

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

Claudiu I. Banea,^a Kahaliah Joseph,^a Evan W. Secor,^a Laurie A. Jones,^a Joseph U. Igietseme,^a Robert L. Sautter,^b Margaret R. Hammerschlag,^c Nancy N. Fajman,^d Rebecca G. Girardet,^e Carolyn M. Black^a

Centers for Disease Control and Prevention, Atlanta, Georgia, USA^a; Carolinas Pathology Group, Charlotte, North Carolina, USA^b; SUNY Downstate Medical Center, Brooklyn, New York, USA^c; Emory University School of Medicine, Atlanta, Georgia, USA^d; The University of Texas—Houston Medical School, Houston, Texas, USA^e

***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 tenax* and *Pentatrachomonas 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-

Received 26 November 2012 Returned for modification 8 January 2013

Accepted 29 January 2013

Published ahead of print 6 February 2013

Address correspondence to Claudiu I. Banea, cbanea@cdc.gov.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.03101-12

TABLE 1 *T. vaginalis* PCR primers and their sensitivities^a

Gene target ^b	Primer	Primer sequence	Size (bp)	Sensitivity ^c	
				PCR	Nested PCR
KENG0E (L23861)	TVK3F	5'-ATT GTC GAA CAT TGG TCT TAC CCT C-3'	262	1	0.01
	TVK7R	5'-TCT GTG CCG TCT TCA AGT ATG C-3'			
	TVC1F	5'-TCA GTT CGC AAA GGC AGT CCT-3'	213	1	
	TVC2R	5'-GTA CTT ACG CTT GGA GAG GAC ATG A-3'			
MURESU (X83109)	TVC3F	5'-GAT GCC ATG AAC GGA AAT GTT-3'	299	1	0.01
	TVC4R	5'-TCT GGA GCA TAT TGG ATC CG-3'			
	TVC11F	5'-CGA ATG GRA TAA CGA ATG CGA C-3'	237	0.1	
	TVC12R	5'-CAA CCT TTC TTG TCA GAC AAC TTG-3'			
PACES (M86482)	TVC5F	5'-AAT TCC CGG ATA ATT GAA ACG GA-3'	190	0.1	0.01
	TVC6R	5'-GAT GTT GGG GAT GTT TTG TAT TCT G-3'			
	TVC7F	5'-GAT AAA GAA AAT GTG TTT AAG TTG ATG GA-3'	148	1	
	TVC8R	5'-TTG TAT TCT GAC ACT GGT TCC AAT TT-3'			
PACES (M86482)	TVOP1F	5'-GTG AAA ATC TCA TTG GGG TAT TAA CTT-3'	580	1	0.01
	TVOP2R	5'-GTT TTA TTT ATC ACT GGA AAA TAA CGC TT-3'			
	TVC9F	5'-AGA ATA CAA AAC ATC CCC AAC ATC TT-3'	358	1	
	TVC10R	5'-CCC ATT CTT TTA GAC CCT TCA GAT T-3'			

^a 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.

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/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* in our nested PCR assay. A subset of specimens, including 8 of the *T. vaginalis*-positive specimens reported in this study, were sent for confirmatory testing using a commercially available APTIMA transcription-mediated amplification assay (courtesy of Gen-Probe Inc., San Diego, CA). Seven of the eight *T. vaginalis*-positive specimens were confirmed as positive; however, due to insufficient specimen quantity, no discrepancy analysis could be performed, which limits the interpretation of the result.

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-

aration 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 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.

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

Primer	Primer sequence
TF1	5'-TCC TAC CGA TTG GAT GAC TCG-3'
TF2	5'-GGA AGG AGA AGT CGT AAC AAG-3'
TF3	5'-GTA GGT GAA CCT GCC GTT GGA T-3'
TR1	5'-TGA GGA GCC AAG ACA TCC ATT G-3'
TR2	5'-ATG CAA CGT TCT TCA TCG TG-3'
TR3	5'-GCG CAA TTT GCA TTC AAA GAT-3'
TR4	5'-GAG ATG CTT CAG TTC AGC GGG T-3'
TR5	5'-CTT TTC CTC CGC TTA TTG AGA TG-3'

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

- Johnston VJ, Mabey DC. 2008. Global epidemiology and control of *Trichomonas vaginalis*. *Curr. Opin. Infect. Dis.* 21:56–64.
- Sutton M, Sternberg M, Koumans EH, McQuillan G, Berman S, Markowitz L. 2007. The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States, 2001–2004. *Clin. Infect. Dis.* 45:1319–1326.
- Van Der Pol B. 2007. *Trichomonas vaginalis* infection: the most prevalent nonviral sexually transmitted infection receives the least public health attention. *Clin. Infect. Dis.* 44:23–25.
- Weinstock H, Berman S, Cates W, Jr. 2004. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. *Perspect. Sex. Reprod. Health* 36:6–10.
- Petrin D, Delgaty K, Bhatt R, Garber G. 1998. Clinical and microbiological aspects of *Trichomonas vaginalis*. *Clin. Microbiol. Rev.* 11:300–317.
- McClelland RS, Sangare L, Hassan WM, Lavreys L, Mandaliya K, Kiarie J, Ndinya-Achola J, Jaoko W, Baeten JM. 2007. Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. *J. Infect. Dis.* 195:698–702.
- Van Der Pol B, Kwok C, Pierre-Louis B, Rinaldi A, Salata RA, Chen PL, van de Wijgert J, Mmiro F, Mugerwa R, Chipato T, Morrison CS. 2008. *Trichomonas vaginalis* infection and human immunodeficiency virus acquisition in African women. *J. Infect. Dis.* 197:548–554.
- Gottlieb SL, Douglas JM, Jr, Foster M, Schmid DS, Newman DR, Baron AE, Bolan G, Iatesta M, Malotte CK, Zenilman J, Fishbein M, Peterman TA, Kamb ML. 2004. Incidence of herpes simplex virus type 2 infection in 5 sexually transmitted disease (STD) clinics and the effect of HIV/STD risk-reduction counseling. *J. Infect. Dis.* 190:1059–1067.
- Zhang ZF, Begg CB. 1994. Is *Trichomonas vaginalis* a cause of cervical neoplasia? Results from a combined analysis of 24 studies. *Int. J. Epidemiol.* 23:682–690.
- Crucitti T, Van Dyck DE, Tehe A, Abdellati S, Vuylsteke B, Buve A, Laga M. 2003. Comparison of culture and different PCR assays for detection of *Trichomonas vaginalis* in self collected vaginal swab specimens. *Sex. Transm. Infect.* 79:393–398.
- Radonjic IV, Dzamic AM, Mitrovic SM, Arsenijevic VSA, Popadic DM, Kranjic ZI. 2006. Diagnosis of *Trichomonas vaginalis* infection: the sensitivities and specificities of microscopy, culture and PCR assay. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 126:116–120.
- Van Der Schee C, van Belkum A, Zwiijgers L, van Der Brugge E, O'neill EL, Luijendijk A, van Rijsoort-Vos T, van Der Meijden WI, Verbrugh H, Sluiter HJ. 1999. Improved diagnosis of *Trichomonas vaginalis* infection by PCR using vaginal swabs and urine specimens compared to diagnosis by wet mount microscopy, culture, and fluorescent staining. *J. Clin. Microbiol.* 37:4127–4130.
- Hardick A, Hardick J, Wood BJ, Gaydos C. 2006. Comparison between the Gen-Probe transcription-mediated amplification *Trichomonas vaginalis* research assay and real-time PCR for *Trichomonas vaginalis* detection using a Roche LightCycler instrument with female self-obtained vaginal swab samples and male urine samples. *J. Clin. Microbiol.* 44:4197–4199.
- Nye MB, Schwebke JR, Body BA. 2009. Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. *Am. J. Obstet. Gynecol.* 200:188.
- Black CM, Driebe EM, Howard LA, Fajman NN, Sawyer MK, Girardet RG, Sautter RL, Greenwald E, Beck-Sague CM, Unger ER, Igietseme JU, Hammerschlag MR. 2009. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. *Pediatr. Infect. Dis. J.* 28:608–613.
- Girardet RG, Lahoti S, Howard LA, Fajman NN, Sawyer MK, Driebe EM, Lee F, Sautter RL, Greenwald E, Beck-Sague CM, Hammerschlag MR, Black CM. 2009. Epidemiology of sexually transmitted infections in suspected child victims of sexual assault. *Pediatrics* 124:79–86.
- Hollman D, Coupey SM, Fox AS, Herold BC. 2010. Screening for *Trichomonas vaginalis* in high-risk adolescent females with a new transcription-mediated nucleic acid amplification test (NAAT): associations with ethnicity, symptoms, and prior and current STIs. *J. Pediatr. Adolesc. Gynecol.* 23:312–316.
- Van Der Pol B, Kraft CS, Williams JA. 2006. Use of an adaptation of a commercially available PCR assay aimed at diagnosis of chlamydia and gonorrhea to detect *Trichomonas vaginalis* in urogenital specimens. *J. Clin. Microbiol.* 44:366–373.
- Kengne J, Veas F, Vidal N, Rey JL, Cuny G. 1994. *Trichomonas vaginalis*: repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. *Cell Mol. Biol.* 40:819–831.
- Paces J, Urbankova V, Urbanek P. 1992. Cloning and characterization of a repetitive DNA sequence specific for *Trichomonas vaginalis*. *Mol. Biochem. Parasitol.* 54:247–255.
- Muresu R, Rubino S, Rizzu P, Baldini A, Colombo M, Cappuccinelli P. 1994. A new method for identification of *Trichomonas vaginalis* by fluorescent DNA in situ hybridization. *J. Clin. Microbiol.* 32:1018–1022.
- Rubino S, Muresu R, Rappelli P, Fiori PL, Rizzu P, Erre G, Cappuccinelli P. 1991. Molecular probe for identification of *Trichomonas vaginalis* DNA. *J. Clin. Microbiol.* 29:702–706.
- Banda CI, Kubota K, Brown TM, Kilmarx PH, Bhullar V, Yanpaisarn S, Chaisilwattana P, Siriwasin W, Black CM. 2001. Typing of *Chlamydia trachomatis* strains from urine samples by amplification and sequencing the major outer membrane protein gene (*omp1*). *Sex. Transm. Infect.* 77:419–422.
- Cepicka I, Hampel V, Kulda J, Flegr J. 2006. New evolutionary lineages, unexpected diversity, and host specificity in the parabasalid genus *Tetratrichomonas*. *Mol. Phylogenet. Evol.* 39:542–551.
- Mantini C, Souppart L, Noel C, Duong TH, Mornet M, Carroger G, Dupont P, Masseret E, Goustille J, Capron M, Duboucher C, Dei-Cas E, Viscogliosi E. 2009. Molecular characterization of a new *Tetratrichomonas* species in a patient with empyema. *J. Clin. Microbiol.* 47:2336–2339.
- Kleina P, Bettim-Bandinelli J, Bonatto SL, Benchimol M, Bogo MR. 2004. Molecular phylogeny of *Trichomonadidae* family inferred from ITS-1, 5.8S rRNA and ITS-2 sequences. *Int. J. Parasitol.* 34:963–970.