Direct Detection of *Chlamydia trachomatis* in Urine Specimens from Symptomatic and Asymptomatic Men by Using a Rapid Polymerase Chain Reaction Assay

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Screening for *Chlamydia trachomatis* infection in men has traditionally been limited to men who present with urethral symptoms, thereby limiting the detection of asymptomatic chlamydia infection in men. In order to effectively screen both symptomatic and asymptomatic men, we evaluated a newly developed polymerase chain reaction (PCR) assay, Amplicor *C. trachomatis*, from Roche Molecular Systems for the detection of *C. trachomatis* in urine specimens in comparison with urethral culture. A total of 530 male urine specimens were collected from 322 symptomatic and 208 asymptomatic men attending two sexually transmitted disease clinics in Baltimore, Md. The prevalence of *C. trachomatis* by culture was 9.8% (10.6% in symptomatic men and 8.2% in asymptomatic men). Compared with culture, the sensitivity of the PCR was 92.8%, the specificity was 94.7%, the positive predictive value was 68.4%, and the negative predictive value was 99.1%. Discrepant results between culture and PCR were further analyzed by direct fluorescent-antibody staining of elementary bodies in urine sediment and in culture transport vials and by major outer membrane protein PCR of transport media for specimens with negative culture. The revised sensitivity and specificity of PCR for urine were 95.0 and 99.8%, respectively, and the positive and negative predictive values were 98.7 and 99.1%, respectively. The sensitivity of culture compared with PCR and/or direct fluorescent-antibody staining was 68.4%. These results indicate that the PCR assay is a highly sensitive and specific assay for the detection of *C. trachomatis* in male urine specimens and provides a noninvasive technique for routine screening of chlamydia infection in both symptomatic and asymptomatic men.

*Chlamydia trachomatis* infections are recognized as the most prevalent sexually transmitted infections in the United States, with an estimated 4 million cases annually (2, 7). *C. trachomatis* is an obligate intracellular bacterium that infects mucosal surfaces of the cervix, urethra, rectum, nasopharynx, and conjunctiva. In males, *C. trachomatis* causes 40 to 50% of cases of nongonococcal urethritis, one of the most common sexually transmitted diseases in heterosexual males (16). Infections ascending from the urethra can result in acute epididymitis. In addition, a large proportion of chlamydia-infected males may remain asymptomatic, representing a large reservoir of infection (15, 17). Screening of males who are asymptomatic has been neglected because urethral sampling is a relatively traumatic and inconvenient procedure.

Detection of *Chlamydia trachomatis* infections in symptomatic males has traditionally been done by urethral swab for culture. Non-culture-based immunologic techniques have been developed to detect *C. trachomatis*, including direct fluorescent-antibody (DFA) tests, which use monoclonal antibodies to detect elementary bodies, and enzyme-linked immunosorbent assays (ELISAs) to detect *C. trachomatis* antigens. While these tests usually require a urethral swab, recent studies have evaluated the reliability of enzyme immunoassays (EIAs) in detecting chlamydia in urine (4).

More recently, a new noninvasive and sensitive diagnostic test to evaluate male urine specimens (Amplicor *C. trachom-

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The presence of inclusion bodies was determined with an epifluorescence microscope (Zeiss, Oberkochen, Germany). The cells in the duplicate plate of each specimen that was negative or toxic or contained less than three inclusions were passaged for an additional 48 h of culture.

PCR. Each urine specimen was mixed thoroughly, and 8 ml was centrifuged in a 15-ml conical tube at 1,000 × g for 15 min at room temperature. The supernatant was discarded, 2 ml of chlamydia urine buffer was added to the pellet, and the resulting suspension mixed in a multiple vortexer for 3 min. The tubes were incubated for 1 h at room temperature and vortexed for 1 min after the addition of 2 ml of specimen diluent. Two milliliters of the treated specimens were then aliquoted, and the aliquots were placed in polypropylene tubes and incubated for 10 min at room temperature prior to PCR amplification. Specimens could then be stored for up to 2 weeks at 4°C before amplification.

A dedicated hood was used for preparing reaction mixtures before amplification by PCR. Pipettors, racks, and tips were dedicated likewise. Positive and negative controls were prepared by adding 750 µl of control diluent to each of the positive and negative control tubes. Unused controls were stored at 4°C for subsequent use for up to 2 weeks. One hundred microliters of AmpErase was added to 1.7 ml of C. trachomatis Master Mix (Roche Molecular Systems). Unused material was stored at 4°C for up to 2 weeks. PCR (96-tube) racks were set up in a microtiter plate format, and 50 µl of C. trachomatis Master Mix with AmpErase was pipetted to each tube with a multichannel pipette. Next, 50 µl each of the treated patient specimens and the previously prepared controls (one positive and three negative) was added to designated tubes.

The sample tray was placed into a programmable model 9600 thermal cycler (Perkin Elmer Cetus, Norwalk, Conn.) for 30 cycles of denaturation at 95°C and annealing at 60°C. The first cycle was held at 95°C for 5 min and at 60°C for 1 min. The following cycles were held at 95°C for 30 s and at 60°C for 60 s. At the end, the temperature was held at 72°C for a minimum of 5 min before the sample tray was removed.

The caps were then carefully removed to avoid aerosolization of the PCR products, and 100 µl of denaturation solution (Roche Molecular Systems) was added to each tube. While the PCR mixture was incubating for 10 min at room temperature, 100 µl of hybridization solution was added to the C. trachomatis microwell plate (Roche Molecular Systems). Twenty-five microliters of denatured PCR product was then added to the microwell plate. The plate was gently tapped, covered, and incubated for 1 h at 37°C. After five washes with 1× wash buffer (Roche Molecular Systems), the plate was tapped dry, and 100 µl of avidin-horseradish peroxidase conjugate was added. The plate was then covered and incubated for 15 min at 37°C. After five washes, 100 µl of substrate A/B (4 parts substrate A to 1 part substrate B; Roche Molecular Systems) was added. The plate was covered and incubated in the dark for 10 min at room temperature. One hundred microliters of stop solution was added last, and the $A_{450}$ was read without blanking (Fig. 1).

Optical density values greater than 0.5 were considered positive; those less than 0.25 were considered negative; those between 0.25 and 0.50 were considered to be in the grey zone and were tested again. Negative controls were required to have optical density values less than 0.25 and the positive control had to have an optical density greater than 1.0 for the test to be considered valid.

Discrepant results. The transport media from the culture tubes were centrifuged at a minimum of 4,000 × g for 15 min. From the resulting pellet, 10 µl was placed on a slide, fixed with methanol for 5 min, and stained with C. trachomatis monoclonal antibody (Syva Microtrak) for the direct detection of elementary bodies (EBs). A positive slide was defined as one that had three or more EBs. Specimens with fewer than three EBs were reevaluated by MOMP-based PCR (1). In the MOMP PCR method, 200 µl of culture medium was desalted by spin dialysis (30,000-nominal-molecular-weight-limit filter; Millipore, Bedford, Mass.), and 50 µl of the
resulting specimen was used for PCR amplification. The ensuing assay method combines a liquid hybridization reaction and an enzyme immunoassay to detect biotinylated DNA-RNA hybrids (1). For DFA testing of urine, 200 µl of urine was centrifuged, and 5 µl of the resulting pellet was stained as described above.

RESULTS

A total of 530 patients attending two Baltimore sexually transmitted disease clinics were evaluated for C. trachomatis infection by a new PCR assay for urine specimens, and the results were compared with those obtained by urethral culture. Three hundred twenty-two (60.8%) men complained of urethral symptoms, and 208 (39.2%) men denied urethral symptoms and were evaluated for other problems. The prevalence of C. trachomatis by urethral culture was 9.8% overall (8.6% in asymptomatic men and 10.6% in symptomatic men).

Fifty-two specimens were PCR-EIA positive and culture positive, and 449 specimens were negative by both tests. There were 29 discrepant tests; 25 were positive by PCR-EIA and negative by culture, and 4 were positive by culture and negative by PCR-EIA. On the basis of discrepant results, the overall sensitivity of the PCR assay was 92.8%, its specificity was 94.7%, its positive predictive value (PPV) was 68.4%, and its negative predictive value (NPV) was 99.1%. After analysis of discrepant results by DFA and/or MOMP PCR, the adjusted sensitivity, specificity, PPV, and NPV increased to 95, 99.8, 98.7, and 99.1%, respectively. Of the 25 specimens which were PCR positive and culture negative, 18 had three or more EBs in DFA of spun culture transport vials. Six had one or two EBs per slide and were PCR positive by the alternative MOMP PCR. The only specimen with a false-positive PCR result which could not be resolved by DFA testing and/or MOMP PCR of the culture supernatant was found to have two EBs per slide by DFA assay of the urine sediment. This specimen was also positive by the Baxter-Bartells Enzyme immunoassay (Baxter-Bartells, McGraw Park, Ill.). Sampling error may explain this result, since the culture was negative by the DFA test and PCR, but the urine specimen was positive by the DFA test and ELISA as well as PCR. Two of the PCR-negative, culture-positive specimens were reamplified and assayed, and the results remained the same, suggesting the presence of inhibitors in the treated urine.

When analyzing the results by symptoms, as compared with culture, the sensitivity, specificity, PPV and NPV were 90, 95.2, 66.7, and 98.9%, respectively, for asymptomatic males and 94.4, 94.4, 68, and 99.3%, respectively, for symptomatic males. After analysis of discrepant results, the adjusted sensitivity, specificity, PPV, and NPV were 92.8, 99.4, 96.3, and 98.9%, respectively, for asymptomatic males and 96.2, 100, 100, and 99.3%, respectively, for symptomatic males. When culture was compared with the PCR and/or the DFA test as the "gold standard," culture detected C. trachomatis in only 54 of 79 specimens found to be positive by the other assays. Thus, the sensitivity of culture was 68.4% (68% for symptomatic males and 68.9% for asymptomatic males).

DISCUSSION

C. trachomatis infections are the most prevalent sexually transmitted bacterial infections in the United States among men and women. Screening for these infections is important not only to identify infected symptomatic individuals for the diagnosis and management of their infections but also to identify asymptomatic infected males who serve as reservoirs for C. trachomatis disease. In this study, asymptomatic males had a prevalence comparable to that of symptomatic males (9 to 10% versus 11 to 13%, respectively), but routine screening of asymptomatic males is problematic because of the unwillingness of asymptomatic patients to routinely submit to sampling of the urethra with a swab. Urine screening offers an alternative method of detection of chlamydiae in such men.

Traditionally, the gold standard for the identification of C. trachomatis infections in symptomatic males is tissue culture of urethral swabs, and patient screening has been limited primarily to symptomatic men because of the invasive nature of the technique. In addition, culture is time-consuming and labor-intensive. It takes 3 to 6 days to complete, and it requires access to specialized facilities and trained personnel. Other non-culture-based immunologic techniques have been developed in an effort to detect C. trachomatis in noninvasive samples. One such test is the DFA test, which utilizes fluorescein-conjugated monoclonal antibodies to detect EBs through microscopic analysis. EBs are difficult to detect in urine specimens because they are usually present in very low numbers and may be masked by a large amount of debris in the urine. Moreover, DFA tests require detection by highly competent personnel and are time-consuming. ELISAs have also been evaluated as screening tests for the rapid identification of infected symptomatic and asymptomatic males by using first-catch urine (3, 6, 12–14). ELISAs are relatively fast and easy to complete, but sensitivities of the tests for urine specimens from asymptomatic men remain relatively low (62.1 to 79.1% for the Abbott Chlamydiazyme test 82.1 to 91.7% for the Syva test, and 80% for the Baxter-Bartells test [8, 11, 13]).

Although the PCR has been evaluated for detection of C. trachomatis in genital specimens (1), it has not been extensively studied for use with male urine specimens. Palmer et al. found that the sensitivity of a MOMP-based PCR was 82% for urine from symptomatic males with nongonococcal urethritis, compared with 95% for urethral swabs, when the reference method was the DFA test (10). When a commercially available DNA amplification kit (Genemed Biotechnologies, San Francisco, Calif.) was used to compare the PCR, ELISA, and culture, the sensitivities of both the PCR and ELISA were 90.9% for male urine compared with symptomatic and asymptomatic urethral culture (18). Overall, for both males and females, the PCR was more sensitive (95.6%) than the ELISA (87.0%). Specificities were 97.7% (99.4% for men and 95.7% for women) for the ELISA and 98.0% (99.4% for men and 96.5% for women) for the PCR (18).

The Roche Amplicor C. trachomatis method combines a PCR and colorimetric detection for the detection of C. trachomatis in first-void male urine specimens. Overall, it is a relatively easy method to process a high volume of specimens (n = 90) in approximately 4.5 h. In this PCR method, the primers are derived from the cryptic plasmid, yielding a 207-bp DNA fragment (9). It is expected that this commercial test will become available within 1 year, pending FDA approval. In our study, it was highly sensitive (92.8%) and specific (94.7%) compared with urethral culture for both symptomatic and asymptomatic males. In processing over 500 specimens, there was only one false-positive PCR result. However, the specimen that yielded this result was positive for chlamydia antigen by EIA and had two EBs by the DFA test of the urine sediment, suggesting that it may have been
a true positive. Since treatment is often based on the specificity and positive predictive value of an assay, the reliability of the PCR assay offers clinicians a clear indication for initiation of treatment. PCR analysis of urine is therefore a highly sensitive and specific noninvasive technique for the diagnosis of \textit{C. trachomatis} and provides a unique opportunity for the early identification of both asymptomatic and symptomatic infected patients.

REFERENCES


