

Detection of *Chlamydia trachomatis* by the Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay (AMP CT) in Urine Specimens from Men and Women and Endocervical Specimens from Women

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Molecular biology-based amplification methods are significantly more sensitive than other methods for the detection of *Chlamydia trachomatis*. The performance characteristics of the new Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay (AMP CT) with endocervical and urine specimens were compared to those of culture for patients attending two Baltimore City sexually transmitted disease clinics and a clinic for adolescents. AMP CT uses transcription-mediated amplification (TMA) and hybridization protection assay procedures to qualitatively detect *C. trachomatis* by targeting a 23S rRNA. Discrepant results between culture-negative and AMP CT-positive specimens were resolved by direct fluorescent-antibody staining of sedimented culture transport medium for elementary bodies and by TMA with 16S rRNA as a target. Following discrepant analysis, for 480 female urine specimens AMP CT had a sensitivity of 93.8% and a specificity of 100%. For 464 male urine specimens, the resolved sensitivity and specificity of AMP CT were 95.6 and 98.7%, respectively. For the 479 endocervical swab specimens the sensitivity of AMP CT was 100% and the specificity was 99.5%. Resolved culture sensitivities of AMP CT for female and male swab specimens were 52.3 and 58.9%, respectively. These results demonstrate that AMP CT is highly sensitive for the detection of *C. trachomatis* in endocervical specimens and in urine specimens from men and women.

Diagnosis of chlamydial infections has, until recently, depended upon cell culture techniques as the “gold standard” for the detection of pathogens in clinical specimens. However, factors such as specimen adequacy due to collection, transport time, and storage of the sample can negatively influence the sensitivity of cell culture (1, 15). Thus, new methods for diagnosis were developed, such as direct immunofluorescence, enzyme immunoassays, and DNA probe techniques (2, 12, 13, 18, 20–22), for use in clinical practice. However, despite the advantages of these assay systems, including ease of transport and lower cost than cell culture, the numbers of infectious organisms in clinical samples were frequently too few to be detected by either culture or antigen or DNA probe assays. The most recent generation of diagnostic techniques, nucleic acid amplification tests such as PCR (4–6, 10, 11), ligase chain reaction (3, 8, 10, 14), and transcription-mediated amplification (TMA) (10, 16–18), are capable of detecting small numbers of microorganisms, and their sensitivities appear to exceed the sensitivity of cell culture.

In this study, the performance characteristics of a new diagnostic nucleic acid amplification assay known as the Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay (AMP CT) (Gen-Probe, Inc., San Diego, Calif.) were evaluated with urine specimens from men and women and endocervical specimens from women. AMP CT couples the Gen-Probe amplification system of TMA with Gen-Probe’s separation and detection system, the hybridization protection assay. Together, these technologies provide an amplification and detection system in a single-

tube format. The TMA system used in this test amplifies a specific 23S rRNA target via DNA intermediates. Use of RNA targets provides a diagnostic advantage because bacterial rRNA is present at many thousands of copies per cell, whereas DNA is present at a much lower copy number. Therefore, the likelihood of initiating amplification is greater when rRNA is targeted than when DNA is targeted. This is particularly important when organisms are present in low numbers, such as in asymptomatic patients.

MATERIALS AND METHODS

Patient population. A total of 485 women and 464 men attending two Baltimore City sexually transmitted disease (STD) clinics and a clinic for adolescents were enrolled following informed consent. The study protocol was approved by the ethical review boards of both the Johns Hopkins University and the Baltimore City Health Department. For women, two endocervical dacron swab specimens were obtained, one for cell culture and the other for AMP CT, along with 15 ml of first-void urine (FVU), which was also tested by AMP CT. The order of collection of the swab specimens was alternated by odd and even patient identification numbers (i.e., for patients with odd patient identification numbers, a swab specimen for culture was obtained first, followed by a swab specimen for AMP CT, and vice versa for patients with even patient identification numbers). The endocervical swab specimen for culture was obtained and placed in chlamydia transport vials containing sucrose-phosphate buffer, 10% fetal bovine serum, and antibiotics. The endocervical swab specimen for AMP CT was obtained and placed in Gen-Probe transport medium, transported at room temperature, then stored at 2 to 8°C until it was processed.

For men, a urethral dacron swab specimen was collected for cell culture, and 15 ml of FVU was obtained for testing by AMP CT. Urethral swab specimens were collected by inserting a narrow-shafted dacron-tipped swab 2 to 3 cm into the urethra, and the swab was then placed in chlamydia transport medium. The 15 ml of FVU was then collected in a sterile 50-ml screw-cap plastic cup. The FVU specimens were transported at room temperature and were then stored at 2 to 8°C until processing. The endocervical and male urethral chlamydia culture transport vials were transported at –20°C and were stored at –70°C for 12 to 24 h until they were processed for culture. Any individuals who had received antibiotics within 21 days prior to sampling were excluded from analysis.

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Cell culture. Chlamydia culture was performed in 96-well microtiter plates with McCoy cell monolayers (American Type Culture Collection, Rockville, Md.). After the cells reached confluent growth at 36°C in 5.0% CO₂, medium from the wells was aspirated two rows at a time. A 100- μ l aliquot of specimens received in 2-sucrose phosphate transport medium was then inoculated into each of two microtiter wells (total volume, 200 μ l) after vortexing. The plates were then centrifuged at 22,000 rpm (Sorvall RT6000B; Sorral, Newton, Conn.) for 1 h at 37°C. After centrifugation, patient samples were aspirated off with a spinal needle, with flaming of the needles performed between aspiration of patient samples to sterilize the needles. Inoculation medium containing cycloheximide (1 μ g/ml) was then added to cover each well, and the plate was incubated at 36°C in 5.0% CO₂ for 48 to 72 h. To stain and read the results for the plate after incubation, the inoculation medium was aspirated off and the plate was fixed with methanol for 1 min. Both wells were evaluated for chlamydia inclusions by immunofluorescence staining. One well was stained with monoclonal antibody to the *Chlamydia trachomatis* major outer membrane protein (Microtrak Chlamydia Culture Reagent; Syva, San Jose, Calif.), and the other well was stained with antilipoplysaccharide monoclonal antibody (Sanofi Diagnostics Pasteur, Chaska, Minn.). Two antibodies were used to ensure the recovery of all serovars of chlamydia (7).

AMP CT. AMP CT was performed with endocervical and urine specimens by using a 23S rRNA-based amplification method from Gen-Probe described, as follows.

(i) **Specimen processing.** After collection at the clinic and transport to the laboratory the swabs for AMP CT, still in the transport tube, were centrifuged at 190 to 425 $\times g$ for 5 min in order to bring all of the liquid to the bottom of the tube. Forty microliters of a reconstituted specimen preparation reagent was added to the transport tube, and the tube was vortexed briefly and then incubated at 60°C for 10 min. After incubation, 20 μ l of specimen was transferred into a polypropylene tube containing 400 μ l of specimen dilution buffer and the tube was then vortexed. For urine, 1.5 ml of the urine specimen was incubated at 36°C for 10 min in order to help eliminate any precipitate which may have existed, and then the specimen was microcentrifuged at 7,500 to 12,000 $\times g$ for 5 min. The supernatant was discarded and 200 μ l of specimen dilution buffer was added to the pellet and the specimen was vortexed. If the samples were not processed directly after transport to the laboratory, they were stored at 2 to 8°C for up to 7 days and then processed.

(ii) **TMA.** TMA is an isothermal, autocatalytic amplification of a 23S rRNA target that uses two enzymes, reverse transcriptase and RNA polymerase. Twenty-five microliters of reconstituted amplification reagent was added to the bottom of a labeled polypropylene tube, and then 200 μ l of oil reagent was added on top to create a barrier against contamination. Then, 50 μ l of the prepared specimen (swab or urine) was added to each tube below the oil layer and the tube was incubated at 95°C for 10 min. The tubes were then transferred to a water bath and incubated at 42°C for 5 min. Twenty-five microliters of reconstituted enzyme reagent was added, and the tubes were swirled to mix their contents and then incubated at 42°C for 1 h. After the 1-h incubation, 20 μ l of the termination reagent was added, and the tubes were swirled to mix their contents and incubated for another 10 min at 42°C.

(iii) **Hybridization protection assay.** The hybridization buffer was warmed at 60°C for 3 to 4 min, and then 6 ml was added to the lyophilized probe reagent. The probe reagent is an acridinium ester (AE)-labeled DNA probe which specifically detects the RNA amplicon. A 100- μ l aliquot of the reconstituted probe reagent was added to each tube, the tube was vortexed, and hybridization to specific target sequences was allowed to take place during incubating at 60°C for 15 min. Separation of hybridized probe from unhybridized probe by chemical hydrolysis of the AE on the unhybridized probe was accomplished by adding 300 μ l of selection reagent to each tube and vortexing the tube vigorously so that there was a homogeneous mixture. The AE labels of hybridized probes are protected within the DNA/RNA double helix and therefore are not hydrolyzed by the selection reagent. The tubes were returned to the 60°C water bath for 10 min and then cooled at room temperature for 5 min. For the detection step, reaction tubes were loaded into an automated LEADER 450i luminometer, which then automatically injected detection reagents into each tube. The detection reagents chemically cleave the AE labels of the DNA-RNA hybrids to emit light. The light emitted from the reactions is detected by a photomultiplier tube and is then converted into a number and expressed as relative light units (RLU).

(iv) **Test interpretation.** A LEADER reading of >500,000 RLU was considered positive for *C. trachomatis*, and a reading of <40,000 RLU was considered negative for *C. trachomatis*. Those samples with readings of between 40,000 and 500,000 RLU were considered equivocal and were retested. For retesting, a cutoff of $\geq 50,000$ RLU was used. Amplification-negative control and amplification-positive control values of <20,000 and >750,000 RLU, respectively, must have been obtained or the test was considered invalid and repeated.

Discrepant analysis. Discrepant results between culture-negative, AMP CT-positive specimens were resolved by direct fluorescent-antibody (DFA) staining of sedimented culture transport medium (2). For those specimens that were negative by DFA staining, TMA with 16S rRNA as an alternate target was performed by the manufacturer. Discrepant analysis was not done with culture-positive, AMP CT-negative specimens because a positive culture was considered to be a true-positive result. Therefore, these latter specimens were considered to have true-positive results and the AMP CT result was considered false negative.

TABLE 1. Analysis of AMP CT performance with specimens from males and females before and after discrepant analysis

Gender, test, and result	No. of samples with the following result:			
	Before discrepant analysis		After discrepant analysis ^a	
	Culture positive	Culture negative	Resolved positive	Resolved negative
Female				
AMP CT of endocervical swab specimens (n = 479)				
Positive	39	28	65	2
Negative	0	412	0	412
AMP CT of urine specimens (n = 480)				
Positive	34	26	60	0
Negative	4	416	4	416
AMP CT of matched endocervical swab and urine specimens (n = 474)				
Positive	35	33	67	1
Negative	3	403	3	403
Male, AMP CT of urine specimens (n = 464)				
Positive	37	33	65	5
Negative	3	391	3	391

^a Discrepant analysis was performed by DFA staining of sedimented culture transport medium and by TMA with 16S rRNA as a target.

RESULTS

Clinical samples from 485 women and 464 men were examined by cell culture and AMP CT for the presence of *C. trachomatis*. Among the female patients, both a swab specimen and a urine specimen were collected from 474 of the patients. Five patients provided only a swab specimen, and six patients provided only a urine specimen. Therefore, a total of 479 individual endocervical swab specimens and 480 individual urine specimens were collected from females. For the male patients, only urine specimens were collected for AMP CT.

Among the 479 endocervical swab specimens, 67 (14.0%) were AMP CT positive, and 39 of these were culture positive. Of the 28 AMP CT-positive, culture-negative specimens, discrepant analysis resolved the results for a total of 26 of these specimens (Table 1) (19 by DFA staining and 7 by TMA with 16S rRNA as a target) yielding a sensitivity of 100% and a specificity of 99.5% (Table 2). Among the 480 urine specimens from females, 60 (12.5%) were AMP CT positive, and 34 of these were culture positive (Table 1). Following discrepant analysis of the 26 AMP CT-positive, culture-negative specimens, all 26 were resolved to be positive, 15 by DFA staining and the remaining 11 by TMA with the alternate target. However, four specimens were AMP CT negative and culture positive, thus yielding a sensitivity of 93.8% and a specificity of 100% (Table 2).

Upon analysis of the 474 female patients from whom matched urine and endocervical specimens were obtained, 38 (8.0%) patients were positive by culture and AMP CT, and 32 (6.8%) of those were positive by AMP CT and negative by culture, but the result was resolved as positive by either DFA staining or TMA with 16S rRNA as a target, yielding a total of

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

Gender and test	Sample type	No. of samples	Resolved performance ^a			
			Sens. (%)	Spec. (%)	PPV (%)	NPV (%)
Female						
AMP CT	Endocervical swab	479	100.0	99.5	97.0	100.0
AMP CT	Urine	480	93.8	100.0	100.0	99.0
AMP CT	Matched urine and endocervical swab	474	95.7	99.8	98.5	99.3
Culture	Endocervical swab	474	54.3	100.0	100.0	92.7
Male						
AMP CT	Urine	464	95.6	98.7	92.9	99.2
Culture	Urethral swab	464	58.8	100.0	100.0	93.4

^a Sens., sensitivity; Spec., specificity; PPV, positive predictive value; NPV, negative predictive value.

70 (14.8%) infected individuals (Table 1). Among those 70 infected patients, 11 (15.7%) were positive by AMP CT of endocervical swab specimens only, 9 (12.9%) were positive by AMP CT of urine specimens only, and 50 (71.4%) were positive by AMP CT of both endocervical swab and urine specimens. By testing both urine and endocervical swab specimens, we were able to detect at least 14.7% more positive patients than if only an endocervical swab specimen was obtained. For example, for patients with matched specimens, infections in 61 women were detected by AMP CT of endocervical swab specimens and infections in 59 women were detected by AMP CT of urine specimens. However, because infections in some patients were detected by AMP CT of an endocervical swab specimen or a urine specimen individually, testing of the combination of specimens detected infections in nine additional women, yielding a 14.7% increase in sensitivity over that from testing only endocervical swab specimens or an 18.6% increase in sensitivity over that from testing only urine specimens.

Among the 464 urine specimens from male patients analyzed by AMP CT, 70 (15.1%) were positive by AMP CT, and 37 of these were culture positive (Table 1). Following discrepant analysis of the 33 culture-negative, AMP CT-positive specimens, 28 were resolved to be positive (16 by DFA staining and 12 by TMA with an alternate target), yielding a sensitivity of 95.6% and a specificity of 98.7% (Table 2).

DISCUSSION

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal to target base pairs and to allow enzymatic amplification of the target nucleic acid strands (9). AMP CT is a nucleic acid amplification system which is sensitive and specific and which has a sensitivity and a specificity comparable to those of currently available amplification tests. Recent studies have demonstrated the ability of several nucleic acid amplification assays to detect *C. trachomatis* in urine with remarkably high sensitivities (3, 8, 14, 18, 19). In our study, the resolved sensitivity of AMP CT with urine samples from females was 93.8%, similar to previous reports on the sensitivity of AMP CT with urine samples (10, 16–18). Unique to this study, however, we tested endocervical swab specimens by AMP CT, which yielded a sensitivity of 100% on a per-specimen basis. For females from whom both an endocervical swab sample and a urine sample were obtained, both specimens were concordantly positive for only 71.4% of the *C. trachomatis*-infected women. If only endocervical swab specimens were collected, infections in 87.1% of all positive patients would have been detected, missing infections in 12.9% of the positive patients. On the other hand, if only female urine had been collected, infections in 84.3% of

all positive patients would have been detected, missing infections in 15.7% of the positive patients. Thus, testing for *C. trachomatis* by using both specimens increased the sensitivity to 100%. In contrast, the sensitivity of culture of endocervical swab specimens was 54.3%. AMP CT also was effective at detecting *C. trachomatis* in urine specimens from males, yielding a resolved sensitivity of 95.6%. The sensitivity of culture of urethral swab specimens was 58.8%. For all specimen types tested, the specificity of AMP CT was quite high, ranging from 98.7 to 100%.

The differences in the sensitivity of AMP CT with urine and endocervical swab specimens could be a result of several different factors. First, sampling discrepancies may have limited the resolution of AMP CT-positive specimens not only due to the simple fact that collection of the samples originates from two different anatomical sites but also from the fact that collection techniques may vary among clinicians. Reliable results are dependent on adequate specimen collection techniques; however, it is impossible to guarantee that there was no variability in the specified collection techniques for this or any other assay. Another factor which may have contributed to the differences in sensitivities could be the use of tampons or douching, neither of which were evaluated for their impact on the detection of *C. trachomatis* in urine. Finally, the finding of a positive culture and a negative AMP CT result may also reflect variance in adequate specimen collection or the presence of inhibitors, or both. It is possible that the presence of inhibitors may cause false-negative results with this product, as is the case for other products. However, for clinical utility purposes, retesting of all AMP CT-negative specimens to assess the presence of such possible inhibitors is not practical and was therefore not performed. It is noteworthy, however, that such an evaluation is an important step in determining the true reliability of an amplification assay, and some manufacturers are considering adding an internal control to tests for each specimen.

In spite of the presence of widely available and accurate tests, the chlamydia epidemic continues throughout the United States, with 4 million to 4.5 million new cases each year. A large number of asymptomatic carriers who are not tested and who therefore continue to spread the disease through sexual contact contribute to this continuing problem. Since the invasive procedures which are usually required to obtain samples often create patient anxieties, people are reluctant to be tested. Hence, urine samples have been investigated as an alternate specimen for testing because of the noninvasive way in which they can be collected. Initially, urine was widely tested in most immunoassays. Unfortunately, since there are generally lower concentrations of *C. trachomatis* in urine compared to the

concentrations in urogenital specimens, the performance of most immunoassays with urine is poor. By targeting rRNA, which is more abundant than DNA in bacterial cells, a larger number of target molecules are available to a TMA reaction, thereby increasing the reliability of amplification initiation. This is an advantage, particularly when organisms are present in very low numbers.

There are several other advantages to using the TMA-based Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay (AMP CT). The single-tube format does not include washing steps or the transfer of amplicon for detection, thus minimizing the possibility of cross contamination and false-positive results. It is also important that carryover contamination is not a major problem due to the labile nature of the RNA amplicon in the laboratory environment. Screening for chlamydia in urine samples for men is particularly important since asymptomatic men rarely seek health care and serve as a reservoir for disease transmission. Screening of male urine would create access to the reservoir of infected individuals. By AMP CT screening for urine from men yielded a 93.8% sensitivity and a 100% specificity, whereas culture of urethral swab specimens from males was only 58.9% sensitive.

These results demonstrate that AMP CT is more sensitive than culture in identifying chlamydial organisms in both endocervical swab specimens and male and female urine specimens. The assay is highly sensitive and specific, has a sensitivity and a specificity comparable to those of other currently available nucleic acid amplification tests, and provides a noninvasive means of screening for *C. trachomatis* through the testing of urine.

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