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Performance of the Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay in Detecting *Chlamydia trachomatis* in Endocervical and Urine Specimens from Women and Urethral and Urine Specimens from Men Attending Sexually Transmitted Disease and Family Planning Clinics

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The Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay (AMP CT) uses transcription-mediated amplification and hybridization protection assay procedures to qualitatively detect *Chlamydia trachomatis* rRNA in urine, endocervical swab, and urethral specimens. The performance of the AMP CT was compared to that of cell culture for endocervical swab and urine specimens from women and urethral and urine specimens from men. Analysis of specimens with discrepant results was performed by a combination of reculture, direct fluorescent-antibody (DFA) staining of specimen sediment, and amplification which targeted a different chlamydial rRNA. A total of 800 urine samples were tested by the AMP CT (607 from women and 193 from men), and 7.1% were positive for *C. trachomatis*, with a sensitivity of 91.2% and a specificity of 99.6% upon discrepant analysis. A total of 926 swab specimens were tested by culture and AMP CT (717 endocervical swab specimens and 209 urethral swab specimens from men), and 7.7% were positive for *C. trachomatis*, with a sensitivity and specificity of 100% upon discrepant analysis. The AMP CT is a sensitive and specific nucleic acid hybridization assay for the detection of *C. trachomatis* in endocervical swab specimens from women, urethral swab specimens from men, and urine specimens from men and women.

The most prevalent sexually transmitted disease in the United States today is caused by *Chlamydia trachomatis* (1, 3, 4). *C. trachomatis* may cause urethritis, epididymitis, proctitis, cervicitis, pelvic inflammatory disease, infant pneumonia, and conjunctivitis. The significance of serious complications related to chlamydial infections has been well established (1, 15). The advent of managed care and shrinking public health budgets have made rapid, early, and accurate diagnosis and treatment even more critical. Until recently, testing capabilities that are noninvasive and that are applicable to various venues were a particular challenge for the diagnosis of chlamydial infections. The laboratory technology available as an aid in the diagnosis of chlamydial infections is currently undergoing rapid evolution.

Traditional cell culture methods have been the "gold standard" for the diagnosis of chlamydial infections. However, cell culture methods are expensive, time-consuming, and subject to laboratory-to-laboratory variation. The advent of enzyme immunoassays (EIAs) and the DNA probe test (Gen-Probe, Inc.) for direct detection of antigen or nucleic acids in patient samples provides an alternative to tissue culture (16). The sensitivities and specificities of EIAs and DNA probe tests are comparable to those of culture (9–11). The performance of the EIA has been reported to be improved with the use of verification testing for samples whose results are in the negative grey zone (6, 7). Recently, amplification methods based on PCR and ligase chain reaction have been reported to offer improved performance over those of culture and nonculture methods (2, 12–14). The newest amplification procedure to be cleared in the United States by the Food and Drug Administration is the AMPLIFIED Chlamydia Trachomatis Assay (AMP CT; Gen-Probe, Inc.).

The purpose of this study was to assess the performance characteristics of AMP CT with endocervical swab and urine specimens from women and urethral swab and urine specimens from men. AMP CT was compared to standard culture methods.

MATERIALS AND METHODS

Study population and sites. Specimens for routine *C. trachomatis* testing were collected from three sites. Site 1 was the San Joaquin County Public Health sexually transmitted disease clinic located in Stockton, Calif., which serves a diverse population. All urethral and urine specimens from men were collected at this site. Sites 2 and 3 were two family planning clinics. One clinic is located in Stockton, Calif., and the other one is located in Lodi, Calif. All endocervical and urine specimens from women were collected at these two sites.

Specimen collection and transport. At Site 1, two urethral swab specimens and a urine specimen were to be collected from each male patient. The patient must not have urinated within 2 h and must have provided a first-catch urine specimen in order to be included in the study. If the patient had urinated within 2 h, a urine specimen was not collected from the patient. Sixteen male patients were unable to provide urine specimens because of this restriction. At Sites 2 and 3, two endocervical swab specimens and a urine specimen were to be collected from each female patient. As for the male patients, if the women had urinated within 2 h a urine specimen was not collected from the patient. One hundred ten female patients were unable to provide urine specimens because of this restriction. At each site the order of swab collection was randomized. One swab sample was used for culture of *C. trachomatis* and one swab was tested by AMP CT. All urine specimens were tested by AMP CT. All specimens tested by culture, swab specimens tested by AMP CT, and urine specimens were transported to the laboratory on cold pack and were held at 4°C until testing.

AMP CT procedure. AMP CT uses transcription-mediated amplification (TMA) and hybridization protection assay procedures to qualitatively detect *C. trachomatis* rRNA in urethral swab and urine specimens from men and endocervical swab and urine specimens from women (8). TMA uses two primers and two enzymes. One primer contains a promoter sequence for RNA polymerase. In the first step of amplification the promoter-primer hybridizes to the target rRNA at

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a predefined site. Reverse transcriptase creates a DNA copy of the target. A second primer then binds to the DNA copy, and a new DNA strand is synthesized by reverse transcriptase, creating a double-stranded DNA molecule. RNA polymerase recognizes the promoter sequence in the DNA template and initiates a transcription. Each of the newly synthesized RNA copies (amplicons) reenters the TMA process and serves as a template for a new round of replication, leading to an exponential expansion of the RNA amplicon. This entire process is autocatalytic and is performed at a single temperature. The RNA amplicons are detected by the hybridization protection assay. An acridinium ester-labeled DNA probe is added and is hybridized to the target amplicons. Separation of hybridized and unhybridized probes is performed by the addition of a selection reagent, and the light emitted is read by a luminometer.

Gen-Probe, Inc., provided swab collection kits and standard urine collection containers for the specimens to be tested by AMP CT. The swab specimens were centrifuged at 300 × *g* for 5 min to bring all fluid to the bottom of the collection tube. Each swab was then treated with specimen preparation reagent and was heated to 60°C for 10 min. A 20-μl portion of treated fluid was further diluted in specimen dilution buffer and tested by AMP CT. Urine specimens were dispensed into 1.5-ml aliquots, warmed to 35°C, and then centrifuged at 9,000 × *g* for 5 min. The pellet was diluted with specimen dilution buffer and tested by AMP CT. A maximum of 48 specimens, in any combination of urine or swab specimens, as well as two controls may be tested at one time. Fifty microliters of each prepared specimen (either swab or urine) was added to a tube containing amplification reagent and oil reagent; the specimen was added below the oil layer. Positive and negative controls were prepared in the same manner. A sealing card was placed over the tubes, and the rack with tubes was placed into a 95°C heat block for 10 min. The tubes were then transferred to a 42°C heat block for 5 min; and 25 μl of enzyme reagent was added to each tube, a sealing card was attached, and the rack with tubes was swirled to mix the contents of the tubes. The tubes were incubated for 1 h at 42°C. At the end of amplification, 20 μl of termination reagent was added to each tube, a new sealing card was attached, and the rack with tubes was swirled to mix the contents of the tubes and incubated for a further 10 min. The rack with tubes was then transferred to the hybridization area. To each tube, 100 μl of probe reagent was added and a sealing card was attached; the contents of the tubes in the rack were then mixed on a multitube vortexer. The tubes were incubated at 60°C in a water bath for 15 min. The tubes were removed, 300 μl of selection reagent was added to each tube, and the tubes were sealed and the contents were swirled mixed as described above. The rack of tubes was then incubated at 60°C for 10 min. The tubes were allowed to cool for 5 min prior to reading of the results on a Gen-Probe LEADER. Any specimen with greater than or equal to 500,000 relative light units (RLUs) was considered positive for *C. trachomatis*, and a reading of less than 40,000 RLUs was considered negative. Any specimen with an RLU value of between 15,000 and 200,000 was retested. The positive control must have greater than or equal to 750,000 RLUs, and the negative control must have less than 20,000 RLUs for the test to be valid. The swab and urine specimens were stored at −70°C after initial testing and until all testing was completed.

Culture procedure. M4 transport medium (MicroTest, Inc., Snellville, Ga.) and collection swabs were provided to each site for culture. The M4 transport medium was vortexed and the swab was removed. A portion of the M4 transport medium was inoculated onto McCoy cell shell vials. The McCoy cell shell vials were centrifuged at 4,000 × *g* for 60 min at 30°C. At the completion of centrifugation the vials were removed and the specimen was aspirated from each vial and replaced with an isolation medium containing cycloheximide. The vials were incubated at 35°C for 48 to 72 h. Each vial was fixed with reagent alcohol for 1 to 10 min and was then stained with fluorescent-antibody stain according to the manufacturer's instructions (Behring Diagnostics, Inc.).

Discrepant analysis. AMP CT-positive, culture-negative specimens were further analyzed by repeat culture, direct fluorescent-antibody (DFA) testing, and/or a supplementary TMA assay which detected a different rRNA target. AMP CT-negative, culture-positive specimens were not further analyzed. Specimens found to be initially positive by culture or positive after discrepant analysis were considered true positives. Repeat culture was performed as previously described in this article. Confirmation by DFA testing was performed with specimens on culture transport medium and urine specimens. The M4 transport medium was centrifuged at 13,000 × *g* for 15 min, the supernatant was decanted, and the pellet was placed on a MicroTrak (Behring Diagnostics, Inc.) slide, air dried, fixed with absolute methanol for 5 min, and then stained by the MicroTrak Direct Specimen Test. The following procedure was used for urine specimens. A 1.5-ml portion of urine was centrifuged at 13,000 × *g* for 5 min, the supernatant was decanted, and the pellet was washed with physiological saline and then centrifuged at 13,000 × *g* for 5 min. The supernatant was discarded and the pellet was resuspended in 10 μl of water. The pellet was placed on a MicroTrak slide, air dried, fixed with absolute methanol for 5 min, and then stained with the MicroTrak Direct Specimen Test. Each slide was examined for the presence of elementary bodies; the observation of one or more elementary bodies constituted a positive test result. Results for AMP CT-positive, DFA assay-negative specimens were further resolved by a supplementary TMA assay which detected a different rRNA target. The assay was performed by Gen-Probe, Inc. A specimen with a false-positive result by AMP CT caused by amplicon contamination will not be positive by the supplementary TMA assay, whereas a specimen with true-positive signal due to *C. trachomatis* rRNA will be positive.

TABLE 1. Comparison of cell culture and AMP CT results for *C. trachomatis* from swab and urine specimens from men and women before and after resolution by discrepant analysis

Time of analysis and sample type	No. of specimens				Total
	Culture of swabs		AMP CT of swabs and urine		
	Positive	Negative	Positive	Negative	
Initial results[a]					
Urine specimens					
Male	18	175	23	170	193
Female	26	581	32	575	607
Swab specimens					
Male	21	188	32	177	209
Female	30	687	39	678	717
After discrepant analysis[b]					
Urine specimens					
Male	26	167	23	170	193
Female	31	576	32	575	607
Swab specimens					
Male	32	177	32	177	209
Female	39	678	39	678	717

[a] Specimens determined to be positive or negative for *C. trachomatis* by culture or AMP CT.
[b] Specimens determined to be positive or negative for *C. trachomatis* by culture, repeat culture, DFA assay, and/or supplementary TMA testing.

RESULTS

A total of 926 swab specimens and 800 urine specimens were tested by AMP CT and 926 swab specimens were tested by tissue culture. Table 1 contains the results of the initial tests for urine and genital swab specimens from men and women. The sensitivity and specificity for urine specimens from men and women before discrepant analysis were 88.5 and 97.5%, respectively. The sensitivity and specificity for urethral swab specimens from men and endocervical swab specimens from women before discrepant analysis were 100 and 97.7%, respectively. These calculations were based on a cell culture sensitivity and specificity of 100% each. Sixteen urine specimens from eight male patients and eight female patients were apparent false positives by AMP CT upon initial testing. However, upon discrepant analysis, 13 urine specimens from eight male patients and five female patients were found to be true positive and urine specimens from three female patients were determined to be false positive by AMP CT. Of these, urine specimens from five male patients and two female patients were DFA assay positive, and urine specimens from three male patients and three female patients were positive by the alternate TMA assay. Eleven urethral swab specimens from men and nine endocervical swab specimens were apparently false positive by AMP CT upon initial testing, but upon discrepant analysis all 20 specimens were determined to be true positive. Of these, four swab specimens from women and two swab specimens from men were positive by the DFA assay and seven swab specimens from men and seven swab specimens from women were positive by the alternate TMA assay.

Five urine specimens from three male patients and two female patients were determined to be false negative by AMP CT. Table 1 also contains the results after discrepant analysis. The sensitivity for urine specimens from men after discrepant analysis was 88.5%, and that for urethral swab specimens was 100%. The specificity for urine and swab specimens from men after discrep-

TABLE 2. Sensitivity, specificity, prevalence, PPV, and NPV for AMP CT before and after resolution by discrepant analysis

Time of analysis and sample type	Sensitivity (%)	Specificity (%)	Prevalence (%)	Predictive value (%)	
				Positive	Negative
Initial results					
Urine specimens					
Male	83.3	95.4	15.3	76.6	99.9
Female	92.3	98.6	5.4	72.8	99.9
Swab specimens					
Male	100.0	94.1	15.3	75.4	100.0
Female	100.0	98.7	5.4	81.5	100.0
After discrepant analysis					
Urine specimens					
Male	88.5	100.0	15.3	100.0	99.9
Female	93.5	99.5	5.4	88.4	99.9
Combined results	91.2	99.6	7.7	95.0	99.9
Swab specimens					
Male	100.0	100.0	15.3	100.0	100.0
Female	100.0	100.0	5.4	100.0	100.0
Combined results	100.0	100.0	7.7	100.0	100.0

ant analysis was 100%. The sensitivity for urine specimens from women after discrepant analysis was 93.5%, and that for endocervical swab specimens was 100%. The specificity for urine specimens from women after discrepant analysis was 99.5%, and that for endocervical swab specimens was 100%. The combined positive predictive value (PPV) and negative predictive value (NPV) for urine specimens from men and women after discrepant analysis were 95.0 and 99.9%, respectively, and were calculated on the basis of an overall prevalence of 7.7%. The PPV and NPV for swab specimens from men and women after discrepant analysis were 100% each and were calculated on the basis of a 7.7% prevalence (Table 2).

DISCUSSION

AMP CT is a sensitive and specific nucleic acid hybridization assay for the detection of *C. trachomatis* in male and female patients (5). The sensitivity for genital swab specimens from men and women after discrepant analysis was 100%, with a specificity of 100% and a PPV and NPV of 100% each. Use of only a urine specimen still gave comparable results, with a sensitivity of 91.2%, a specificity of 99.6%, a PPV of 94.5%, and an NPV of 99.3%. Use of a noninvasive specimen such as urine has its advantages if the patient is reluctant to allow a swab sample to be collected or if the collection venue does not permit invasive genital sampling procedures with a swab.

The assay can be performed in 1 day, thus eliminating the 3- to 4-day turnaround for a culture result. AMP CT does not require specialized equipment such as a thermal cycler. The swab specimens for AMP CT may be held at room temperature (<25°C) for up to 7 days. Urine specimens may be held at room temperature (up to 30°C) for 24 h after collection and during transport or up to 7 days if it is stored at 2 to 8°C. Culture specimens usually must be tested within 3 days and must be held at 4°C prior to being tested and during transport. This also lends more flexibility to the use of AMP CT compared to other amplification procedures, which require more stringent specimen handling.

The sensitivities of cell culture compared to the results after discrepant analysis of AMP CT (true-positive results) for urine specimens from men and women and for urethral from men and endocervical specimens from women were 77.2 and 71.8%, respectively. The sensitivity of cell culture can vary greatly from laboratory to laboratory. Even in experienced laboratories, culture has a reported sensitivity of only 70 to 85% (1, 10, 12). The advantage of culture is the ability to visualize the inclusion body, and confirmation assays are not necessary. The advantages of nonculture methods over culture methods include the need for less stringent transport times and transport temperatures. The time from specimen receipt to result can be reduced by one-half compared to the times for culture. The ability to use a specimen collected by a noninvasive procedure such as urine for the diagnosis of chlamydial infection has the potential to broaden screening efforts to nontraditional venues.

In conclusion, AMP CT performs very well compared to the performance of tissue culture. AMP CT has performance characteristics with urethral and endocervical swab specimens which are equal to or superior to those published for comparisons of EIA and nonamplification nucleic acid probe assays to tissue culture (16). AMP CT has performance characteristics which are comparable to those published for comparisons of tissue culture to other amplification assays such as LCR and PCR (2, 12–14).

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REFERENCES

1. **Black, C.** 1997. Current methods of laboratory diagnosis of *Chlamydia trachomatis* Infections. Clin. Microbiol. Rev. **10:**160–184.
2. **Bulmer, M., G. J. J. van Doornum, S. Ching, P. G. H. Peerbooms, P. K. Plier, D. Ram, and H. H. Lee.** 1996. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by ligase chain reaction-based assays with clinical specimens from various sites: implications for diagnostic testing and screening. J. Clin. Microbiol. **34:**2395–2400.
3. **Centers for Disease Control.** 1985. *Chlamydia trachomatis* infection policy guidelines for prevention and control. Morbid. Mortal. Weekly Rep. **35:**535–574.
4. **Centers for Disease Control and Prevention.** 1993. Recommendations for the prevention and management of *Chlamydia trachomatis* infections. Morbid. Mortal. Weekly Rep. **42**(No. RR-12)**:**1–39.
5. **Crotchfelt, K., B. Pare, C. Gaydos, and T. Quinn.** 1998. 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. J. Clin. Microbiol. **36:**391–394.
6. **Dean, D., D. Ferrero, and M. Mc Carthy.** 1998. Comparison of performance and cost-effectiveness of direct fluorescent-antibody, ligase chain reaction, and PCR assays for verification of chlamydial enzyme immunoassay results for populations with a low to moderate prevalence of *Chlamydia trachomatis* infection. J. Clin. Microbiol. **36:**94–99.
7. **Ferrero, D. V., S. A. Willis, and M. M. McCarthy.** 1996. Verification of Micro Trak II *Chlamydia* enzyme immunoassay reactive and negative grey zone results by PCR, abstr. C41, p. 21. *In* Abstracts of the 96th General Meeting of the American Society for Microbiology 1996. American Society for Microbiology, Washington, D.C.
8. **Hill, C. S.** 1996. Gen-Probe® transcription-mediated amplification: system principles. Gen-Probe, Inc., San Diego, Calif.
9. **Hill, C. S.** 1995. DNA probe assays for the detection of sexually transmitted diseases. Am. Clin. Lab. **10:**8.
10. **Kellogg, J. A.** 1989. Clinical considerations of culture vs. antigen assays for detection of *Chlamydia trachomatis* from genital specimens. Arch. Pathol. Lab. Med. **113:**453–460.
11. **McCarthy, M., and D. MacColloch.** 1993. MicroTrak II *Chlamydia* EIA blocking reagent versus MicroTrak *Chlamydia trachomatis* Direct Specimen Test for verification of *Chlamydia* EIA nonreactive grayzone results, abstr. C-412, p. 519. *In* Abstracts of the 93rd General Meeting of the American Society for Microbiology 1993. American Society for Microbiology, Washington, D.C.
12. **Ostergaard, L., S. Birkelund, and G. Christiansen.** 1990. Use of the poly-

merase chain reaction for detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **28:**1254–1260.
13. **Pasternack, R., P. Vuorinen, A. Kuukankorpi, T. Pitkajarvi, and A. Miettinen.** 1996. Detection of *Chlamydia trachomatis* infection in women by Amplicor PCR: comparison of diagnostic performance with urine and cervical specimens. J. Clin. Microbiol. **34:**995–998.
14. **Quinn, T. C., L. Welsh, A. Lentz, K. Crotchfelt, J. Zenilman, J. Newhall, and C. Gaydos.** 1996. Diagnosis by Amplicor PCR of *Chlamydia trachomatis* infection in urine samples from women and men attending sexually transmitted disease clinics. J. Clin. Microbiol. **34:**1401–1406.
15. **Schachter, J.** 1992. Chlamydial infections, p. 817–822. *In* S. L. Gorbach, J. G. Bartlett, and N. R. Blacklow (ed.), Infectious diseases. The W. B. Saunders Co., Philadelphia, Pa.
16. **Waren, R., B. Dwyer, M. Plackett, K. Pettit, N. Rizvi, and A.-M. Baker.** 1993. Comparative evaluation of detection assays for *Chlamydia trachomatis*. J. Clin. Microbiol. **31:**1663–1666.