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Diagnosis of *Chlamydia trachomatis* Endocervical Infections by a Commercial Polymerase Chain Reaction Assay

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We evaluated a prototype polymerase chain reaction (PCR)-based assay for *Chlamydia trachomatis* developed by Roche Molecular Systems to detect endocervical infection in women. Of 587 endocervical samples obtained from women attending the Harborview Medical Center sexually transmitted diseases clinic, 58 (10%) were positive for *C. trachomatis* by cell culture. Compared with culture, the PCR method had a sensitivity of 88% (51 of 58) and a specificity of 99.2% (525 of 529). The positive and negative predictive values were 92.7% (51 of 55) and 98.7% (525 of 532), respectively. After resolution of discrepant results whereby true positives were considered to be either culture-positive patients (58 patients) or culture-negative patients positive upon PCR analysis using both plasmid- and major outer membrane protein-based primers (4 patients), the resolved sensitivities of the PCR and culture were 89 and 93%, respectively. We subsequently performed a second analysis of 362 women, comparing the proposed commercial PCR assay from Roche Molecular Systems with chlamydia cultures. Thirty (8%) women were infected with *C. trachomatis*. Compared with culture, the assay had a sensitivity of 60% (18 of 30) and a specificity of 99% (328 of 332). Repeat PCR assay done 2 to 5 days later subsequently yielded positive results for 7 of 11 PCR-negative samples from culture-positive women. We conclude that the Roche Molecular Systems PCR assay provides highly specific results compared with culture in a high-risk population of women. Further study is needed, however, to more clearly define the sensitivity of the PCR assay in detecting endocervical *C. trachomatis* infection in women and to identify factors that may compromise sensitivity.

Chlamydia trachomatis frequently causes urethritis, epididymitis-orchitis, cervicitis, and pelvic inflammatory disease (2). In many instances, these infections may be asymptomatic or minimally symptomatic and hence are difficult to recognize clinically. Of the available laboratory tests, culture for chlamydiae using McCoy cells and a fluorescein-conjugated monoclonal antibody for identification of intracellular inclusions is considered the "gold standard." However, culture requires careful specimen collection and stringent transport conditions, is labor-intensive, and requires at least 48 h to perform (18, 22). Antigen detection tests, such as direct fluorescent antibody or enzyme immunoassay and nucleic acid hybridization assays, are easier to perform and require much less time and technical expertise than culture (8, 17, 20).

Recently, the polymerase chain reaction (PCR) has been used to detect *C. trachomatis*-specific nucleotide sequences in clinical specimens (3, 5, 9, 12–15). Theoretically, the amplification provided by PCR should considerably enhance sensitivity, while the inherent specificity of a DNA probe should ensure a high specificity for the assay. Standard PCR technology, however, requires significant expertise in DNA extraction, gel electrophoresis, and/or nucleic acid hybridization utilizing radioactive materials and demands meticulous laboratory techniques to prevent false-positive results. It is unlikely that such demanding technology would be easily carried out in most clinical microbiology laboratories. For this reason, Roche Molecular Systems has developed a commercial method that uses PCR and DNA probe hybridization followed by an enzyme-linked immunosorbent assay (ELISA)-like detection of amplified *C. trachomatis* plasmid DNA to detect endocervical chlamydial infection (9). In this study, we have compared the prototypic assay and the proposed commercial assay with a sensitive cell culture system for 587 women and 362 women, respectively, attending sexually transmitted diseases clinic.

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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 endocervical specimens were obtained from 949 patients attending the clinic between July 1991 and September 1992 and were sent for *C. trachomatis* culture and PCR assay.

Specimen collection and transport. Following removal of excess endocervical mucus, a cytologic brush (Medical Packaging Corporation, Carmarillo, Calif.) or Dacron swab (Hardwood Products, Guilford, Minn.) was used to collect an endocervical specimen for culture. The specimen collected by swab or brush was placed in 1.5 ml of 0.2 M sucrose phosphate chlamydia transport medium prepared according to the method of Gordon et al. (7) and refrigerated for 6 to 24 h until inoculated. A Dacron-tipped, plastic swab (Hardwood Products) was then used to obtain an additional specimen, which was placed in a PCR transport tube provided by Roche Molecular Systems which contained 1 ml of transport medium. The PCR specimens were stored for 12 to 95 h at room temperature (prototypic assay) or refrigerated (proposed commercial assay) until assayed. Furthermore,

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swabs were left in PCR transport media during storage for the prototypic assay while swabs were discarded by the clinicians following inoculation of PCR transport media during testing of the proposed commercial assay.

Laboratory methods. PCR analysis was performed at Harborview Medical Center by using a rapid, nonradioactive, PCR-based assay developed by Roche Molecular Systems according to the manufacturer's instructions. Specimen tubes were briefly centrifuged (to remove liquid from the caps), 1 ml of a specimen diluent solution was added, and the tubes were mixed thoroughly by vortexing. Patient samples or controls (50 μl) were then added to PCR tubes containing 50 μl of the chlamydia PCR mix. The PCR mix contained primers labelled with biotin, *Taq* polymerase, and dNTPs (including dUTP substituted for TTP) in a buffered solution. UNG (uracil-N-glycosylase) was added to the PCR Master Mix prior to amplification to prevent carryover contamination. 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.

After amplification, the PCR products were denatured with a weak sodium hydroxide solution and then added to a microtiter plate containing a hybridization solution and *C. trachomatis*-specific DNA probe immobilized in the wells. After a 1-h incubation at 37°C, the plates were washed to remove unbound material and an avidin-horseradish peroxidase conjugate was added. The conjugate was allowed to bind for 15 min at 37°C, the plates were washed to remove unbound conjugate, and a peroxidase substrate solution containing tetramethylbenzidine was added. The reaction mix was 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. The prototypic assay materials were provided by Roche as individual reagents, whereas the proposed commercial product was manufactured elsewhere and received as a kit.

Chlamydia cultures were performed at the University of Washington Chlamydia laboratory by using cycloheximide-treated McCoy cells in microtiter plates and fluorescein-conjugated monoclonal antilipopolysaccharide antibody for enumeration of inclusions as previously described (18, 22). All culture-positive isolates were then serotyped by the recently described microtiter plate method (19). 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 their culture results.

Analysis of discrepant results. In the preclinical study of 587 patients, analysis of PCR and culture specimens with discrepant results was performed at Harborview Medical Center and Roche Molecular Systems. For analysis of specimens from PCR-positive, culture-negative patients, an alternate PCR assay that amplifies a 129-bp sequence of the *C. trachomatis* major outer membrane protein (MOMP) gene and uses primers CT 1 and CT 2 of Dutilh et al. (6) was developed. 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 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 product.

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

Result	No. with result by culture[a]	Result of PCR[b]	
		No. positive	No. negative
Positive	58	51	7
Negative	529	4	525
Total	587	55	532

[a] Prevalence was 9.9% by culture.
[b] The sensitivity of the PCR was 87.9%, and the specificity was 99.2%.

All PCR-positive specimens from culture-negative patients were analyzed by MOMP PCR. A positive result with MOMP PCR showed that the specimen contained MOMP DNA in addition to cryptic plasmid DNA, excluding the possibility that the original PCR-positive result was due to contamination with the amplification products from previous PCRs.

PCR was used to analyze culture transport medium from patients with negative cultures but positive PCR results. DNA was purified from 200-μl aliquots from the original culture transport vials by phenol extraction and ethanol precipitation. The precipitated nucleic acids were resuspended in 100 μl of PCR transport medium. One hundred microliters of the specimen diluent solution was added, and PCR was performed with 50-μl aliquots, as described above.

True positives were considered to be all culture-positive patients (58 patients) plus those PCR-positive, culture-negative patients with *C. trachomatis* DNA also detected by MOMP PCR (4 patients), giving a total of 62 positives. Analysis of discrepant results was not routinely performed in the study of the proposed commercial assay except that PCR of samples from PCR-negative, culture-positive women was repeated.

RESULTS

Preclinical evaluation of the prototypic assay. Five hundred eighty-seven duplicate endocervical specimens were obtained for culture and PCR assay. Of these, 58 (9.9%) were culture positive for *C. trachomatis*. For 576 patients, the results of culture and the PCR assay were concordant (Table 1). Of the 11 discordant specimen pairs, 7 were culture positive and PCR negative and 4 were culture negative and PCR positive. Compared with culture, the sensitivity of the PCR assay was 88% and the specificity was 99%.

The PCR specimens from the four PCR-positive, culture-negative patients were retested by both the standard PCR test and the alternate PCR test directed against the MOMP gene. All four specimens were positive by both PCR systems, confirming the presence of *C. trachomatis* DNA in the specimens. For three of these patients, the specimens sent for cell culture testing were available for PCR analysis. *C. trachomatis* DNA was detected in two of these three cell culture specimens by PCR (Table 2). After resolution of these discrepant results, the resolved sensitivities of the PCR and culture were 89 and 92%, respectively.

Samples from seven culture-positive patients were initially negative by the PCR assay. Four of these specimens were

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

Patient no.	Culture result	PCR result for PCR specimen		PCR result for culture specimen	
		Plasmid	MOMP	Plasmid	MOMP
170	−	+	+	NA	NA
306	−	+	+	−	+
477	−	+	+	+	NP
528	−	+	+	−	−

[a] Plasmid and MOMP refer to the standard PCR test and the alternate PCR test directed against the MOMP gene, respectively. NA, specimen not available; NP, test not performed.

TABLE 4. Performance of the (proposed commercial) *C. trachomatis* PCR test compared with culture

Result	No. by culture[a]	Result of PCR[b]	
		No. positive	No. negative
Positive	3	18	12
Negative	332	4	328
Total	362	22	340

[a] Prevalence was 8% by culture.
[b] The sensitivity of the PCR was 60%, and the specificity was 99%.

PCR positive on repeat analysis and two were positive after phenol extraction of the specimen, suggesting that the false-negative PCR results were due to inhibition of PCR by these specimens or to technical error (Table 3). One culture-positive, PCR-negative specimen (no. 558) remained PCR negative on repeat analysis. However, after phenol extraction, plasmid PCR was negative and MOMP PCR was positive (Table 3).

Clinical evaluation of the proposed commercial assay. Three hundred sixty-two duplicate endocervical swabs were obtained for culture and PCR assay. Of these, 30 (8%) were culture positive for *C. trachomatis*. For 346 patients, the results of culture and the PCR assay were concordant (Table 4). Of the 16 discordant specimen pairs, 12 were PCR negative and culture positive and 4 were PCR positive and culture negative. Three of these four specimens were analyzed by MOMP PCR, and all were positive. Thus, the resolved sensitivities of the PCR and culture were 64% (21 of 33) and 91% (30 of 33), respectively. Seven of the 12 PCR specimens from PCR-negative, culture-positive women were subsequently PCR positive on repeat PCR assay.

DISCUSSION

The PCR has been a major breakthrough in molecular biology, but clinical application of this technology has thus far been limited. In earlier studies of chlamydial infection, amplification of chromosomal DNA or plasmid DNA followed by nucleic acid assays to detect *C. trachomatis* in urethral, cervical, and urine specimens was found to be at least as sensitive as culture or antigen detection assays (1a, 5, 12–15). However, these early PCR assay detection methods had limited clinical application because they involved DNA extraction, gel electrophoresis, and Southern blot or dot spot hybridization. Bobo et al., however, reported successful detection of *C. trachomatis* in clinical specimens by using PCR amplification of a 280-bp segment of the *Chlamydia* MOMP genome in which enzyme immunoassay detection of amplified DNA greatly simplified the indicator system (1a).

We evaluated a prototype PCR-based assay system, provided by Roche Molecular Systems, to identify *C. trachomatis* endocervical infection. The assay is simple to perform, does not utilize isotopes, and requires minimal technical expertise. One technician can run 92 clinical samples (and 4 controls) at one time, with a turnaround time of 4 to 5 h. All of the serovars present in the isolates reported here (specifically, D, D−, E, F, G, H, Ia, J, and K) were detected by the PCR assay (data not shown), and the assay has also been reported to detect serovars A, Ba, C, I, L1, L2, and L3 (9).

In the preclinical study of the prototype PCR assay, the assay compared favorably with culture, providing a sensitivity of 88% and a specificity of 99%. If the four PCR-positive, culture-negative patients are reclassified as true positives, on the basis of repeat demonstration of the presence of both the MOMP and plasmid target sequences, the sensitivity of the PCR assay was 89% (55 of 62) and the positive and negative predictive values were 100% (55 of 55) and 99% (525 of 532), respectively. These results compare favorably with those obtained with other nonculture *C. trachomatis* detection methods studied at our center (4, 10, 11, 16). Seven culture-positive patients were negative by the prototype PCR test, possibly because of inhibition of PCR by the specimens or technical error. Six of these seven were positive upon repeat analysis using plasmid and MOMP primers on either the original specimen or a phenol extract of it. Two of the false-negative PCR results were obtained from patients with very low inclusion counts and may have been due to a very small amount of chlamydial DNA in the specimen.

Of the four specimens that were PCR positive and culture negative, two were most likely culture false negatives on the basis of analysis of discrepant results and review of patient histories. Patient 477 was found to have had *C. trachomatis* on 30 May but was not treated until 3 September. She was then treated with doxycycline, but the treatment status of her regular partner was unknown. She had unprotected sexual intercourse with her partner and with a new partner between the time she was treated and the time the PCR specimen was obtained. This patient may have been reinfected by her regular partner or by her new partner. The PCR result of the original cell culture specimen was positive for *C. trachomatis* plasmid DNA. Patient PMPC 306 had sexual contact with a male who had nongonococcal urethritis, and the patient had evidence of pelvic inflammatory disease but was culture negative for *C. trachomatis*. PCR

TABLE 3. Analysis of PCR-negative, culture-positive patients

Patient no.	Culture inclusions/well	Result of initial PCR (plasmid)	Result of repeat PCR			
			Original specimen		Phenol-extracted specimen	
			Plasmid	MOMP	Plasmid	MOMP
205	3,450	−	+	+	NP[a]	NP
256	15,000	−	−	+	+	
270	8	−	+	+	+	
481	1,625	−	+	+	NP	NP
486	13,000	−	−	−	+	
529	450	−	+	+	NP	NP
558[b]	4	−	−	−	+	

[a] NP, not performed.
[b] PCR analysis of culture specimen 558 was positive for *C. trachomatis* plasmid and MOMP DNA.

testing of her original cell culture specimen was negative for plasmid DNA but positive for MOMP DNA.

The other two PCR-positive, culture-negative specimens (PMPC 170 and PMPC 528) may have contained nonculturable organisms. Specimen PMPC 528 was taken from a female who had sexual contact with a male who had gonorrhea. She was asymptomatic and had received recent treatment with doxycycline at the time the specimen was obtained for PCR assay. Her pretreatment and posttreatment *C. trachomatis* cultures were negative. Specimen PMPC 170 was taken from a female who had been diagnosed as having pelvic inflammatory disease and a positive culture for *C. trachomatis*. She had been treated with ceftriaxone and doxycycline. Despite completing the antimicrobial therapy, the patient had clinical evidence of persistent disease at follow-up. The specimen obtained at the follow-up visit was culture negative but PCR positive and may have contained nonculturable *C. trachomatis* due to the antibiotic therapy.

The concern that PCR may be too sensitive for routine clinical use and would frequently detect nonviable organisms has been raised. Claas et al., however, studied 32 patients who had been treated with doxycycline for *C. trachomatis* infection (3). Their PCR assay detected no *C. trachomatis* DNA in specimens taken 1 week after therapy. In another study, Workowski et al. evaluated the presence of *C. trachomatis* DNA by using two different PCR assays with endocervical specimens from 20 patients who had just completed doxycycline therapy for culture-proven *C. trachomatis* cervicitis (21). Although all patients were culture negative at the completion of therapy, 10 of 20 patients were PCR positive immediately posttherapy and 3 of 10 and 1 of 10 were positive at 1 and 2 weeks after completing therapy, respectively. All patients were PCR and culture negative at 3 weeks after the completion of therapy. Such cases presumably represent the presence of nonculturable *C. trachomatis* DNA after the completion of successful therapy.

Following this preclinical study using the prototypic PCR assay, we evaluated the proposed commercial assay. The lower sensitivity (60%) of this assay was unexpected and has not been satisfactorily explained. The same experienced group of clinicians collected both sets of specimens, and the same clinic and patient populations were used. Similarly, the same laboratory personnel performed the PCR assays and cultures. Two changes in specimen handling were made after the preclinical study. First, because the manufacturer had data indicating that the swab itself may act as an inhibitor of the PCR assay, it was elected not to leave the swab in the PCR transport media as had been done in the first study. However, for a small group of study patients, we found that leaving the endocervical swab in the transport medium (versus inoculation of the transport media followed by swab removal and discard) had no impact on the sensitivity of the assay (data not shown).

Second, the swabs were stored at room temperature after collection and prior to assay in the preclinical study but were refrigerated during this period in the second trial. Upon analysis of discrepant results, we discovered that 7 of 11 initially PCR-negative specimens were PCR positive on repeat testing several days after the initial run, suggesting that inhibition of the PCR may have occurred. It is possible that certain components within endocervical mucus inhibit the PCR assay. Slow inactivation of these inhibitors in the transport media could explain our findings that some initially PCR-negative specimens were PCR positive on repeat analysis several days later. This phenomenon may not have been a significant problem with the prototypic assay because the PCR specimens were held at room temperature rather than being refrigerated, perhaps allowing degradation of inhibition to occur more rapidly. Alternatively, other factors such as technical errors, the fact that swab order was not randomized, or small numbers of inclusion-forming units in these specimens may have contributed to this phenomenon.

In conclusion, the Roche PCR assay greatly simplifies the performance of PCR compared with conventional methods. Compared with culture, the PCR assay is simpler to perform, requires less technical expertise, and can be performed by one technician, with a turnaround time of 4 to 5 h. The specificity of this PCR assay compared favorably with that of culture for this relatively high prevalence group of largely symptomatic women. For the women studied here, however, culture was more sensitive than the current PCR assay when used on endocervical specimens. Additional studies are needed to more clearly define the actual sensitivity of the assay and the nature of inhibitors present in endocervical specimens that may influence the sensitivity of this assay. Interestingly, the sensitivity of the same PCR assay was much higher (93 to 96%) when the assay was used for urine or urethral specimens from men (1).

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