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Diagnosis of *Chlamydia trachomatis* Urethritis in Men by Polymerase Chain Reaction Assay of First-Catch Urine

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To determine the accuracy of a recently developed polymerase chain reaction (PCR) urine assay to detect *Chlamydia trachomatis* urethral infection in men, we obtained urethral swabs and first-catch urine from 365 men attending a sexually transmitted diseases clinic. Thirty-three (9%) of the 365 men were infected with *C. trachomatis* as defined by urethral culture. Thirty-two of the 33 men with culture-positive urethral swabs also had PCR-positive urine assays. Of 332 patients with culture-negative urethral swabs, 325 had PCR-negative urine. Compared with chlamydia culture of urethral specimens, PCR assay of urine samples thus had a sensitivity of 97% and a specificity of 98%. The positive predictive value of the urine PCR assay was 82%, and the negative predictive value was 99%. Analysis of discrepant results indicated that six of seven PCR-positive, urethral culture-negative patients probably had chlamydial urethritis. All six patients had symptoms of urethritis and had either a positive urethral swab PCR or a positive urine PCR with a different amplification target. After resolution of discrepant results, (defining true positives as the 33 culture-positive patients and the 6 PCR-positive, culture-negative patients just described), the sensitivity and specificity of culture were 85% (33 of 39) and 100% (326 of 326), respectively. The revised sensitivity and specificity of PCR were 97% (38 of 39) and 99.7% (325 of 326), respectively. We conclude that this urine PCR assay provides a highly sensitive, noninvasive alternative method for the detection of *C. trachomatis* urethral infection in high-risk men attending a sexually transmitted diseases clinic. This assay could greatly facilitate the testing of larger numbers of male patients for chlamydial infection and should be studied in other settings.

Chlamydia trachomatis is the major cause of symptomatic nongonococcal urethritis in the United States and frequently causes asymptomatic urethral infection in sexually active men as well (1, 2). Furthermore, recent studies suggest that asymptomatic chlamydial urethritis in men constitutes an important reservoir for this infection in the community from which *C. trachomatis* may be transmitted to women, in whom serious sequelae may arise (19, 20). A rapid, easily performed, and accurate diagnostic test would facilitate early recognition and treatment of these infections in men and thus ultimately reduce infections in women. Because urethral swabs are painful and 10 to 30% of swabs are inadequately obtained, a noninvasive collection procedure, such as urine testing, would be particularly valuable for screening men for infection (3, 9, 26). In previous studies, urine testing using enzyme immunoassay or direct fluorescent antibody to detect *C. trachomatis* antigen have shown various degrees of sensitivity, sometimes as low as 45% (3, 4, 8, 11, 15, 26, 27). Since the amplification component of the polymerase chain reaction (PCR) assay (17) should increase the sensitivity of urine testing, we compared a PCR method that detects *C. trachomatis* DNA in urine with standard urethral cultures as a means for diagnosing *C. trachomatis* urethritis.

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MATERIALS AND METHODS

Patient population. The Harborview Medical Center sexually transmitted diseases clinic provides low-cost care to approximately 20,000 patients per year. Duplicate urethral specimens and 30 to 50 ml of first-catch urine were obtained from 365 men attending the clinic between January 1992 and September 1992. Patients who had taken antibiotics within the previous 2 weeks or patients who had urinated within the previous 2 h were not eligible for the study.

Specimen collection and transport. Following milking of the urethra, a dacron-tipped male urethral swab (Baxter Scientific Products, Seattle, Wash.) was advanced 2 cm into the urethra and then removed. This swab was placed in 1.5 ml of 0.2 M sucrose-phosphate buffer chlamydia transport medium (prepared according to the method of Gordon et al. [7]) and refrigerated for 6 to 24 h until inoculated.

An all-plastic, dacron-tipped male urethral swab (provided by Roche Molecular Systems) was similarly used to obtain a second urethral sample from each of the initial 200 patients. This swab was streaked onto an agar plate (for *Neisseria gonorrhoeae* culture), smeared onto a glass microscope slide (for Gram's stain), swirled in the Roche PCR transport medium for several seconds, and discarded. The PCR samples were stored at 4°C for 12 to 96 h prior to processing.

After the urethral swabs were obtained, the initial 200 patients were asked to provide 30 to 50 ml of first-catch urine. Following analysis of the first 200 patients, urine samples were obtained from 165 additional men in order to assess the accuracy of the urine PCR in a larger sample size. The urine samples were stored at 4°C.

Laboratory methods. PCR analysis of urine specimens was performed at Harborview Medical Center. The urine samples were transported to the PCR laboratory, where, after

mixing, 5 to 8 ml was centrifuged for 10 min at 1,500 × *g*. The supernatant was discarded, and the pellet was resuspended with 2 ml of Urine Response Buffer (Roche Molecular Systems) and incubated for 1 h at room temperature. An equal volume of Urine Diluent (Roche Molecular Systems) was added immediately after incubation. The tubes were vortexed thoroughly and allowed to sit for 10 min at room temperature prior to removal of 50 μl for PCR analysis.

PCR analysis of the urethral swab samples was also performed at Harborview Medical Center by using a rapid, nonradioactive, PCR-based assay developed by Roche Molecular Systems according to the manufacturer's instructions (10). Specimen tubes were briefly centrifuged (to remove liquid from the caps), and 1 ml of a specimen diluent solution was added. Fifty microliters of the patient samples or controls then underwent PCR analysis.

The 50-μl urine or urethral aliquots were added to PCR tubes containing 50 μl of the chlamydia PCR mix. The PCR mix contained primers labelled with biotin and all other components required for PCR. PCR amplification was carried out for 30 two-temperature cycles with the GeneAmp System 9600 thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.). The PCR assay amplifies a 207-bp sequence of the *C. trachomatis* cryptic plasmid. To control contamination, dUTP was substituted for TTP in the reaction mix. Amplification reaction mixes contained uracil-*N*-glycosylase.

After amplification, the PCR mixes were denatured with a weak sodium hydroxide solution and then added to a microtiter plate containing an immobilized *C. trachomatis*-specific DNA probe and a hybridization solution. After a 1-h incubation at 37°C, the plates were washed to remove unbound material and an avidin horseradish peroxidase solution was added. Following a 15-min incubation at 37°C, the plates were washed. Peroxide substrate solution containing tetramethylbenzidine was then added, and the plates were incubated for 10 min at room temperature. The reactions were stopped by the addition of a weak acid solution, and the optical density of the reaction mix was measured with a Titertek Multiscan MC spectrophotometer (ICN Flow, Costa Mesa, Calif.). Absorbance readings greater than 0.250 (at 450 nm) indicated the presence of *C. trachomatis* DNA. The total time for PCR analysis and detection was approximately 4 h.

Chlamydia cultures were performed at the University of Washington chlamydia laboratory by using cycloheximide-treated McCoy cells in microtiter plates and fluorescein-conjugated antilipopolysaccharide monoclonal antibody for enumeration of inclusions as previously described (25). The PCR assay was performed in a different laboratory at Harborview Medical Center, and the results of the PCR assay were not available to either the clinicians or the laboratory technician doing chlamydia cultures. All patients were treated on the basis of the culture results.

Analysis of discrepant results. Analysis of PCR and culture specimens with discrepant results was performed at Roche Molecular Systems. For patients with PCR-positive urine specimens and culture-negative urethral swabs, an alternate PCR assay that amplifies a 129-bp sequence of the *C. trachomatis* major outer membrane protein (MOMP) gene was performed (6). The assay conditions for MOMP PCR were similar to those used for the standard chlamydia PCR test (plasmid PCR), except that biotinylated primers specific to the MOMP gene were used and the amplification was carried out for 40 cycles. Detection of amplification products was carried out as described above, except that the DNA probe immobilized on the microtiter plates was specific for the MOMP PCR products.

TABLE 1. Performance of the *C. trachomatis* urine PCR test compared with culture

Result	No. by swab culture[a]	Result of urine PCR[b]	
		No. positive	No. negative
Positive	33	32	1
Negative	332	7	325
Total	365	39	326

[a] Prevalence was 9% by culture.
[b] Positive predictive value, 82%; negative predictive value, 99%; sensitivity, 96%; specificity, 98%.

RESULTS

Three hundred sixty-five duplicate urethral swabs and first-catch urine specimens were obtained for culture and PCR assay. Of the 365, 33 (9%) were culture positive for *C. trachomatis*. Thirty-two of the thirty-three culture-positive patients also had PCR-positive urine tests. Three hundred twenty-five culture-negative patients also had PCR-negative urine tests (Table 1). Compared with culture, the sensitivity of the PCR assay was thus 96% and the specificity was 98%.

Among the 33 culture-positive patients, separate urethral swabs for PCR were obtained from 21. Nineteen (95%) of the 21 swabs were also PCR positive. Urethral swabs for PCR were obtained from 166 of 177 culture-negative men, and 162 of the 166 were PCR negative.

Of the seven urine PCR-positive, urethral culture-negative patients, four had urethral swabs that were also PCR positive. Two other patients had urine samples that were also positive for MOMP DNA (Table 2). In light of these test results and the clinical findings for these six patients (see below), the patients were felt to have false-negative cultures.

All six patients with PCR-positive urine but culture-negative urethral swabs had symptoms of urethritis and/or contact with a female with mucopurulent cervicitis. Two of the patients had gonorrhea and were treated with cefixime and doxycycline. Three patients had urethral discharge, and Gram's stain of the discharge demonstrated no intracellular gram-negative diplococci but more than 15 polymorphonuclear leukocytes per high-power oil immersion field, thus meeting the diagnostic criteria for nongonococcal urethritis. These patients were treated with doxycycline. A sixth patient was an asymptomatic contact of a female with muco-

TABLE 2. Analysis of PCR-positive, culture-negative patients

Patient no.	Result of:			
	Urine PCR	Urethral culture	Urethral PCR	Urine MOMP PCR
51	+	−	NA[a]	−
65	+	−	−	+
100	+	−	+	+[b]
114	+	−	NA	+
119	+	−	+	−[b]
161	+	−	+	+[c]
166	+	−	+	+[c]

[a] NA, not available (not collected).
[b] MOMP PCR of urethral swab was positive.
[c] MOMP PCR of urine was not performed; MOMP PCR of urethral swab was positive.

purulent cervicitis. Gram's stain of a urethral smear from this patient demonstrated 5 polymorphonuclear leukocytes per high-power oil immersion field, and he had been treated with doxycycline. The final patient had a urine sample that was PCR positive for plasmid DNA but not MOMP DNA. The urethral culture was negative, and no urethral swab was available for PCR testing. This was the only patient among the seven for whom results were discrepant that was considered to be PCR false positive. A review of his record showed that this patient had been initially seen for nongonococcal urethritis and treated with doxycycline for 1 week. The pretreatment chlamydia urethral culture was positive. He returned 17 days later, desiring a test of cure, and was asymptomatic. Gram's stain of a urethral smear demonstrated more than 15 polymorphonuclear leukocytes per high-power oil immersion field, and the patient received an additional 14 days of doxycycline for persistent nongonococcal urethritis. He returned 7 days later (1 week after completing a second course of doxycycline), at which time urine PCR was positive and urethral culture was negative. There has been no further follow-up since that visit.

After resolution of discrepant results, in which true positives were considered to be the 33 culture-positive patients plus the 6 culture-negative patients with PCR-positive urine described above, the sensitivity and specificity of culture were 84.6% (33 of 39) and 100% (326 of 326), respectively. The revised sensitivity and specificity of PCR were 97.4% (38 of 39) and 99.7% (325 of 326), respectively.

DISCUSSION

C. trachomatis is the most common bacterial sexually transmitted disease in the United States. The infection can be successfully treated with nontoxic antibiotics, but control efforts have been hampered by the fact that many patients, both men and women, have mild symptoms or are asymptomatic carriers. These infected individuals may harbor the organism for months or years, transmitting the disease to their sexual partners (24).

Because of the fact that urine cultures for chlamydia are highly insensitive, urethral swab cultures have been the preferred diagnostic test for men (4, 21). Alternatively, antigen tests done with urethral swabs can be used. However, these tests have several attendant disadvantages. Swab insertion is painful and often leads to collection of an unsatisfactory specimen due to the brief insertion period. Ten to 30% of swabs may be inadequately obtained, particularly in patients without urethral discharge (3, 11, 26). Furthermore, male urethral infections are characterized by relatively small numbers (often less than 100) of inclusion forming units and may thus be missed by culture, especially under less-than-ideal transport circumstances (22). Similarly, antigen tests often perform poorly with urethral swab specimens because of the smaller number of organisms present (23).

For these reasons, rapid, noninvasive diagnostic tests such as enzyme immunoassay, direct fluorescent antibody, and leukocyte esterase testing of male urine samples have been proposed as possible alternatives to urethral culture (3, 4, 7, 11, 15, 26, 27). However, the sensitivities of these approaches compared with those of tests directly performed on urethral samples have often been suboptimal, particularly when these tests are compared with sensitive urethral culture techniques (8, 9, 18). Recently, the PCR has been used to detect *C. trachomatis*-specific nucleotide sequences in clinical specimens (10). Amplification of chromosomal DNA or plasmid DNA, followed by hybridization, to detect *C. trachomatis* in urethral and urine specimens has been found to be at least as sensitive as culture or antigen detection assays (12, 13, 16). However, widespread application of the techniques used in these studies has been limited because the PCR detection assay methods employed require expertise in DNA extraction, gel electrophoresis, and/or nucleic acid hybridization utilizing radioactive materials.

In this study, we evaluated a new detection assay provided by Roche Molecular Systems to identify *C. trachomatis* urethral infection by using first-catch urine. With a population of largely symptomatic men, we found the test to be at least as sensitive as a highly sensitive urethral culture system and highly specific. Discrepant results between urine PCR and urethral culture were obtained for eight patients. One patient had a culture-positive urethral swab, with 10 inclusion-forming units per well, and PCR-negative urine. Seven patients had urine that was PCR positive but urethral swabs that were culture negative. Analysis of the discrepant results demonstrated that six of these seven patients likely had false-negative cultures, and clinical data obtained from review of the charts of these seven patients supported this conclusion.

The seventh patient had recently received doxycycline therapy and may have had *C. trachomatis* DNA but no viable organisms still present in the urethra after therapy. This last case suggests that PCR may occasionally be so sensitive that it identifies nonviable chlamydiae in urine in the posttreatment period. Ten copies of plasmid DNA exist for each copy of MOMP DNA, possibly explaining why the PCR assay for plasmid DNA was positive and the PCR assay for MOMP DNA was negative for this patient (14). Workowski et al. reported that, among women with *C. trachomatis* endocervical infection that were treated with doxycycline, the PCR assay may be positive for 2 weeks after therapy but is uniformly negative by 4 weeks after therapy (28). Other investigators have reported similar findings for men (5).

In conclusion, our data indicate that PCR assay of male urine samples appears to be a sensitive, noninvasive method to identify *C. trachomatis* urethral infections in high-risk, untreated men with urethritis. The assay is simple to perform, does not utilize isotopes, requires minimal technical expertise, and detects all serovars of *C. trachomatis* (10). If true positives are defined as culture-positive patients and culture-negative, PCR-positive patients with clinical evidence strongly suggesting *C. trachomatis* infection, then the urine PCR was actually more sensitive (97%) than urethral cultures (85%). Record review indicated that approximately 50% of our high-risk sexually transmitted diseases clinic study population had clinical evidence of urethritis. The prevalence of chlamydial infection in the nonurethritis group was so low (5%) that we were not able to assess the utility of the urine PCR assay for this group of patients. Thus, other studies should define its role as a screening method for men without symptoms of urethritis. Furthermore, the significance of PCR-positive urine in men following appropriate antimicrobial therapy should also be addressed in other studies.

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