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Diagnosis by AMPLICOR PCR of *Chlamydia trachomatis* Infection in Urine Samples from Women and Men Attending Sexually Transmitted Disease Clinics

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Screening of urine specimens from men for *Chlamydia trachomatis* infection by a commercial PCR assay (AMPLICOR *C. trachomatis* Test; Roche Diagnostic Systems, Inc., Branchburg, N.J.) is a sensitive and specific noninvasive diagnostic assay. Since screening of women for *C. trachomatis* infection with the AMPLICOR *C. trachomatis* Test has been limited to use with endocervical swab specimens, we conducted an evaluation of the AMPLICOR *C. trachomatis* Test for the detection of *C. trachomatis* using female urine samples and compared the results with those obtained by in vitro culture and PCR of endocervical swab specimens. For 713 men we compared the performance of AMPLICOR *C. trachomatis* Test with urine specimens with that of culture of urethral specimens. For specimens that were PCR positive and culture negative, two additional tests were used to resolve the discrepancies: direct fluorescent-antibody assay (DFA) of sediment from a spun endocervical specimen culture vial and major outer membrane protein-based PCR of the sediment from the endocervical specimen culture vial. Of 525 urine specimens from females, 67 (12.8%) were PCR positive, and 41 (7.8%) endocervical specimens from the 525 women were culture positive. After resolution of the discrepancies, the resolved sensitivity of the urine PCR was 93.3%, whereas the sensitivity of endocervical swab specimen culture was 67.3%. Of 468 female endocervical swab specimens, 47 (10.0%) had a positive PCR result and 33 (7.0%) were culture positive. The resolved sensitivity of the endocervical swab specimen PCR was 86%. Of 415 matched female urine and endocervical swab specimens, there were 49 confirmed infections; 30 (61.2%) specimens were positive by culture of the endocervical swab specimen, 40 (81.6%) were positive by confirmed endocervical swab specimen PCR, 43 (87.8%) were positive by confirmed urine PCR, and all 49 (100%) were positive by either endocervical swab specimen PCR or urine PCR. For men, the resolved sensitivity of the urine PCR was 88%, and the sensitivity of culture was only 50.7%. These results indicate that urine PCR is highly sensitive for the detection of *C. trachomatis* in both women and men and provides a noninvasive technique for routine screening for chlamydial infection.

Chlamydia trachomatis is the most common sexually transmitted pathogen in the United States, with an estimated 4.5 million new cases each year (9). It is a common cause of urethritis and cervicitis, and serious sequelae include pelvic inflammatory disease (PID), infertility, ectopic pregnancy, epididymitis, proctitis, and arthritis. Conjunctivitis and pneumonitis can develop in infants exposed during passage through a chlamydia-infected birth canal. Direct and indirect costs for chlamydial infections have been estimated to exceed several billion dollars annually (7, 43). Consequently, the Centers for Disease Control and Prevention has recently recommended that young, sexually active individuals at risk, particularly women, be routinely screened for chlamydial infection (9). Upon identification of an infected individual, partner notification should be undertaken, with contact tracing if necessary, so that both sexual partners can be effectively treated.

Treatment for chlamydial infection with a 7-day course of doxycycline is highly effective and inexpensive. With the advent of efficacious single-dose azithromycin therapy (27), treatment has been further simplified and compliance has been markedly improved, resulting in a more cost-effective treatment for chlamydial infection. However, routine screening and identification of asymptomatic carriers of *C. trachomatis* remain major obstacles to a more complete and effective control program. Since 30 to 70% of all chlamydial infections may be asymptomatic, routine, noninvasive screening of individuals at risk for chlamydial infection is highly desirable. In the past, in vitro tissue culture has been the laboratory standard for the diagnosis of chlamydial infection (33, 39), but this technique requires the insertion of a swab into the urethras of men and requires that an endocervical swab specimen be obtained from women during a pelvic examination in order to obtain an adequate sample. In addition, the sensitivity of culture is less than 85% in most laboratories and is subject to inadequate sampling and transport (1, 20, 25, 33, 39). With the development of nucleic acid amplification assays such as PCR and ligase chain reaction (LCR), it has been possible to screen urine samples from men for *C. trachomatis* with remarkably high sensitivity and specificity (3, 4, 13, 14, 18, 42). More recently, several studies have demonstrated that the LCR assay could also be used to screen first-void urine (FVU) samples from women for chlamydial infections with sensitivities significantly higher than those of in vitro cultivation of endocervical swab specimens (2, 12, 21). We therefore conducted an evaluation of a commercially available PCR assay (AMPLICOR *C. trachomatis* Test; Roche Diagnostics Systems, Branchburg,

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N.J.) previously approved for use in screening male urine specimens to determine the sensitivity and specificity of this assay with urine samples from men and women compared with those of culture of male urethral and female endocervical swab specimens and PCR of endocervical swab specimens.

MATERIALS AND METHODS

Patient population. From July 1993 through June 1995, patients attending one Baltimore City sexually transmitted disease (STD) clinic were prospectively enrolled in Project RESPECT, a multicenter randomized trial for the evaluation of prevention and counseling for human immunodeficiency virus (HIV) infection. The primary objective of the study was to compare the effectiveness of various models of HIV infection prevention counseling or education in reducing the incidence of HIV and other STDs. One of the target outcome measures was detection of a new STD during the study's 12-month follow-up interval. STDs included new episodes of gonorrhea, syphilis, chlamydia, PID, or trichomoniasis and seroconversion to HIV, herpes simplex virus type 2, and hepatitis B virus positivity. To be eligible for the study, participants had to be English speaking, sexually active heterosexuals; women had to have had vaginal or anal sex in the previous 3 months. Participants also had to attend the STD clinic for a full examination and had to agree to confidential HIV testing. The study was approved by The Johns Hopkins University Joint Committee on Clinical Investigation.

After informed consent was obtained, a questionnaire regarding sexual behavior and symptoms was administered to each patient; this was followed by a physical examination and collection of genitourinary specimens. For men, an urethral swab specimen for chlamydia was collected by inserting a narrow-shafted, Dacron-tipped swab 2 to 3 cm into the urethra, and the swab was placed into a chlamydia transport vial containing sucrose-phosphate buffer, 10% fetal bovine serum, and antibiotics. Thirty millimeters of FVU was then collected in a sterile 50-ml screw-cap plastic cup. For women, one endocervical swab specimen was collected, and the swab was placed into a two sucrose-phosphate chlamydia transport vial. An additional endocervical swab specimen was then obtained and was placed into a vial containing AMPLICOR Specimen Transport Medium. An FVU sample was collected from the women before the completion of their pelvic examination and the collection of all other specimens. The FVU specimens were transported at room temperature and were later stored at 4°C for up to 96 h before the urine was processed. Chlamydia transport vials were stored at −70°C for 12 to 24 h until they were processed for culture. Following the patient's baseline visit, subsequent specimens were obtained whenever the patient returned to the clinic for a visit because of the presence of symptoms or was referred because of contact with a known STD.

Chlamydia culture. Tissue culture was done in 96-well microtiter plates with McCoy cell monolayers pretreated with DEAE-dextran as described previously (40). A 100-μl aliquot of transport medium was inoculated into each of three microtiter wells (total volume, 300 μl). After 48 h two wells were evaluated by immunofluorescence staining for chlamydia inclusions. One well was stained with monoclonal antibody to *C. trachomatis* major outer membrane protein (MOMP; Microtrak Chlamydia Culture Reagent; Syva, San Jose, Calif.), and the other well was stained with antilipopolysaccharide monoclonal antibody (Sanofi Diagnostics Pasteur, Chaska, Minn.).

PCR. PCR analysis was performed by a commercial, rapid, and nonradioactive PCR-based assay (Roche Diagnostics Systems) according to the manufacturer's instructions. For endocervical specimens, 1 ml of Specimen Diluent was added, the tubes were mixed thoroughly by vortexing, and the specimens were incubated for 10 min at room temperature. After overnight storage at 4°C, 50 μl of the patient or control sample was then added to each PCR tube containing 50 μl of the chlamydia PCR Master Mix. The PCR Master Mix contains primers for the 207-bp sequence of the *C. trachomatis* cryptic plasmid. The primers are labeled with biotin, *Taq* polymerase, and deoxynucleoside triphosphate in a buffered solution. Uracil-*N*-glycosylase was added to the PCR Master Mix prior to amplification to prevent carryover contamination (24). PCR amplification was carried out for 30 cycles with the GeneAMP 9600 thermocycler (Perkin-Elmer, Norwalk, Conn.).

For the urine samples from both men and women, 8 ml was centrifuged at 1,500 × *g* for 10 min. The supernatant was discarded, the pellet was resuspended in 2 ml of urine Resuspension Buffer (Roche Diagnostic Systems), and the mixture was incubated for 1 h at room temperature. Two milliliters of Urine Diluent (Roche Diagnostic Systems) was added immediately after incubation. The tubes were vortexed thoroughly, incubated at room temperature for 10 min, and allowed to sit overnight at 4°C prior to the removal of 50 μl for PCR analysis.

After amplification, the PCR mixtures were denatured with a weak sodium hydroxide solution and were incubated at room temperature for 10 min, and then 0.25 ml of the mixture was added to a microwell plate containing an immobilized *C. trachomatis*-specific DNA probe. 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 mixture was measured with a spectrophotometer at 450 nm. Optical density values greater than 0.5 nm were considered positive; those less than 0.25 nm were considered negative. Reaction mixtures with optical density values of between 0.25 and 0.50 were retested in duplicate and were interpreted as positive if two of the three absorbance values were greater than 0.25 nm. Negative controls were required to have an optical density value of <0.25, and the positive control had to have an optical density value of >2.0 for the test to be considered valid.

Analysis of discrepant results. For specimens that were PCR positive and culture negative, two additional tests were used to resolve the discrepancies: direct fluorescent-antibody assay (DFA) of sediment from a spun endocervical specimen culture vial and MOMP-based PCR of sediment from the endocervical culture vial. This expanded reference standard has been used in the past to help resolve discrepancies, particularly among evaluated tests that might be more sensitive than culture (12, 17, 21, 26, 30, 37). For evaluation by DFA, the transport media from the culture tubes were centrifuged at 12,000 × *g* for 15 min. A 10-μl sample of the resulting pellet 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. A slide was defined as positive if three or more elementary bodies were present. Specimens with fewer than three elementary bodies were evaluated by MOMP-based PCR at Roche Molecular Systems. By the MOMP-based PCR method, 100 μl of culture medium was diluted 1:10 and was placed into 1 ml of AMPLICOR Specimen Transport Medium, which was then treated by adding 1 ml of Specimen Diluent. Fifty microliters of the resulting specimen was used for PCR amplification by using primers to amplify a 129-bp sequence of the *C. trachomatis* MOMP as described previously (23).

RESULTS

Between July 1993 and June 1995, 713 matched urethral and urine specimens from male participants were available for evaluation, and 576 genital specimens from women were cultured for *C. trachomatis*. The individuals were predominantly young, with a median age of 23 and 24 years for men and women, respectively, and with an age range of 14 to 67 years. The majority of participants were African Americans (96%). Eighty-six percent of the men and 71% of the women presented to the STD clinics with genital symptoms, whereas the remainder were asymptomatic and were seeking routine screening for STDs or were referred to the clinic because a sexual partner had a diagnosis of an STD. A history of an STD was reported by 72.7% of men, a frequency similar to that reported by women (72.8%). A past history of chlamydial infection was higher in women (35.7%) than in men (15.8%) ($P < 0.01$). However, 32.1% of the men reported a history of nongonococcal urethritis. Within the previous 6 months, 21.5% of the men and 48.8% of the women reported having only one sexual partner, with 78.5% of the men and 51.2% of the women reporting two or more sexual partners during the same time period.

Neisseria gonorrhoeae was diagnosed at the baseline in 30% of the men, syphilis was diagnosed in 1.7% of the men, and 76.8% of the men had a clinical diagnosis of nongonococcal urethritis. Gonorrhea was diagnosed in 14.1% of the women at the baseline, syphilis was diagnosed in 3.0% of the women, and trichomonas was diagnosed in 19% of the women. Mucopurulent cervicitis, defined as cervical discharge, was present in 48.2% of the women, and 10.6% of the women had evidence of PID by clinical criteria.

C. trachomatis was detected by culture in 38 (5.3%) of 713 male urethral specimens and in 45 (7.9%) of 570 endocervical specimens. Eighty-five male urine specimens were positive for *C. trachomatis* by PCR; 29 of these were culture positive and 9 were culture positive and urine PCR negative (Table 1). Following analysis of the 56 PCR urine-positive, culture-negative specimens with discrepant results, 23 were found to be positive by DFA and 14 were found to be positive by MOMP-based PCR of the culture transport medium. MOMP-based PCR and DFA of the urine specimens could not be performed since the urine specimens had been discarded. The resolved sensitivity

TABLE 1. Comparison of PCR with culture for *C. trachomatis* detection for 713 male and 576 female specimens following analysis of discrepant results[a]

Group, test, and result	No. of specimens with the indicated result			
	Culture		Resolved infection status[b]	
	Positive	Negative	Positive	Negative
Men				
Urine PCR (n = 713)				
Positive	29	56	66	19
Negative	9	619	9	619
Women				
Urine PCR (n = 525)				
Positive	37	30	56	11
Negative	4	454	4	454
Endocervix PCR (n = 468)				
Positive	26	21	43	4
Negative	7	414	7	414

[a] Not all women had matching urine and endocervical specimens. Analysis of discrepant results was performed for PCR-positive, culture-negative samples.
[b] Resolved infection status was defined as a positive culture, positive DFA, or positive MOMP-based PCR result for samples that had discrepant results by AMPLICOR PCR and culture.

of urine PCR for men was 88.0%, with a specificity of 97.0% (Table 2). In contrast, the sensitivity of culture after analysis of samples with discrepant results was 50.7%. By using culture and resolved PCR results, the number of *C. trachomatis*-positive specimens increased from 38 (5.3%) to 75 (10.5%) ($P < 0.001$). From among the 576 women enrolled in the study for whom endocervical specimen culture results were available, there were 525 urine specimens for PCR and 468 endocervical swabs for PCR, but there were only 415 matched endocervical swab and urine specimens. Of the 525 female urine specimens for which PCR results were available, 67 were PCR positive, and 37 of these 67 specimens were also culture positive (Table 1). Four specimens were culture positive but were negative by urine PCR. Of the 30 culture-negative, urine PCR-positive specimens, 10 were positive by DFA and 9 were positive by MOMP-based PCR of endocervical swab in the specimen transport vial. The resolved sensitivity of urine PCR was 93.3% and the resolved specificity was 97.8%. The sensitivity of endocervical swab specimen culture was 68.3%. By using culture and confirmed urine PCR results, the number of specimens positive for *C. trachomatis* increased from 41 (7.8%) to 60 (11.4%) ($P < 0.05$).

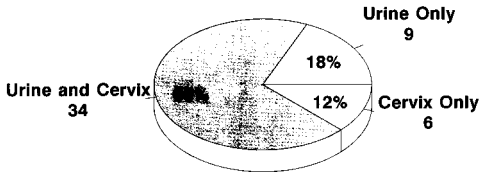

FIG. 1. Distribution of PCR-positive samples among 415 matched female urine and endocervical specimens.

Of the 468 endocervical swab specimens obtained for PCR, 47 were PCR positive, and 26 of these 47 specimens were culture positive. Seven specimens were culture positive but PCR negative. Of the 21 specimens that were positive by PCR but negative by culture, 9 were DFA positive and 8 were positive by MOMP-based PCR of the culture transport medium. The resolved sensitivity and specificity of endocervical swab specimen PCR were 86.0 and 99.0%, respectively.

Of the 415 matched urine and endocervical swab specimens, 59 specimens had either a positive endocervical swab specimen PCR result or a positive urine PCR result. Only 30 specimens were culture positive, and all of these were PCR positive. Of the 29 specimens with PCR-positive and culture-negative results, 10 were DFA positive and 9 were MOMP-based PCR positive; the results for 10 specimens remained unconfirmed. Of the 19 culture-negative, confirmed PCR-positive specimens, 13 were positive by PCR of both cervical and urine specimens, 3 were positive by only urine PCR, and 3 were positive by only endocervical swab specimen PCR. Of the 10 specimens with unconfirmed results, 3 were endocervical swab specimen PCR positive, 6 were urine PCR positive, and one was positive by both urine and endocervical swab specimen PCR. Of the 49 specimens from patients with confirmed infections, 30 (61.2%) were positive by culture, 40 (81.6%) were positive by endocervical swab specimen PCR, 43 (87.8%) were positive by urine PCR, and 49 (100%) were positive by either endocervical swab specimen PCR or urine PCR (Fig. 1).

DISCUSSION

Infections caused by *C. trachomatis* are among the most common sexually transmitted infections in the world, with an estimated 50 million new cases occurring worldwide each year (45). Since the prevalence of chlamydial infections ranges from 3 to 5% among asymptomatic men and women to 10 to 20% among adolescents attending STD clinics (31, 32, 35) and because of its associated sequelae of PID, diagnosis and treatment of this infection and contact tracing for this infection have been recommended by the Centers for Disease Control and Prevention (8, 9, 34). Although syndromic treatment of

TABLE 2. Resolved performance characteristics for detection of *C. trachomatis* by PCR for gender and specimen type

Group and test	Sample	No. of samples tested	Sensitivity (%)	Specificity (%)	PPV (%)[a]	NPV (%)[b]
Male						
PCR	Urine	713	88.0	97.0	88.2	98.6
Culture	Urethral	713	50.7	100	100	94.5
Female						
PCR	Endocervical	468	86.0	99.0	91.5	98.3
PCR	Urine	525	93.3	97.6	84.4	99.1
Culture	Endocervical	525	68.3	100	100	96.1

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

nongonococcal urethritis, mucopurulent cervicitis, and PID is effective for treating some chlamydial infections, this approach is insensitive since a large number of asymptomatic individuals would not be treated (33–35). Therefore, laboratory-based screening has been recommended (22, 23). Until recently, the laboratory standard for diagnosis of *C. trachomatis* infection was in vitro cell culture isolation (1, 33, 39). However, following the development of molecular amplification assays such as PCR and LCR, the sensitivity of culture has been estimated to range from 50 to 85% in laboratories whose personnel are experienced with these procedures (2, 12, 13, 18, 21, 37, 42). In addition, cell culture has several other disadvantages. An endocervical or urethral swab specimen must be obtained, requiring either a pelvic examination or the insertion of an urethral swab. Chlamydia culture requires isolation of the organism, which may take from 3 to 7 days before results are known. Since only viable organisms can be detected by culture, a special transport medium must be used to process the samples and storage temperature requirements are stringent, particularly in maintaining a cold chain in transport and storage (1, 25, 33). The adequacy of the sample is another limitation, particularly those from males (20). Because of the small numbers of cells that can be collected from the male urethra, the sensitivity of chlamydia culture with urethral swab specimens from males has been reported to be even lower than that for culture of endocervical swab specimens (12, 13, 18, 42).

Alternative tests for the chlamydia detection have included enzyme immunoassays, DFAs, and rapid antigen tests, in addition to the newer molecular amplification assays (1, 11, 16, 30). Screening for chlamydia by enzyme immunoassay or DFA of urine specimen has been proved to have a low degree sensitivity and a low degree of specificity, and these assays are not routinely recommended for use with urine (11, 22, 36, 41). In contrast, PCR and LCR assays with urine samples have proven to be highly sensitive and specific and provide a method for the noninvasive screening of both symptomatic and asymptomatic individuals (10, 15, 30, 44). PCR of urine samples from men with the commercially available AMPLICOR test (Roche Diagnostic Systems) has been cleared by the U.S. Food and Drug Administration and is commercially available. Various studies have demonstrated sensitivities of PCR of male urine samples following analysis of discrepant results to range from 93 to 97%, with specificities of 99% or greater (3, 4, 14, 18). In our study, the sensitivity and specificity of urine PCR were 88 and 97%, respectively. These results are slightly lower than those published previously because resolution of discrepancies for 19 PCR-positive, culture-negative samples could not be performed since the urine samples or pellets were not saved. Because resolution of discrepant results was limited to only the culture transport specimen, sampling variation may have prevented confirmation of the results for some specimens, resulting in an underestimation of both the sensitivity and the specificity of PCR. For males, since the amount of the urethral specimen is often limited, the culture transport specimen may not contain adequate material to allow for a positive analysis. Nevertheless, the sensitivity of urine PCR obtained by using our results was still dramatically higher than that of culture, which was 50.7%, similar to that in previous studies comparing culture and nucleic acid amplification assays (4, 14, 18). As a result of this differential in sensitivity, the prevalence of chlamydia in male participants doubled from 5.3 to 10.4% when resolved urine PCR results were included.

To our knowledge this is the first report of the results of the AMPLICOR *C. trachomatis* Test for detecting *C. trachomatis* in urine samples from women. Recent studies have demonstrated the utility of screening of urine samples from women by LCR for *C. trachomatis* with sensitivities ranging from 87.5 to 96.3% (2, 12, 21, 42). In our study the resolved sensitivity of PCR of FVU specimens was 93.3%, similar to that for LCR. Similarly, PCR of the endocervical swab specimens resulted in a resolved sensitivity of 86%. In contrast, the sensitivity of culture was only 68.3% for samples from women. As in the case of specimens from males, the sensitivity (93.3%) and specificity (97.6%) may have been higher for samples from women in the case of urine PCR, because none of the culture-negative, PCR-positive urine specimens could be directly retested by DFA or MOMP-based PCR since the original urine specimen had been discarded. In other PCR and LCR studies of urine, resolution of discrepant results was based on DFA and MOMP-based PCR of the excess urine specimens (2–4, 6, 12, 13, 19, 21, 42). In the present study, resolution of the results for the samples was restricted to tests with the sample remaining in the culture transport vial. Sampling differences between two different anatomic sites may have limited the resolution of some PCR-positive specimens, resulting in an underestimation of the assay's sensitivity and specificity. Although confirmatory testing with the culture transport media rather than the PCR swab specimen may also have affected the resolution for the culture-negative, endocervical swab specimen-positive PCR samples, the difference was less dramatic since the same anatomical site was being sampled.

In the analysis of the matched urine and endocervical specimens from females, the addition of screening of both samples by PCR increased the sensitivity to 100%. Thirty-four specimens were positive by PCR of both urine and endocervical swab specimens, whereas 9 were positive by PCR of urine and negative by PCR of endocervical swab specimens and 6 specimens were positive by PCR of endocervical swab specimens and negative by PCR of urine specimens. The finding that some individuals may be urine PCR positive or endocervical swab specimen PCR positive may reflect the finding that 10 to 20% of infected women may have a localized urethral infection or a localized endocervical infection without involvement of the other site (4, 5, 17, 28, 29, 33, 39). Screening of both endocervical swab and urine specimens by a molecular amplification assay will undoubtedly increase the number of *C. trachomatis*-infected individuals who are identified, but at an increased cost.

The use of nucleic acid amplification technology to detect infected males and females by examination of their FVU is a newly recognized, noninvasive screening method that can be easily implemented in population-based control efforts (38). The advantages of urine-based screening include the ease of collection, simple transport and storage requirements, and the avoidance of a sampling bias during urethral or endocervical examination. Urine-based screening also affords access to large populations, particularly asymptomatic men and women who may not frequent health clinics where chlamydia-based screening is more routine. The results of the present study as well as previously published results of LCR of urine from women for *C. trachomatis* (6, 12, 21, 42) demonstrate the high degrees of sensitivity and specificity of urine-based screening by assays that are semiautomated and that provide results within 6 to 8 h.

With the development of nucleic acid amplification assays, clinicians now have the advantage of using highly sensitive assays for the mass screening of patients for *C. trachomatis* infection. Because of the high degrees of sensitivity of these assays, genital specimens, ocular specimens, and noninvasive urine specimens can be easily obtained and screened for *C. trachomatis*. While further evaluations are required to accurately measure the specificity of this assay, which could not be determined in the present study, it is likely that urine-based

screening will be used more frequently, particularly in nonhealth care clinic settings. The costs of PCR and LCR are twice those of chlamydia antigen enzyme immunoassays and nonamplified probe assays, but are half the cost of culture. With the marked increase in sensitivity and the use of noninvasive samples, molecular diagnostic assays should prove to be highly cost-effective. Further studies are also required to evaluate the positive and negative predictive values of these new assays in populations with low, medium, and high prevalences of chlamydial infections. By implementing broad, population-based screening programs and with the use of single-dose therapy such as that with azithromycin, it is now possible to enact better and more effective programs for controlling chlamydial infections.

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