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Diagnosis of *Chlamydia trachomatis* Infections in Asymptomatic Men and Women by PCR Assay

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A PCR assay was evaluated for its ability to detect genital chlamydial infection in asymptomatic men and women. Urethral swab specimens were collected from 472 men for culture and PCR assay, and first-void urine (FVU) specimens were collected from 379 of these men for enzyme immunoassay (EIA) and PCR assay. Cervical swab specimens were collected from 242 women for culture, EIA, and PCR assay. Patients were considered infected if they were culture positive or positive by PCR with both plasmid- and major outer membrane protein-based primers. By using this extended "gold standard," the prevalence of infection in this population was 7.6% for men and 7.9% for women. For men, the sensitivities of urethral swab specimen culture and PCR and FVU specimen EIA and PCR were 61, 72, 55, and 91%, respectively. All assays had specificities of ≥99.8%. The positive and negative predictive values for PCR testing of FVU specimens were 100 and 99.4%, respectively, compared with values of 96.3 and 97.8%, respectively, for PCR of urethral swab specimens. The sensitivities of cervical swab specimen culture and PCR testing were 42 and 90%, respectively, with corresponding specificities of 100 and 99.3%. All cervical swabs were negative by EIA. Molecular techniques such as PCR assays are valuable tools for the detection of asymptomatic genital chlamydial infection. In particular, PCR assays of FVU specimens from men offer a highly sensitive, noninvasive screening tool that will likely improve patient compliance for diagnostic testing.

Chlamydia trachomatis infections are recognized as the most prevalent bacterial sexually transmitted disease in Canada and the United States. Unlike gonococcal infections, genital chlamydial infections often result in milder symptoms or asymptomatic carriage. Up to 25% of men with urethral infection and 76% of women with cervical infection have no symptoms (11, 22). As a result, asymptomatically infected individuals represent a large reservoir for further disease transmission in the community (6). In addition, asymptomatically infected women may be at risk of serious reproductive sequelae. Therefore, screening for genital chlamydial infection is a high public health priority.

Screening of asymptomatic women can be performed during routine pelvic examinations by obtaining a cervical swab specimen. In contrast, detection of asymptomatic infections in men has been difficult since proper urethral swabbing is invasive and uncomfortable, which often results in the collection of inadequate specimens. As a result, a first-void urine (FVU) specimen serving as a urethral washout has been used as an alternative specimen for men since it can be obtained in a noninvasive manner and is more acceptable to patients. Nonculture methods such as enzyme immunoassay (EIA) have proven to be useful in the detection of symptomatic infection. The sensitivity of EIA testing of urethral or cervical swab specimens has ranged from 70 to 98% for symptomatic patients (6, 13) and from 76 to 91% for testing of FVU specimens from symptomatic men (7, 18). However, because asymptomatically infected individuals may shed fewer organisms, testing of swab specimens by these antigen detection methods has not performed as well for the detection of asymptomatic infections (6, 13). This is especially true for men, for whom sensitivities of EIA testing of urethral swab specimens as low as 48% have been reported (19). The sensitivity of EIA testing of FVU specimens from asymptomatic men has also been variable, ranging from 42 to 96% (14, 20).

Nucleic acid amplification techniques such as PCR involve exponential amplification of well-defined DNA targets, resulting in enhanced sensitivity of detection compared with the sensitivities of other nonculture methods. The introduction of a commercial PCR assay, Amplicor *Chlamydia trachomatis* test (Roche Molecular Systems, Branchburg, N.J.), has allowed clinical laboratories the opportunity to use this technology for routine work. This assay uses biotinylated oligonucleotide primers for target amplification, detection of amplified product (amplicon) with a specific capture oligonucleotide probe bound to the microwells, and dUTP with uracil-*N*-glycosylase to prevent carryover DNA contamination. Previously published reports indicate that this assay performs well compared with cell culture and EIA (1–4, 9, 12, 15, 21, 25). However, most of the evaluations have been with symptomatic patients or with populations with high prevalences of chlamydial infections (1–4, 9, 12, 25).

The objective of the study described here was to evaluate the Amplicor *Chlamydia trachomatis* test with specimens from asymptomatic men and women from populations with chlamydial culture rates of approximately 5%. This assay, performed with swab and FVU specimens, was compared with urethral swab specimen culture and FVU specimen EIA for men and with cervical swab specimen culture and EIA for women.

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MATERIALS AND METHODS

Patient population. The study included consecutive asymptomatic men who attended the Sexual Health Centre in Ottawa and who gave informed consent. The median age of these men was 27 years, and approximately 15% of the men tested were contacts of individuals with sexually transmitted diseases. Two urethral swab specimens were obtained on Dacron swabs from 472 men with no urethral symptoms and who had not voided in the previous 2 h. The first specimen was placed immediately into sucrose phosphate chlamydia transport medium (2-SP) and was stored at 4°C until it was processed, and the second specimen was obtained for *Neisseria gonorrhoeae* culture as part of the clinic protocol. FVU specimens (20 ml) were obtained from a subset of 379 men after the urethral swab specimens were obtained. Urine was stored at 4°C until it was processed.

The asymptomatic women included patients being investigated for infertility at the Ottawa Civic Hospital infertility clinics who required a pelvic examination. The median age of these women was 31 years, and 22% had a history of a previous sexually transmitted disease or pelvic inflammatory disease. The women seen at these clinics include those referred for in vitro fertilization, and approximately one-quarter of the women enrolled in the study had tubal infertility. After cleansing the cervix, two endocervical swab specimens were obtained from 242 women. In randomized order, one swab specimen was obtained and placed into Chlamydiazyme EIA transport medium (Abbott Laboratories, North Chicago, Ill.), and the other specimen was obtained on a Dacron cytobrush, which was placed immediately into 2-SP transport medium and which was stored at 4°C until it was processed.

Cell culture assay. All specimens for culture were transported in 2-SP transport medium at 4°C, maintained at this temperature, and processed by cell culture within 48 h of collection. Culture was performed in duplicate by using DEAE-dextran and cycloheximide-treated HeLa 229 cells on 13-mm-diameter glass coverslips in shell vials as described previously (23). Confluent 24-h-old cell monolayers were pretreated for 20 min with DEAE-dextran (30 μg/ml) prior to inoculation of the specimen. After inoculation, the cultures were incubated for 48 h in culture medium containing 1 μg of cycloheximide per ml at 35°C in a humidified atmosphere of 5% CO_2. The monolayers of one of the two coverslips were fixed in methanol and stained with fluorescein-conjugated monoclonal antichlamydial antibodies (MicroTrak Culture Confirmation Kit; Syva Co., Palo Alto, Calif.). If this coverslip was negative, the same sample in the second coverslip was passaged once.

The number of inclusion bodies in each shell vial culture was determined by counting the number of fluorescent inclusions on the entire coverslip or by the method described by Furness et al. when the number of inclusions exceeded 100 per coverslip (10).

FVU samples. Urine samples that formed precipitates on storage at 4°C were briefly warmed to 37°C to allow for clarification. The urine samples were mixed thoroughly and were divided into two aliquots: one of 7 to 8 ml for PCR testing and the rest for EIA testing. Each aliquot was centrifuged at 3,000 × *g* at room temperature for 10 min. The urine sample sediments were assayed by EIA and PCR as described below.

EIA. Cervical swab specimens and FVU specimen sediments were processed by the Chlamydiazyme EIA (Abbott Laboratories) according to the manufacturer's instructions. All specimens which were positive on initial testing by the Chlamydiazyme EIA were further analyzed by the Chlamydiazyme blocking assay for confirmation of the results. A Chlamydiazyme EIA result was considered positive only if it was confirmed by the blocking assay.

PCR. The Amplicor *Chlamydia trachomatis* test uses primers directed at the cryptic plasmid. Cervical and urethral swab specimens received in 2-SP transport medium were assayed by PCR by a protocol provided by Roche Molecular Systems. Briefly, after vortexing, 20 μl of the specimen in 2-SP medium was diluted with 180 μl of Amplicor STM. After 20 min of incubation at room temperature, 200 μl of Amplicor specimen diluent was added. A 50-μl aliquot of this diluted sample was amplified, and amplicons were detected according to the manufacturer's instructions. The optical densities of the microtiter wells were read at 450 nm by using a Bio-Rad enzyme-linked immunosorbent assay plate reader, with absorbance readings greater than 0.25 considered positive.

The FVU specimen sediment was resuspended in 2 ml of Amplicor urine resuspension buffer. Two milliliters of urine diluent was then added, and 50 μl of this diluted sample was amplified and processed as described above.

Pre- and post-PCR areas in the laboratory were physically separated, and the work flow was unidirectional. Dedicated pipettes, equipment, and supplies, including aerosol-resistant pipette tips and frequent changes of gloves, were used.

Analysis of discrepant results. All specimens with discordant results (culture-positive, PCR-negative and culture-negative, PCR-positive results or when the results for urine and swab specimens did not agree) were reassayed by using the Amplicor PCR kit. All specimens with discordant results as well as several specimens with positive and negative concordant results were sent to Roche Molecular Systems in a blinded manner for further analysis. To eliminate possible inhibitors in the specimens, the specimens negative on repeat Amplicor PCR testing were reamplified after phenol-chloroform extraction and ethanol precipitation. Positive PCR samples were confirmed by a second PCR assay with primers directed at the major outer membrane protein (MOMP) gene (15). A positive result by the MOMP PCR indicated that the specimen contained MOMP DNA in addition to plasmid DNA, which confirms the specificity of the assay for chlamydial DNA and excludes the possibility that the original PCR-positive result was due to amplicon contamination (16).

The results for all culture-positive coverslips from patients who provided PCR-negative specimens were confirmed by an independent reader who was unaware of the initial culture or PCR results. A patient was considered to be infected if the urethral or cervical swab specimen was culture positive or if the urethral or cervical swab specimen or FVU specimen sediment was repeatedly Amplicor PCR positive and was confirmed to be positive by MOMP PCR.

Statistical analysis. Comparisons of sensitivities were performed by χ^2 analysis with Yates' correction. Median inclusion counts were compared by the Mann-Whitney test.

TABLE 1. Comparison of urethral swab and FVU specimens from asymptomatic men[a]

No. of specimens	Test result			
	US[b] culture	US PCR	FVU PCR	FVU EIA
8	+	+	+	+
3	+	+	+	−
1	+	+	−	−
1	+	−	−	−
1	−	+	+	+
1	−	+	+	−
1	−	+[c]	−	−
3	−	−	+	+
4	−	−	+	−
356	−	−	−	−

[a] A total of 379 specimens were tested.
[b] US, urethral swab specimen.
[c] False-positive result because the MOMP PCR result was negative.

RESULTS

Of the urethral swab specimens from the 472 asymptomatic men tested, 22 (4.7%) were positive by culture and 27 (5.7%) were positive by PCR; among these swab specimens, 19 were positive by both methods. All three culture-positive, PCR-negative specimens remained PCR negative after phenol-chloroform extraction. All but one of the eight culture-negative, PCR-positive specimens were confirmed to be positive by MOMP PCR.

The results for the 379 asymptomatic men from whom urethral swab and FVU specimens were obtained are summarized in Table 1. Thirteen (3.4%) urethral swab specimens were culture positive and 15 (4.0%) were PCR positive. PCR testing of FVU specimen sediments was positive for 20 (5.3%) specimens, and EIA testing of FVU specimen specimens was positive for 12 (3.2%) specimens. PCR-negative urine or swab specimens from culture-positive patients remained PCR negative after phenol-chloroform extraction. Among the culture-negative patients, all nine FVU specimen sediments which were Amplicor PCR positive and two of the three urethral swab specimens which were Amplicor PCR positive were confirmed to be positive by MOMP PCR. All 12 FVU specimen sediments which were EIA positive were also PCR positive.

A patient was considered to be infected if the urethral swab was culture positive or if the urethral swab or FVU specimen sediment was repeatedly Amplicor PCR positive and was confirmed to be positive by MOMP PCR. By this definition, 7.6% (36 of 472) of the men were infected. Table 2 compares the sensitivity, specificity, and positive and negative predictive values of culture and PCR assay of urethral swab specimens and PCR and EIA testing of FVU specimen sediments after analysis of the discrepant results.

Of the cervical swab specimens from 242 asymptomatic women, 8 (3.3%) were culture positive, 19 (7.9%) were PCR

TABLE 2. Comparison of culture, PCR, and EIA of urethral swab and FVU specimens from asymptomatic men[a]

Test and result	No. of patients		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	Infected[b]	Uninfected				
Culture of urethral swab specimen						
Positive	22	0	61.1	100	100	96.9
Negative	14	436				
PCR of urethral swab specimen						
Positive	26	1	72.2[c]	99.8	96.3	97.8
Negative	10	435				
PCR of FVU specimen (n = 379)						
Positive	20	0	90.9[d]	100	100	99.4
Negative	2	357				
EIA of FVU specimen (n = 379)						
Positive	12	0	54.5[e]	100	100	97.3
Negative	10	357				

[a] A total of 472 specimens were tested.
[b] Culture positive and/or swab or urine specimen PCR positive and with the positive result confirmed by MOMP PCR.
[c] $P = 0.45$ compared with culture of urethral swab specimen.
[d] $P = 0.03$ compared with culture of urethral swab specimen.
[e] $P = 0.8$ compared with culture of urethral swab specimen.

positive, and none were EIA positive. Both specimens which were culture positive, PCR negative remained negative by PCR after phenol-chloroform extraction. Eleven of 13 Amplicor PCR-positive, culture-negative specimens were positive by the MOMP PCR. After analysis of the discrepant results, 7.9% (19 of 242) of the women were considered to be infected, and a comparison of the cervical swab culture and PCR results is provided in Table 3.

Combining both groups of patients, of the 25 infected patients who were culture negative but who were positive by PCR testing of either swab or FVU sediment specimens, only 2 had been treated with antimicrobial agents in the month prior to testing. One was being treated with tetracycline for acne, and the other was receiving penicillin. Among the 29 culture-positive patients whose specimens were positive on the first coverslip without the need for passage, the median number of inclusions per coverslip was 25 among the men (range, 1 to 500) and 12 among the women (range, 1 to 219) ($P = 0.9$). Specimens from all five infected patients who were culture positive but negative by PCR testing of swab specimens had three or fewer inclusions per coverslip.

DISCUSSION

The introduction of a commercial nucleic acid amplification assay that can be incorporated into the routine clinical microbiology laboratory is an important advance in our ability to diagnose asymptomatic chlamydial infections. Previously, asymptomatic infections have not been adequately detected because of the lower sensitivity of antigen detection assays, which have been more widely available to clinicians than culture. Even culture (which in the past as been considered the "gold standard" for the diagnosis of chlamydial infections) fails to detect many infections, presumably for technical reasons as well as its stringent transport conditions. In the present study, we found that PCR testing of FVU specimens was the most sensitive and specific method for detecting asymptomatic chlamydial infection in men. It had improved sensitivity over EIA testing of FVU specimens as well as culture or PCR testing of urethral swab specimens. Despite the small numbers of specimens, in asymptomatic women PCR testing of cervical swab specimens was clearly superior to culture or EIA.

Antigen detection assays of FVU specimens for the diagnosis of urethral *C. trachomatis* infections have been used but have produced variable results (18, 20). The lower than desired test sensitivities that have been reported have been attributed to the inclusion of a significant number of asymptomatic men and those with low-inclusion-count infections (20). Since large-scale screening programs by EIA of FVU specimens for males would focus on these type of patients, concerns have been raised about whether the use of antigen detection assays to

TABLE 3. Comparison of culture and PCR of cervical swab specimens from asymptomatic women[a]

Test and result	No. of patients		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	Infected[b]	Uninfected				
Cervix culture						
Positive	8	0	42.1	100	100	95.3
Negative	11	223				
Cervix PCR						
Positive	17	2	89.5[c]	99.3	89.5	99.1
Negative	2	221				

[a] EIA of cervical swab specimens was negative for specimens from all patients. A total of 242 specimens were tested.
[b] Culture positive and/or PCR positive and with the positive result confirmed by MOMP PCR.
[c] $P = 0.006$ compared with culture of specimen from the cervix.

reduce the reservoir of chlamydial infections would be effective. Talbot and Romanowski (24) report that EIA of FVU specimens had a sensitivity of 93.1% for detecting *C. trachomatis* in men with acute urethritis but a sensitivity of only 58.3% for those without signs of urethritis. Our study population was exclusively asymptomatic, and culture-positive patients had low inclusion counts. Our results show that PCR testing of FVU specimens appears to overcome these concerns raised about screening asymptomatic men by EIA testing of FVU specimens. Other studies with populations with higher prevalences of chlamydial infections have also reported similar findings (9, 12, 25). Initial experience with another DNA amplification technique, the ligase chain reaction, also indicates that it is highly sensitive when it is used on FVU specimens from men, even if they are asymptomatic (8). Thus, DNA amplification appears to be the optimal method of testing FVU specimen sediments to detect chlamydial infections in men, especially if they are asymptomatic.

The results of the present study show that in our patient population, FVU is the preferred specimen for diagnosing asymptomatic chlamydial infections in men. The majority of asymptomatic men are reluctant to undergo urethral swabbing because of the discomfort of the procedure. In addition, specimens are inadequately obtained on up to 30% of such swabs if there is no urethral discharge (2, 5). This may account for the improved detection rate of PCR testing of FVU specimen sediments that we found compared with the detection rate obtained by testing urethral swab specimens, even if the urine is collected after the swabs have been taken. Most of our patients had low-inclusion-count infections, and the number of organisms contained in a poorly obtained specimen which is diluted 10-fold prior to testing may even be below the level of detection of the PCR, whereas an urethral washout in a FVU specimen might remove enough organisms that can be concentrated by centrifugation.

The present study extends the findings of previous studies which have found PCR testing of cervical specimens to be a sensitive and specific method of detecting *C. trachomatis* infections, even in asymptomatic women (1, 3, 4, 21). What was most striking was the inability of the Chlamydiazyme EIA to detect any of the asymptomatic infected women. This poor performance is likely due to the lower sensitivity of antigen detection in a group of women who shed very few organisms. We do not recommend EIA testing as a means of screening for *C. trachomatis* infection in such patients. One study has also used PCR testing of FVU specimens from asymptomatic women and found this to have a sensitivity of 91.7% (21). These results need to be confirmed, but FVU specimens may prove to be useful because they can be obtained by noninvasive means and can be used to diagnose genital chlamydial infections in women. Their use would increase the ease of screening for chlamydial infections in women who are not presenting for a Pap smear or gynecologic examination.

A concern with the use of DNA amplification in a clinical laboratory is the presence of inhibitors in the specimen. Several studies have noted specimens from culture-positive patients which were initially PCR negative but which were PCR positive on repeat testing or after phenol-chloroform extraction (1, 3, 15, 25). Some of these initial false-negative results may be due to technical factors, but PCR inhibitors may also play an important role. Although certain substances such as heme are known to inhibit PCR, the exact nature of all of the inhibitors present in genital or urine specimens remains unclear. Nonetheless, dilution, heating to greater than 95°C, or storage at −75°C has been successfully used to remove inhibitors (1, 4, 15). Our swab specimens were transported and stored in 2-SP medium and were diluted prior to amplification, which may have contributed to the lack of inhibition found in the present study. However, PCR amplification of five false-negative swab specimens from culture-positive patients in our study failed, despite phenol-chloroform extraction. It is interesting that all five specimens were from infected patients with very low inclusion counts.

A second concern with the use of PCR in a clinical laboratory is the potential for false-positive results because of cross-contamination and the high degree of sensitivity of the assay itself. The Amplicor PCR assay has been reported to have a high degree of specificity (98 to 100%), and our results confirm this (1–4, 9, 12, 21, 25). Carryover cross-contamination is prevented in the assay by the incorporation of uracil-*N*-glycosylase (AmpErase). Since dUTP is used in place of dTTP during primer extension, the AmpErase will inactivate any previously amplified DNA which may be present in the new sample as a result of carryover. Despite this, separation of preamplification and postamplification areas and unidirectional work flow procedures must still be adhered to in order to reduce the likelihood of false-positive results (17). Further experience in routine clinical laboratories is required to determine how PCR assays will perform in laboratories which handle large volumes of chlamydial specimens.

In conclusion, PCR testing of cervical swab specimens from asymptomatic women will detect a greater number of infected patients than either culture or EIA. PCR testing of FVU specimens is a noninvasive, sensitive, and specific method of diagnosing asymptomatic chlamydial infections in men. If it can be shown to be cost-effective, PCR testing of FVU specimens may prove to be useful as a method of screening asymptomatic men to improve case finding and to reduce the reservoir for transmission of infection.

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