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Detection of *Chlamydia trachomatis* in symptomatic and asymptomatic populations with urogenital specimens by AMP CT (Gen-Probe Incorporated) compared to others commercially available amplification assays

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Abstract

The present study was designed to evaluate the sensitivity and specificity of AMP CT (Gen–Probe Incorporated, San Diego, CA, USA) on urogenital specimens taken from symptomatic patients and on first void urine (FVU) specimens from asymptomatic patients. In symptomatic patients, 618 specimens from 140 men (140 urethral swabs and 140 FVU) and 202 women (202 endocervical swabs and 136 FVU) were tested by using cell culture, AMP CT and Amplicor *Chlamydia trachomatis* MWP™ (microwell plate) (Roche Diagnostics, Somerville, NJ, USA) on genital samples, and AMP CT and Amplicor on FVU. A clinical specimen was considered to be truly positive if either the cell culture was positive and/or both AMP CT and Amplicor were positive. In the asymptomatic population, a total of 300 FVU (136 women and 164 men) were tested by four amplification methods, AMP CT, LCx™ (Abbott, Abbott Park, IL, USA), Amplicor MWP, and Cobas Amplicor. A subject was considered to be infected when two or more amplification methods were positive. In the symptomatic population (prevalence 13%), concordant results were observed in 320/342 cases (93.5%). After analysis of discordant results, the sensitivity of AMP CT, Amplicor, and culture was 100%, 95.5%, 68.8%, respectively, and the specificity was 98.3%, 99.3%, 100% respectively. The number of false negative results by AMP CT in urine, probably due to labile inhibitors, was 3/276 (1%). In the asymptomatic population, the results were concordant in 298/300 (99.3%), seven positive and 291 negative. Two results were considered false positives, one by Cobas Amplicor, one by AMP CT. Compared to other amplification methods, AMP CT is at least as sensitive for the identification of chlamydial infection in symptomatic and asymptomatic men and women on genital or urine specimens.

1. Introduction

Chlamydia trachomatis is a leading cause of sexually transmitted diseases and due to the morbidity associated with these infections, laboratory diagnosis of chlamydial infection requires highly sensitive methods (Taylor–Robinson & Robinson, 1998). Detection methods developed for *C. trachomatis* include direct staining of infected tissue, cell culture, antigen detection and nucleic acid hybridization (Black, 1997). Using amplification technology, very low numbers of organisms can be detected (Bianchi et al., 1998) and tested in specimens such as urine for which traditional methods are inadequate.

Gen–Probe Incorporated has developed a transcription mediated amplification (TMA) test based on amplification of rRNA from *C. trachomatis,* the amplified products being detected by hybridization with a DNA probe labeled with an acridinium ester using the hybridization protection assay (HPA) technique. The new amplification procedure is the AMPLIFIED™ *C. trachomatis* assay (AMP CT).

The objective of this study was to evaluate the AMP CT method by comparison with either culture and Amplicor *C. trachomatis* MWP™ (microwell plate) (Roche Diagnostics)

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on urogenital specimens taken from symptomatic patients or Amplicor *C. trachomatis* MWP™ and Cobas Amplicor™ (Roche Diagnostics), and LCx™ (Abbott) on urine samples taken from asymptomatic subjects.

2. Patients and Methods

2.1. Patients population and specimen collection

Patients from two population types, symptomatic and asymptomatic, were enrolled. The first population consisted of 342 symptomatic patients, 202 women and 140 men, seen in the gynecology or sexually transmitted diseases departments of Hôpital Pellegrin in Bordeaux, France. Either one urethral swab from male patients or one endocervical swab from female patients was collected in 2SP medium and tested by using cell culture, AMP CT, and Amplicor *C. trachomatis* MWP tests. After the genital sampling, first catch urine specimens were collected in sterile collection cups and tested by using AMP CT and Amplicor MWP tests. In all, 276 urine samples were examined, 136 from women and 140 from men. Patients were instructed not to urinate in the 2 h preceding specimen collection.

The second population consisted of 300 asymptomatic persons, 136 women and 164 men, attending the center for voluntary and anonymous HIV testing. Clinical background (age, use of condom, STD history) was noted. A first void urine was collected in sterile collection cups and tested by using the four commercialized nucleic acid amplification techniques, AMP CT, LCx, Amplicor MWP, and Cobas Amplicor.

All specimens were transported to the laboratory within 4 h. Once at the laboratory, genital specimens in 2SP and four aliquots of the unprepared primary urine specimens were frozen at −80°C and a part of urine specimens were prepared for the Amplicor MWP test as instructed by the manufacturer before freezing.

2.2 Chlamydia cell culture

The 342 genital swab specimens from symptomatic patients were cultured for 48 h with monolayers of cycloheximide-treated McCoy cells in two shell vials each after 1 h centrifugation at 3000 × *g*. *C. trachomatis* inclusions were detected using a fluorescein-conjugated monoclonal antibody raised against the major outer membrane protein (Syva). Specimens were positive when at least one inclusion was detected on either the initial or blind pass cultures.

2.3. AMP CT assay (Gen-Probe Incorporated)

The AMP CT assay was performed on all specimens according to manufacturer's instructions except the use of 2 SP as transport medium rather than Gen-Probe transport medium. A 100 μl sample of each cervical or urethral swab specimen, collected in 2SP medium was added to 1 ml of transport medium and 50 μl of specimen preparation reagent, both furnished in the kit. The specimens were incubated at 60°C for 10 min, and 20 μl were transferred to the bottom of the polypropylene tube containing 400 μl of specimen dilution buffer. These were the "prepared specimens" to be used for RNA amplification.

Frozen urine samples were thawed and incubated for 10 min at 37°C followed by centrifugation of 1.5 ml of the specimen at 10 000 *g* for 5 min. The supernatant was decanted and the pellet was resuspended in 200 μl of specimen diluent buffer. These were the "prepared specimens" to be used in amplification.

A 50 μl aliquot of each prepared specimen was added to a tube containing 25 μl of reconstituted amplification reagent provided in the kit and 200 μl of silicone oil. The tubes were incubated at 95°C for 10 min and then 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. After addition of 100 μl of probe reagent, the tubes were vortexed and incubated for 15 min at 60°C. After 300 μl of selection reagent was added, the tubes were incubated for 10 min at 60°C and cooled down for 10 min at room temperature. Hybridization results were read with a luminometer (Leader 50, Gen-Probe) and specimens exhibiting more than 50 000 relative light units were considered positive. Positive and negative controls were included in every run.

2.4. Amplicor C. trachomatis *MWP (Roche Diagnostics)*

The Amplicor MWP test was performed on 342 genital specimens collected in 2SP medium and on the pellet of 8 ml of 276 matched urine samples centrifuged at 2500 g for 10 min at room temperature, according to the manufacturer's instructions. This procedure has been described in a previous report (Barbeyrac et al., 1994). The PCR was carried out using a TC 9600 thermocycler (Perkin Elmer Cetus). The amplicons were detected using target-specific DNA probes. The resulting enzyme reaction was measured with a spectrophotometer. Specimens with an A_{450} >0.5 were considered positive, those with an A_{450} <0.25 negative. Specimens with an absorbance value of 0.25 to 0.5 (grey zone) were tested again.

2.5. Amplicor C. trachomatis *Cobas (Roche Diagnostics)*

The Cobas Amplicor test was performed on 300 urine samples from asymptomatic subjects in a combined automatic thermocycler/ELISA apparatus as described by the manufacturer. Wash buffer (0.5 ml) was added to urine sample (0.5 ml) in a reaction tube. The mix was incubated for 15 min at 37°C, followed by centrifugation at 14 000 × *g* for 5 min at room temperature. After discarding the supernatant, the pellet was resuspended with lysis buffer

Table 1
Analysis of discrepant test results by the different assays in the symptomatic population according to specimen source

	No. of specimens presenting the following results by:				
	AMP CT		Amplicor MWP		Culture
Women	cervix	urine	cervix	urine	cervix
False+	3	0	2	0	0
False−	0	2	1	0	6
Men	urethra	urine	urethra	urine	urethra
False+	2	0	0	0	0
False−	0	3	3	2	8

(0.25 ml), vortexed and incubated for 15 min at room temperature. An equal volume of specimen diluent buffer was added, vortexed, centrifuged at 4000 × *g* for 10 min. Fifty microliters of the supernatant were added to 50 μl of amplification mix containing primers for *C. trachomatis*, nucleotides, the internal control and DNA polymerase. The internal control was a sequence of plasmid DNA with primer-binding regions identical to those of the *C. trachomatis* target sequence but a different probe-binding region. The internal control was coamplified with the target DNA from the clinical specimen in each amplification reaction. The amplicons for the internal control and *C. trachomatis* were automatically detected using target-specific DNA probes coated on magnetic particles. The optical density was measured A_{660} using the built-in spectrophotometer.

2.6. *LCx* C. trachomatis *assay (Abbott)*

The LCx test was performed on 300 urine samples of asymptomatic subjects according to the manufacturer's instructions. A pellet from 1 ml of urine sample centrifuged at 15 000 *g* for 15 min was resuspended with 1 ml of urine resuspension buffer and incubated at 95°C for 15 min. After cooling to room temperature, 100 μl of the processed urine was transferred to individual LCx unit-dose tubes containing 100 μl of the amplification mixture. The samples were processed in a thermocycler (PE 480) for 40 cycles of 1 s at 95°C, 1 s at 55°C, and 50 s at 65°C. The controls, calibrators and samples were then centrifuged at 8000 × *g* for 1 s and the tubes were transferred to LCx reaction cells. Amplification products were detected in an LCx analyser.

2.7. *Analysis of results*

For the symptomatic population, patients with a positive culture of a genital swab specimen were considered to have *C. trachomatis* infection. Patients whose genital swab specimens were negative by cell culture, were considered to have a *C. trachomatis* infection if their swab specimens or urine tested positive by both amplification methods. When the results obtained with both amplification methods were discordant, the specimens were tested again using the same procedures. A positive result obtained by only one amplification method was considered as false positive. For the asymptomatic population, a subject was considered to be infected by *C. trachomatis* when two or more amplification methods were positive. If only one amplification method was positive, the result was considered as false positive result.

3. Results

In symptomatic patients, a total of 342 swab specimens (202 women and 140 men) and 276 matched urine specimens (136 women and 140 men) were tested by AMP CT and Amplicor MWP and the 342 swab specimens were tested by tissue culture. Before analysis of discrepancies, the results were concordant in 320/342 patients (93,5%). Among them, the test results were uniformly positive for 30 patients and negative for 290 patients. Out of 22 patients presenting discordant results, 14 were considered trully infected according to our definition (one with a culture positive and 13 with two amplification methods positive) and for eight patients, specimens required retesting. The analysis of discrepant results according to the specimen tested is detailed in Table 1. A second run showed five nonreproducible positive results by AMP CT and two by Amplicor probably due to a contamination during the first analysis process. Three of five urine specimens initially AMP CT negative, and three of four genital specimens and two urine specimens initially Amplicor negative became positive after repeat testing suggesting the presence of polymerase labile inhibitors. After retesting, one out of eight patients was considered trully infected. Forty-five patients had chlamydial infection according to our criteria. The overall prevalence of chlamydial infection in this symptomatic population was 13% (10.8% in women and 16.4% in men).

The performance characteristics of the different assays after analysis of the discordant results are presented in Table 2. In this study, AMP CT is the most sensitive method (100%) compared to Amplicor (95.5%) and culture (68.8%) but its specificity (98.3%) is slightly lower than that of Amplicor (99.3%). The sensitivities of AMP CT and Amplicor MWP assays for swab specimens from women were 100 and 95.4% and from men 100 and 86.9%, respectively.

Table 2
Performance of the three methods after analysis of discrepancies in the symptomatic population

	Infected patients		Sensitivity %	Specificity %	Predictive value %	
	yes	no			positive	negative
AMP CT						
+	45	5	100		90	
−	0	292		98.3		100
Amplicor						
+	43	2	95.5		95.5	
−	2	295		99.3		99.3
Culture						
+	31	0	68.8		100	
−	14	297		100		95.4

The sensitivities of AMP CT and Amplicor MWP assays for urine specimens from women were 91 and 100% and from men, 86.9 and 91.3%, respectively.

In asymptomatic persons attending the centre for HIV detection, a total of 300 urine specimens (136 women and 164 men) were tested by the four commercialized amplification methods. The results were concordant in 298/300 samples (99.3%), with seven positive and 291 negative cases. Two results were discordant, one positive only by AMP CT and one only by Cobas Amplicor. These two results were considered to be false positive according to our criteria. The prevalence in this population was 2.3% and the positive predictive value of Cobas Amplicor and AMP CT was 87.5%. The mean of age of infected subjects was 21.2 years for men and 21.6 years for women whereas the mean age of the total asymptomatic population was 28.2 years for men and 25.7 for women. None of the patients reported using condoms or were HIV seropositive. The presence of inhibitors in urine was detectable by using the internal control (IC) of Cobas Amplicor. The IC detection was negative in 15/300 urine specimens (5%), more often in women (9.5%) than in men (1.2%).

4. Discussion

In this study, the AMP CT test, a TMA assay based on the isothermal amplification of rRNA, was evaluated by comparison with cell culture and the Amplicor PCR test using urogenital specimens and urine specimens from symptomatic patients. This AMP CT assay was also compared with two commercially available amplification assays using urine specimens from asymptomatic patients.

In the symptomatic population, the prevalence of 13% was comparable to the prevalence of 12.3% (9.5% in women and 16.1% in men) found in a similar population at Bordeaux (Barbeyrac et al., 1995) in 1995. All 45 chlamydial infections were detected by the AMP CT assay in urogenital specimens (sensitivity 100%). The AMP CT assay was less sensitive using urine specimens with a sensitivity of 91% in women and 86.9% in men. Repeat testing of the urine specimens with false negative results yielded three additional specimens with positive results and increased the sensitivity of the AMP CT assay on urine specimens to 95.4% for women and 95.6% for men. The reason for the initial false-negative results by AMP CT may have been the presence of inhibitors of RNA polymerase as already described (Pasternack et al., 1997). The reason why two urine specimens remained negative when they were positive by Amplicor, remains unknown and may have been due to the labile nature of the RNA. The diagnostic sensitivity of AMP CT with male or female urine is comparable to that reported in five other studies, with ranges between 84.5% to 93.5% for women and 88.5% to 100% for men (Pasternack et al., 1997; Mouton et al., 1997; Goessens et al., 1997; Crochfelt et al., 1998; Ferrero et al., 1998). In the present study, the differences in the sensitivity of the AMP CT assay with urine and urogenital swab specimens could be a result of the collection methods. In both men and women, urine specimens were collected after swab specimen collection to increase the sensitivity of culture. In women, swab specimens were collected after the removal of secretions and discharge from the cervix as recommended by Black (Black, 1997). Ideally, to achieve maximum assay sensitivity in urine samples, the perineum should not be cleaned before urinating.

In this study, the sensitivity of the AMP CT assay was better than that of culture (68.8%) and the Amplicor MWP assay (95.5%). Furthermore, in one endocervical specimen, Amplicor was initially negative although culture and AMP CT were positive. The AMP CT assay had no false-negative results with endocervical specimens. The specificity of AMP CT was very high (98.3%) and was comparable to others studies (98.8 to 100%). Automation of this technique could improve the specificity by eliminating contamination during the manual process. The method we used to evaluate the AMP CT assay in a symptomatic population was somewhat different from the reported in most other publications. First, as suggested by Hadgu (1997), we did not use an additional method to resolve the discordant results between

culture and the AMP CT assay. Instead, a second amplification method was used on all specimens as a "tie braker". Goessens et al (1997) have proposed that new amplification techniques should be validated against two or more other amplification assays in combination with cell culture. Secondly, the genital swabs were collected in the same 2SP transport medium. The cell culture, Amplicor and AMP CT tests were performed on the same sample eliminating the discordant results due to sample-to-sample variation. Previous studies have used swabs collected in 2SP medium for the Amplicor *C. trachomatis* test (Dubuis et al., 1997). To our knowledge, this is the first time that this transport medium has been used for AMP CT.

In the asymptomatic population, 300 urine specimens were analysed by four amplification methods and all seven chlamydial infections were detected by all methods. The prevalence rate is similar to that of 2.6% found in a similar population in Paris, France (Bianchi et al., 1996). These results show that urine specimens can be used to screen for chlamydial infection using a variety of commercialized amplification techniques. The use of urine specimens for screening raises questions about inhibitors. Recently, Mahony et al. (1998) showed that in women, the nature of amplification inhibitors was different for PCR, LCR, and TMA. Storage and dilution were shown remove most of the inhibitors. In this study, urine specimens were frozen and thawed before amplification. Most results were concordant and the specimens with a negative IC result were negative by all four methods. This suggests that the results were probably true negatives because the probability that the specimens contained a substance inhibiting all amplification methods is very low. Results from two patients were considered to be false positives because they were positive only by one method. The most probable explanation is contamination during processing or samples. The different methods had approximately the same performance in terms of sensitivity and specificity using urine specimens.

AMP CT is well suited for use in a clinical microbiology laboratory. This system is simple, and testing can be completed in 2.5 h. The system is not automated and is more convenient for few tests. In conclusion, AMP CT is a very sensitive method when performed on genital specimens taken from symptomatic patients. AMP CT is concordant with other amplification methods using urine specimens taken from asymptomatic patients. AMP CT can be recommended for detection and screening for *C. trachomatis* infections in men and women.

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