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Evaluation of the Gen-Probe *Chlamydia trachomatis* Transcription-Mediated Amplification Assay with Urine Specimens from Women

RAFAEL PASTERNACK,* PAULI VUORINEN, AND ARI MIETTINEN

Department of Clinical Microbiology, Tampere University Hospital, FIN-33521, Tampere, Finland

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We evaluated the Gen-Probe *Chlamydia trachomatis* transcription-mediated amplification (TMA) assay with urine specimens for the detection of *C. trachomatis* infections in women. The novel test, based on the isothermal amplification of chlamydial RNA, was compared with the Roche Amplicor PCR with urine and cell culture with endocervical specimens. First-catch urine and endocervical swab specimens were collected from a total of 561 patients, of whom 70 (12.3%) were confirmed to have chlamydial infection. The diagnostic sensitivity and specificity of TMA with urine were 91.4 and 99.6%, respectively, and those of Amplicor PCR were 97.1 and 99.8%, respectively. By repeated analysis of the specimens with discrepant results, the sensitivity of TMA could be increased to 99%, indicating that some methodological improvements in the assay are still to be expected. The sensitivity of PCR could be increased to 100% by the elimination of DNA polymerase inhibitors in a repeated analysis. The sensitivity and specificity of cell culture with cervical specimens were 85.7 and 100%, respectively. The results indicate that TMA with urine specimens from women is a sensitive and specific assay for the detection of *C. trachomatis*, providing a new noninvasive technique for the screening of chlamydial infections in women.

Chlamydia trachomatis is the major cause of genitourinary infections in developed countries. It has been estimated that some 10 to 40% of inadequately treated patients with chlamydial cervicitis develop pelvic inflammatory disease (2), which is an important cause of infertility, chronic pelvic pain, and ectopic pregnancy (14). According to a recent study the screening and treatment of *C. trachomatis* in an asymptomatic group of young women reduced the incidence of pelvic inflammatory disease by more than 50% (11), indicating that the screening of asymptomatic risk groups for chlamydial infection would be beneficial. So far, the inconvenience of cervical specimen collection has made the screening less tempting for women.

Nucleic acid amplification methods have increased the sensitivity of diagnostic tests for *C. trachomatis* considerably and have made it possible to use urine specimens for the detection of *C. trachomatis* in both men and women (1, 3–6, 12). Two different commercial applications of nucleic acid amplification are available for this purpose: PCR (Roche Amplicor PCR) and ligase chain reaction (LCR; Abbott LCx). Both techniques are based on the amplification of plasmid DNA specific for *C. trachomatis.*

An alternative method, transcription-mediated amplification (TMA), based on the isothermal amplification of rRNA (Gen-Probe AMTD), has been used in clinical practice for the detection of *Mycobacterium tuberculosis*, with good results (8, 13). Recently, TMA has also been applied for the detection of *C. trachomatis*. We evaluated this new application of TMA, the Gen-Probe *Chlamydia trachomatis* TMA assay, with urine specimens for the diagnosis of chlamydial infection in a high-risk female population. The novel test was compared with Amplicor PCR with urine specimens and cell culture with cervical specimens.

* Corresponding author. Mailing address: Department of Clinical Microbiology, Tampere University Hospital, Box 2000, FIN-33521, Tampere, Finland. Phone: 358-3-2475296. Fax: 358-3-2475260.

MATERIALS AND METHODS

The patients consisted of 561 women. They were examined and treated at the Policlinic for Sexually Transmitted Diseases, Community Health Centre of the City of Tampere, and at the policlinic of University Students' Health Foundation, Tampere, Finland, between January 1995 and April 1996. The study protocol had been approved by the ethical committees of Tampere University Hospital and the City of Tampere. Informed consent was obtained from all patients verbally.

First-catch urine specimens were collected in clean cups prior to the cervical sampling and were transferred to sterile plastic containers. Patients were expected not to have urinated in the 2 h before specimen collection. The specimens were transported to the laboratory within 24 h. Once at the laboratory, urine specimens for Amplicor PCR assay were prepared as instructed by the manufacturer. An aliquot of the unprepared primary urine specimen was frozen at −70°C for the TMA assay.

The Gen-Probe TMA assay (Gen-Probe, Inc., San Diego, Calif.) was performed with the primary urine specimens frozen at −70°C, according to the manufacturer's instructions. Briefly, 1.5 ml of urine was pipetted into a microcentrifuge tube. The tubes were incubated at 37°C for 10 min and were centrifuged at 10,000 × *g* for 5 min. The supernatants were decanted, and 200 μl of specimen dilution buffer was added. The pellets were resuspended, and a 50-μl aliquot of the suspended specimen was added to a tube containing amplification reagent and silicon oil. The tubes were incubated at 95°C for 10 min and were cooled to 42°C. The amplification enzyme mixture was added, and the tubes were incubated at 42°C for 1 h. Termination reagent was added, and the incubation was continued for 10 min at 42°C. A specific labelled probe was added to the hybridization assay. The tubes were then incubated at 60°C for 15 min. The selection reagent was added, and the tubes were incubated at 60°C for 10 min. Hybridization results were read with a luminometer (Leader 50; Gen-Probe, Inc.), and specimens exhibiting >50,000 relative light units were considered positive.

The Roche Amplicor PCR assay (F. Hoffmann-La Roche Ltd., Basel, Switzerland) with urine specimens was performed according to the manufacturer's instructions. The PCR was accomplished by using the Thermocycler TC 9600 instrument (Perkin-Elmer Cetus, Norwalk, Conn.). After the amplification, the amplified nucleotide sequences were detected by using target-specific DNA probes. The resulting enzyme reaction was measured with a spectrophotometer (Multiskan; Labsystems Ltd., Helsinki, Finland). Specimens with an A_{450} of ≥0.250 were considered positive.

Endocervical swab specimens were obtained by using TS/19-CW aluminum wire swabs with viscose tips (Technical Service Consultants Ltd., Lancaster, United Kingdom). After removal of the mucus from the cervix, the swab was revolved in the endocervix to collect epithelial cells. The swab was then shaken vigorously with the glass beads in chlamydia transit tubes (Labsystems Ltd.) and compressed toward the tube wall to free the specimen from the swab. The specimens were transported to the laboratory within 24 h. The cell culture was performed by inoculation of 500 μl of the specimen in sucrose-phosphate me-

TABLE 1. Analysis of discrepant test results by Gen-Probe TMA, Roche Amplicor PCR, and chlamydial cell culture

No. of specimens	Result by:				Final result; conclusion
	Gen-Probe TMA with urine	Amplicor PCR with urine	Cell culture with cervical swab	Amplicor PCR with cervical swab	
9	Positive	Positive	Negative	Negative	Positive; the infection was not detected in cervix by cell culture or Amplicor PCR
1	Positive	Positive	Negative	Positive	Positive; the infection was not detected in cervix by cell culture, but the cervical specimen was positive by Amplicor PCR
5	Negative/positive[a]	Positive	Positive	ND[b]	Positive; the infection was not detected in urine by TMA in the first run; when the specimen was reanalyzed, the TMA result was positive
1	Negative/negative	Positive	Positive	ND	Positive; the infection was not detected in urine by TMA
2	Positive	Negative/positive	Positive	ND	Positive; DNA polymerase inhibitors were present in urine
2	Positive	Negative	Negative	Negative	Negative; the positive results by TMA could not be confirmed
1	Negative	Positive	Negative	Negative	Negative; the positive result by PCR could not be confirmed

[a] Initial result/result from reanalysis.
[b] ND, not done.

dium onto a monolayer of McCoy cells in flat-bottom, plastic shell vials with coverslips. The specimens were centrifuged (3,000 × *g*) at 35°C for 1 h. The medium was then changed to a medium containing 1 μg of cycloheximide per ml (10). After an incubation of 3 days, the cells were stained with iodine and were screened for inclusions. If one or more typical inclusions were seen, the result for the specimen was interpreted as positive.

Cell culture-negative endocervical specimens from patients whose urine was positive by either of the amplification assays were tested by the Roche Amplicor PCR, which was performed according to the manufacturer's instructions.

A positive culture result for a cervical swab specimen was taken to indicate a *C. trachomatis* infection. In addition, patients whose endocervical swab specimens were negative by cell culture were determined to have *C. trachomatis* infection if their urine tested positive by both amplification methods.

RESULTS

The results of all three tests were identical for 541 (96.4%) of the 561 patients. Altogether, 70 (12.5%) of the patients had chlamydial infection by definition. Among them, the test results were uniformly positive for 52 patients. The discrepant results for 21 patients are presented in Table 1. Of these 21 patients, 18 could be confirmed to be positive by definition. Cervical specimens were negative by cell culture for 10 patients who were positive by both the Amplicor PCR assay and the Gen-Probe TMA assay with urine specimens. Only 1 of these 10 cervical specimens was positive by the Amplicor PCR, indicating that the organism actually was not detectable in the endocervix in 9 of the 10 patients.

The result obtained by the TMA assay with urine was negative for six patients who were positive by both cell culture and Amplicor PCR. They were considered to be false negative by definition. However, when the TMA assay was repeated with the primary specimens, five of the six became positive. The Amplicor PCR was negative for two urine specimens from patients whose specimens were positive by the other methods. When the prepared specimens were reanalyzed after freezing and thawing, both turned positive, indicating the presence of DNA polymerase inhibitors. Two urine specimens from patients who were negative by the other methods were positive by the TMA assay. These results could not be confirmed and were taken to be false positive by definition. Similarly, one urine specimen was repeatedly weakly positive by the Amplicor PCR only and was taken to be a false-positive result. The diagnostic sensitivities and specificities of the different assays are presented in Table 2.

DISCUSSION

Methods based on the amplification of *C. trachomatis*-specific plasmid DNA by DNA polymerase (PCR) and by a combination of DNA polymerase and DNA ligase (LCx) have been used for the detection of chlamydia infection for some time. These methods have been shown to be both sensitive and specific, and they have made it possible to use urine specimens from women, replacing cervical and urethral sampling. An alternative nucleic acid amplification method, TMA, detects pathogen-specific rRNA. For the detection of *M. tuberculosis* in respiratory specimens, this method has been shown to be as rapid, sensitive, and specific as Amplicor PCR (8, 13). The recently introduced Gen-Probe *C. trachomatis* TMA assay is based on the isothermal amplification and detection of *C. trachomatis*-specific rRNA. In the first step of the TMA assay, reverse transcriptase creates a complementary DNA copy of the target rRNA. In the second step, RNA polymerase synthesizes RNA amplicons, with cDNA as a template. Each of the newly synthesized RNA amplicons reenters the TMA process and serves as a template for a new round of replication, resulting in a theoretical 10^9 amplification of the target *C. trachomatis*-specific rRNA. RNA amplicons are detected by a luminometer after a hybridization protection assay with specific chemiluminescence-labeled DNA probes.

TABLE 2. Comparison of Gen-Probe TMA and Roche Amplicor PCR with urine specimens and cell culture with cervical swab specimens for the detection of genital *C. trachomatis* infections in 561 women

Test and result	No. of samples in which *C. trachomatis* infection was:		% Sensitivity	% Specificity
	Present (n = 70)	Absent (n = 491)		
Gen-Probe TMA (urine)				
Positive	64	2	91.4[a]	99.6
Negative	6[a]	489		
Amplicor PCR (urine)				
Positive	68	1	97.1[b]	99.8
Negative	2[b]	490		
Cell culture (cervical swab)				
Positive	60	0	85.7	100
Negative	10	491		

[a] In the reanalysis, five of the six urine specimens became positive, increasing the sensitivity to 98.6%.
[b] In the reanalysis, both urine specimens became positive, increasing the sensitivity to 100%.

The theoretical advantage of TMA over PCR or LCR comes from detecting the rRNA present at about 2,000 copies per cell, thus presumably offering a good sensitivity for specimens with a low target sequence number. Another benefit could be that the carryover contamination of specimens is not a major problem for TMA due to the labile nature of the RNA in the laboratory environment. A plasmid-free strain of *C. trachomatis* has been described (7), although its pathogenic role is arguable. Whether or not it is of clinical significance, TMA should be able to detect plasmid-free *C. trachomatis*, too.

In this study we evaluated the Gen-Probe TMA assay for the detection of chlamydia in urine specimens from women and compared the results with those of Amplicor PCR with urine specimens and cell culture with cervical specimens. The results support earlier findings indicating that urine is superior sample material for the detection of *C. trachomatis* infections in women (5, 6, 9). All 70 chlamydial infections could be detected by the amplification assays with urine. In contrast, 10 (14%) of the 70 infections were not found by cell culture with endocervical specimens. Of the 10 cervical swab specimens, 9 were confirmed to be negative by Amplicor PCR performed with the same specimens, indicating that the endocervix actually was not infected in these patients. The current results indicate that more infections can be detected by using urine specimens than by using cervical specimens.

The results obtained by the TMA assay were very promising. The TMA assay detected all but 6 of the 70 chlamydial infections in the material tested, yielding a sensitivity of 91%. Repeat testing of the specimens with false-negative results yielded five additional specimens with positive results and increased the sensitivity of TMA to 99%. The reason for the initially false-negative results by TMA remains unknown. It is possible that the differences in the sample preparation procedures for TMA and PCR account for the observed differences in performance between the two tests. For practical reasons, specimens for TMA were freshly frozen and were then prepared and analyzed later, whereas the PCR was performed with specimens that had first been prepared and then frozen. Studies with fresh specimens and identical sample preparation protocols for all techniques are warranted to finally resolve the sensitivity of TMA in clinical practice in comparison with those of other amplification techniques. The false-negative results might also be explained by inhibition of the amplification step of the assay. DNA polymerase inhibition is a well-recognized problem for both PCR and LCR techniques that use DNA amplification (5, 6). Whether inhibitors of RNA amplification exist is still unknown. If the false-negative results obtained with the current material were due to inhibition, the results indicate that specimens inhibitory to TMA are different from those inhibitory to PCR.

The sensitivity of the Amplicor PCR in this study population was 97.1%. Two urine specimens initially Amplicor PCR negative became positive when the prepared specimens were reanalyzed in the discrepancy analysis. The specimens were TMA positive and were interpreted as inhibitory to PCR. If it would have been possible to detect and eliminate the inhibition of DNA polymerase by an internal control protocol, all infections in this material would have been detected by Amplicor PCR with urine specimens.

Besides the increased sensitivity, the standardized commercially available gene amplification techniques are likely to make chlamydia detection more reliable and less laborious for use in routine clinical microbiology laboratories. TMA was well suited for use in a clinical microbiology laboratory with experienced technicians. The TMA system requires only a single tube for the preparation of the urine specimen, and the prepared specimen is then transferred to the reaction tube for isothermal amplification and detection. Amplicor PCR is a more complex system, requiring separate laboratory areas for sample preparation, amplification, and detection. The Gen-Probe TMA had an assay time of 4.5 h and proved to be slightly faster than the Amplicor PCR, which had an assay time of 6 h.

To conclude, we found that Gen-Probe TMA is a sensitive and specific assay for the detection of *C. trachomatis* in urine specimens from women. The use of urine specimens analyzed by Gen-Probe TMA or Amplicor PCR can, without doubt, be recommended for screening programs for *C. trachomatis* infections in women.

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