \) \( \text{vaginalis} \) by PCR but negative by culture had a positive urine sample on testing by PCR.

There was a high degree of concordance between PCR and culture when vaginal samples were tested for the presence of

![Image](http://jcm.asm.org/)
–20°C, however, did degenerate over time (H. Mayta, personal observation).

In this study, primers Tv1 and Tv2 were also able to detect *T. vaginalis* in urine samples. PCR of urine samples gave results comparable to those obtained by culturing vaginal specimens. In contrast, PCR of urine samples rarely detected *T. vaginalis* from patients who had *T. vaginalis* PCR-positive and culture-negative vaginal specimens. If all PCR-positive specimens are considered (whether culture positive or negative), then the sensitivity of the urine PCR was 80%. When urine is used to detect trichomonas infection in large-scale population samples, the decrease in urine PCR sensitivity needs to be taken into consideration.

Molecular techniques for the diagnosis of *T. vaginalis* have been previously reported but are not as sensitive or specific as the PCR we described in the present study. The use of a DNA probe (1, 17, 23) has the disadvantage of cross-reacting with DNA from *Pentarichomonas hominis* and also being relatively insensitive, since its detection limit is 200 axenically cultivated protozoa. The first PCR described for *T. vaginalis* (22) has a similar sensitivity to culture (7, 28) and also misses some axenically cultivated strains (15). Targeting another repetitive sequences, Kegne et al. (9) could amplify one axenically cultured parasite, but this PCR has not been tested under clinical conditions. A nested PCR and a colorimetric nested PCR also were described which used as their target a repetitive DNA sequence (13, 18, 26). Both PCR protocols produce nonspecific bands, which may cause false-positive results. Nested PCR techniques, although generally sensitive, have a higher cost, are more labor intensive and are also more prone to contamination than a simple PCR. More recently, a PCR based on the β-tubulin genes (15) was described. However, due to the high degree of variation among *T. vaginalis* strains, it lacks sensitivity since it misses some culture-positive strains. The β-tubulin gene PCR also lacks specificity, since it cross-reacts with *T. tenax*.

The PCR described here is highly sensitive. All culture-positive specimens were detected by PCR. This technique is also highly specific, as demonstrated by the lack of cross-reaction with the closely related trichomonad *T. tenax*. The high sensitivity of primer set Tv1 and Tv2 may permit the detection of small numbers of *T. vaginalis* organisms which may not grow in culture. Moreover, culture of *T. vaginalis* may not be successful, since between 300 and 500 organisms are required to obtain a positive result (4). The PCR using primer set Tv1 and Tv2 was indeed reproducible and, as demonstrated by restriction enzyme analysis (REA), the product obtained from clinical samples was concordant with those obtained from *T. vaginalis* axenic strains. The PCR assay for the detection of *T. vaginalis* that we have designed is simple, easy to perform, and highly sensitive and specific. While it is optimal when using vaginal secretions, the test will perform, albeit at lower levels of sensitivity, with urine samples, permitting an easy and non-invasive method of specimen collection.

While culturing of samples takes at least 7 days, the PCR assay described here takes about 4 to 5 h from the time of sample collection until electrophoresis of enzyme-restricted PCR products. Restriction analysis of PCR products is not a necessary step after it has been performed once; thus, on a routine basis, PCR for the diagnosis of *T. vaginalis* can be performed within a 3-h period.

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**REFERENCES**


