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Improved Detection by DNA Amplification of *Trichomonas vaginalis* in Males

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Trichomoniasis is a sexually transmitted infection that is highly prevalent worldwide and has been linked to preterm birth and human immunodeficiency virus acquisition. In females, trichomoniasis causes vaginitis, while in males, it is frequently asymptomatic but can be a cause of urethritis. Control efforts have been hampered by the lack of a sensitive diagnostic technique for this infection in males. Men attending a sexually transmitted disease (STD) clinic for a new complaint were screened for *Trichomonas vaginalis* by culture and by PCR analysis of urine and urethral-swab specimens. The prevalence of *Trichomonas* determined by culture was 5% (15 of 300 specimens), compared to 17% (52 of 300) determined by PCR. Urine specimens yielded a greater number of positive results by PCR than did urethral-swab specimens. The sensitivity of PCR analysis of urine specimens in comparison to that of culture was 100%. The use of PCR techniques in urine specimen-based detection of *T. vaginalis* was highly sensitive and revealed a prevalence of infection more than three times that revealed by culture for men at high risk for STDs.

Bacterial sexually transmitted diseases (STDs), such as syphilis, gonorrhea, and chlamydia, are declining in the United States; however, infections caused by *Trichomonas vaginalis* have not evidenced similar declines. Despite the fact that vaginal trichomoniasis has been linked to preterm birth and the acquisition of human immunodeficiency virus (HIV) (3, 11), increased screening efforts have not been made. For women, the most commonly used diagnostic test for *Trichomonas* is a direct microscopic examination of the vaginal fluid. Although it is highly specific, the sensitivity of this technique in comparison to that of culture ranges from 50 to 80% (9). Culture of vaginal specimens for the organism is the current "gold standard"; however, the use of PCR techniques for detection in females has been studied, with varied results, and current techniques do not appear to have greater sensitivity than culture does (4, 12, 13, 15). Diagnostic techniques for *Trichomonas* in males are not routinely used, as the current gold standard (culture of urine and urethral swabs) (10) is cumbersome and likely suboptimal. Few studies have addressed the utility of PCR testing for *Trichomonas* in males. We compared PCR results for urethral and urine specimens to those of culture techniques for the diagnosis of *Trichomonas* in men attending an STD clinic.

MATERIALS AND METHODS

Heterosexual males attending the Jefferson County Health Department STD Clinic in Birmingham, Ala., for STD screening or the treatment of its symptoms, were invited to participate in this diagnostic study. The study was approved by the Institutional Review Boards of the Jefferson County Health Department and the University of Alabama at Birmingham. Men were excluded from participation if they had urinated in the previous hour or had taken antibiotics during the preceding 14 days. Reported symptoms of urethral discharge and dysuria were recorded, as well as the number of polymorphonuclear leukocytes on a urethral Gram stain. Two urethral swabs, one for PCR and one for on-site inoculation, were collected into TVInPouch culture medium (BioMed Diagnostics, San Jose, Calif.) (1). A first-fraction voided urine specimen was collected and processed on-site. Fifty-milliliter sterile conical tubes were marked at 20 ml in order to control the voided sample amount. Ten milliliters of urine was processed by centrifugation at 100 × *g* for 5 min. The specimen was decanted, and the pellet was resuspended in 250 μl of the *Trichomonas* culture medium, 50 μl of which was inoculated into a second culture pouch. The remainder of the urine pellet was sent to the laboratory for PCR testing. Culture pouches were incubated at 37°C and examined daily for up to 5 days.

PCR for *T. vaginalis*. Urine pellets were washed in 5 ml of sterile water and centrifuged at 2,000 rpm for 10 min, decanted, and extracted with polyethylene glycol (30% PEG 8000; Fisher Scientific, Pittsburgh, Pa.) in 3 M NaCl (14). Five hundred microliters of PEG was used to resuspend the pellet, which was then incubated on ice for 30 min, centrifuged at room temperature for 15 min at 12,000 × *g*, decanted, and frozen at −20°C for DNA extraction (Wizard DNA purification kit; Promega, Madison, Wis.). Urethral swabs were not extracted with PEG but were agitated in 500 μl of sterile water, centrifuged at 12,000 × *g* for 5 min, decanted, and frozen for DNA extraction. DNA was extracted as previously described (12). The presence of DNA was confirmed in each sample by electrophoresis prior to PCR amplification. *T. vaginalis*-specific primers (TV 3/7) (7) were used for PCR amplification, which was performed as previously described (12). The amplified product was electrophoresed on a gel consisting of 2% agarose and 0.5 μg of ethidium bromide per ml, viewed on a UV light box, and photographed. Samples containing a 300-bp fragment were considered positive for *T. vaginalis*. The specificity of the PCR was confirmed by sequence analysis of the 300-bp PCR product from random samples, as previously described (17). For samples with discrepant results (culture positive, PCR negative), we performed PCR analysis a second time, with threefold more *Taq* polymerase in the reaction mixture, to check for inhibition.

RESULTS

Specimens were collected from 300 men. The prevalence of *Trichomonas* determined by culture was 5% (15 of 300), compared with a prevalence of 17% (52 of 300) determined by PCR. The background prevalence of *T. vaginalis* among women attending the STD clinic was 25%. Table 1 shows the sensitivities and specificities of both culture and PCR, stratified by specimen type for the diagnosis of *T. vaginalis* in men. The sensitivity and specificity of PCR for any urethral-swab specimens that were positive by culture were 80 and 93%, respectively, while those for urine specimens that were culture positive were 100 and 88%, respectively. PCR and culture results were significantly correlated (*P* value, <0.001). All culture-

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TABLE 1. Comparison of diagnostic tests for *T. vaginalis* in males

Diagnostic method and specimen[a]	No. of specimens				Sensitivity		Specificity		Predictive value (%)	
	True positive	False positive	False negative	True negative	%	95% CI[b]	%	95% CI	Positive	Negative
PCR										
Urine	15	35	0	250	100	74.7–100	88	83.2–91.2	30	100
Urethral swab	12	19	3	266	80	51.4–94.7	93	89.6–95.8	38.7	98.9
Culture										
Urethral swab	11	0	4	285	73	44.8–91.1	100	98.3–100	100	98.6
Urine pellet	8	0	7	285	53	27.4–77.7	100	98.3–100	100	97.6

[a] The gold standard is positive culture results for urethral-swab and/or urine specimen.
[b] CI, confidence interval.

positive specimens were PCR positive. Fifty of 52 (96%) urine specimens were PCR positive, whereas only 31 of 52 (59.6%) urethral-swab specimens from the same subjects were. Only five subjects whose urethral-swab specimens were PCR positive had negative PCR results when urine specimens were tested. It was determined that the latter were true false negatives after threefold more *Taq* polymerase was used in the follow-up PCR check for inhibition. Overall, inhibition was seen in 5 of 301 (2%) urine specimens and 4 of 301 (1%) urethral-swab specimens.

There was no significant association between urethral symptoms or inflammation (defined as ≥5 polymorphonuclear leukocytes per oil immersion field) and positive PCR or culture. Among males who reported having sexual contact with a woman with *Trichomonas* infection, the concordance rate was 27% (13 of 49); however, this was not statistically different from the rate among those males not reporting such contact.

DISCUSSION

T. vaginalis has been shown to be a cause of urethritis in males (10). It is known to be sexually transmitted and may be associated with preterm birth and HIV acquisition in women (3, 11). Cross-sectional studies have shown a significant association between *Trichomonas* infection and preterm birth (3). However, a recent prospective treatment trial examining the benefit of treating women with asymptomatic trichomoniasis with higher than usual doses of metronidazole yielded negative results (8), and thus the association remains controversial. The relationship of HIV to trichomoniasis seems clearer. Laga et al. reported a significant association between incident trichomoniasis and HIV seroconversion among women enrolled in a prospective study in Zaire (11). Buve et al. reported significantly higher rates of vaginal trichomoniasis among women residing in cities with high prevalences of HIV than among those residing in cities with low prevalences and suggested that trichomoniasis may be an important factor in determining rates of HIV infection (2). Control of trichomoniasis could result in significant public health benefits; however, control efforts have been hampered by the lack of a sensitive and convenient diagnostic test for males.

Reported prevalence rates of urethral infection with *T. vaginalis* in males have varied depending on the population studied and the diagnostic techniques used. In his series of sentinel studies using cultures of urine, urethra, coronal sulcus, and semen specimens, Krieger et al. found a prevalence of 11% among men attending an STD clinic. Among men with *Trichomonas* as the sole urethral pathogen, half of them had urethritis (10). In a similar study conducted at an STD clinic in Denver, Colo., investigators used a urine sediment culture, which produced a prevalence of 2.8% (6). In an interesting study reported by Saxena and Jenkins (16), the prevalence of *Trichomonas* among inner-city males from 16 to 22 years of age in a job training program was an astounding 58%. This group also had high prevalences of other STDs. *Trichomonas* was detected by a combination of methods, including urine sediment culture and direct fluorescent antibody testing. This study also found that only half of men with *Trichomonas* infections were symptomatic (16).

The use of PCR for diagnosis of *Trichomonas* in males has been previously reported in only one study, conducted by Hobbs et al. (5). Wet-mount microscopy and urethral culture, as well as PCR detection with urethral swabs, were used in Malawi to study both men who attended an STD clinic and men who went to a dermatology clinic. The prevalence of infection among symptomatic men was 21%, compared to 12% in asymptomatic males. The sensitivity and specificity of the PCR assay were 82 and 95%, respectively. The investigators speculated that the lower-than-expected sensitivity of the PCR assay may have been the result of the long-distance transport of the specimens.

The results of our study differ from those of Hobbs et al. We found that PCR for the detection of *Trichomonas* in males was significantly more sensitive than culture. The prevalence of infection detected by PCR was 17%, a rate which was anticipated considering the high rates among women attending the clinic. The yield was greatest from urine specimens, which were not examined in the Malawi study. Although inhibition is a potential problem with these techniques, the rate of inhibition in the present study was only 2%. As with other studies comparing DNA amplification techniques to culture, the specificity of PCR appears artificially low due to the lower sensitivity of the present gold standard; however, the inherent nature of the PCR technique suggests that the true specificity of the assay is much higher.

In summary, for men attending an STD clinic, PCR analysis of urine specimens was far more sensitive than culture for the detection of *Trichomonas* and prevalence rates were high. Screening for *Trichomonas* among men at risk for STDs should

be considered part of any public health initiative to control trichomoniasis.

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