Detection of *Chlamydia trachomatis* in Male and Female Urine Specimens by Using the Amplified *Chlamydia trachomatis* Test

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The amplified *Chlamydia trachomatis* test (AMP-CT; Gen-Probe), a new diagnostic test for the detection of *Chlamydia trachomatis*, was evaluated with urine specimens from 1,000 patients visiting the outpatient department for sexually transmitted diseases at the University Hospital Rotterdam, Rotterdam, The Netherlands, by comparing the results to those of cell culture. From February 1996 to July 1996, urine samples for the AMP-CT test and urethral swabs for cell culture were collected from 544 men, while cervical swabs from 456 women were also taken for cell culture. Positive test results were obtained for 130 (13%) of the patients. AMP-CT test and cell culture results were discordant for 70 (7%) specimens. Analysis of the samples with discordant results was performed by an in-house PCR. After resolution of the discordant results, the sensitivity, specificity, and positive and negative predictive values of the AMP-CT test were 84.3, 98.8, 89.6, and 98%, respectively, for samples from females and 100, 99.2, 93.1, and 100%, respectively, for samples from males, while for cell culture these values were 72.5, 99.2, 92.5, and 98%, respectively, for samples from females and 57.4, 99.0, 86.1, and 95.4%, respectively, for samples from males. We conclude that the AMP-CT test is a fast and reliable test for the detection of *C. trachomatis* in urine specimens from females and, in particular, males.

Infection with *Chlamydia trachomatis* is one of the most important sexually transmitted diseases and has potentially serious sequelae (9). *C. trachomatis* can cause infections of the cervix, urethra, and upper genital tract in women, infections of the urethra and epididymis in men, and conjunctivitis and pneumonia in newborns (23). The most sensitive method for the diagnosis of a genital *C. trachomatis* infection was, until recently, based on culture of the microorganism on HeLa 229 or McCoy cells, which requires extensive laboratory facilities. In addition, circumstances with regard to transport and storage of the sample may influence the reliability of the cell culture result considerably (17). A second major disadvantage is that it takes several days before the test result is known. These disadvantages of the cell culture method have led to the search for alternative techniques for the detection of *C. trachomatis*. Apart from the development of antigen detection techniques, such as direct fluorescent-antibody tests and enzyme immunoassays (3, 11, 21, 22), nucleic acid detection techniques have been developed (7, 16, 18). These nucleic acid amplification tests have generally been more sensitive than traditional tests for the detection of *C. trachomatis*. The disadvantage of all these tests still remained that the majority of assays had to be performed with samples obtained from cervical or urethral smears. A sensitive, reliable, and inexpensive assay for the detection of *C. trachomatis* in specimens that can be easily collected was urgently needed. Two such assays have been marketed recently: the urine LCx assay from Abbott Laboratories based on the ligase chain reaction (LCR) (10) and the AMPLICOR assay from Roche based on PCR (13). These assays are based on the detection and amplification of *C. tra-

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0.1 ml of complete growth medium (Eagle minimal essential medium with Glutam- 
tan [Flow Laboratories Inc., Paisley, Scotland]) containing 10% fetal calf 
serum (Flow), 1% vitamins (Flow), 20 mg of gentamicin per liter, 5 mg of 
ampicillin per liter, and 25 mg of amphotericin B per liter, 25 mg of vancomycin 
per liter, 4.5 g of glucose per liter, and 1 mg of cycloheximide (pH 7.5; Sigma 
Chemical Co., St. Louis, Mo.) per liter. The plates were incubated at 37°C in 5% 
CO2 for 48 h. Thereafter, the specimens were fixed with ethanol (96%) for 10 min 
and stained with a fluorescein monoclonal antibody (Microtrak, Syva Co., Palo Alto, Calif.) 
for C. trachomatis and examined for inclusions. Culture results were scored 
as follows: 0, no inclusions per two wells; 1, 1 to 5 inclusions per two wells; 2, 6 to 
20 inclusions per two wells; and 3, >20 inclusions per two wells.

**AMP-CT test.** The whole AMP-CT test procedure was carried out 
according to the instructions of the manufacturer. The urine samples were incubated for 10 
min at 37°C. After centrifuging the specimens at 8,000 × g for 5 min, the 
supernatant was decanted. The pellet was resuspended in 200 μl of specimen 
diluent buffer, and 25 μl of amplification reagent was added to the propylene 
reaction tubes. After adding 200 μl of oil reagent to the reaction tube, 50 μl of 
processed specimen was pipetted under the oil reagent. The tubes were 
incubated for 10 min at 95°C in a heating block (Diagnostics Grifols S.A., 
Barcelona, Spain). After cooling down in a heating block at 42°C for 5 min, 25 μl of enzyme 
reagent was added. After incubation for 1 h at 42°C in the same heating block, 
20 μl of termination reagent was added and the mixture was incubated for 10 min 
at 42°C in the heating block. After the addition of 100 μl of probe reagent, the 
tubes were briefly vortexed and incubated for 15 min at 60°C in a water bath. 
After adding 300 μl of selection reagent, the tubes were incubated for 10 min 
at 60°C in the water bath and were cooled down for 10 min at room temperature. 
The hybridization protection assay with acridinium-ester-labeled DNA probes 
(2) which are specific for the target organism was used for detection. Positive and 
negative controls were included in every run.

**PCR.** (i) Urine specimens. The urine specimens were thawed at room 
temperature and vortexed, and 1.5 ml was transferred to a clean tube and incubated 
for 15 min at 37°C. The specimens were then centrifuged at 14,000 × g for 30 min 
at 37°C. The supernatant was removed and the pellet was treated with 80 μl of 
lysis buffer (50 mM Tris-HCl [pH 7.5], 1% Triton X-100, 1 mM EDTA, 250 μg 
of proteinase K per ml). After incubation at 37°C for 1 h, the DNA in the lysates 
was extracted and purified with 4 M guanidine isothiocyanate and Cetyl by the 
method of Boom et al. (8). At the final step the DNA was eluted in 100 
μl of 10 mM Tris-HCl (pH 8.0).

(ii) Cervical and urethral swabs. A 400-μl sample of specimen was centrifuged 
at 14,000 × g for 30 min, and the pellet was treated with 40 μl of lysis buffer (50 
mM Tris-HCl [pH 7.5], 1% Triton X-100, 1 mM EDTA, 250 μg of proteinase K 
per ml). After incubation at 37°C for 1 h, the lysates were boiled for 10 min and 
centrifuged briefly. From each lysate, 8 μl was added to 92 μl of the PCR 
mixture.

**Primers and PCR protocol.** PCR was performed in a 100-μl volume 
under standard conditions with 10 μl of DNA sample. The following primer set, 
generating a 517-bp fragment, was used: T1, GGACAAATCTGGATACTCGG; T2, 
GAAACCAATTCTACGCTG. PCR was performed in 100 
μl of PCR solution containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl2, 200 μM 
(each) deoxynucleoside triphosphate, 50 pmol of each primer, 0.1 mg of gelatin 
per ml, 0.2 U of Taq DNA polymerase (Sphaero-ta, Qielen, The Netherlands), 
and 10 μl of sample. The reaction mixture was overlaid with 100 
μl of mineral oil (Sigma) and centrifuged for 5 min at 94°C for DNA 
denaturation. Thirty-five cycles of amplification were performed with a PCR 
processor (Biomed GmbH, Theres, Germany). Each cycle consisted of a dena- 
turation step at 94°C for 1 min, a primer annealing step at 55°C for 1 min, and 
a chain elongation step at 74°C for 2 min. After 35 cycles, a temperature delay 
step of 5 min at 74°C completed the elongation. A 40-μl volume of the amplified 
PCR product was analyzed by agarose (1.5%; wt/vol) gel electrophoresis.

**Southern blot analysis of PCR products.** DNA was transferred from 
agarose to Hybond plus nylon filters (Amersham International, plc, Amersham, United 
Kingdom) by electrophoretic transfer. The PCR products were analyzed with a 
*C. trachomatis*-specific probe (GGCGGCCTCTAGGGCGGCTTATTAGTA 
T).

**Analysis of results.** Analysis of samples with discordant results was performed 
as follows. A result was considered to be true positive when cell culture results 
were comparable to the AMP-CT test results. The results for all samples with 
discrepant results were confirmed by using the in-house PCR. The in-house PCR 
was performed with FVU, US, and CS, if applicable. A cell culture-negative and 
AMP-CT test-positive result was considered to be true positive if the PCR with 
FVU was positive. If the PCR result was negative, the AMP-CT test result was 
considered to be false positive. A cell culture-positive and AMP-CT test-negative 
result was considered to be false negative if the result with PCR FVU was 
positive. If the PCR result with FVU was negative and the US and/or CS 
result was positive, the AMP-CT test was considered to be false negative 
due to sample-to-sample variation. DNA was isolated from cells in the 
incubation of cell culture monolayers, which was followed by PCR if the PCR FVU 
and US (and CS, if applicable) remained negative by PCR. The cell culture result 
was considered true positive if the inoculated monolayer was PCR positive.

The SAS statistical program, version 6.08 (20), was used for analysis of 
the results. The Fisher test was used for statistical evaluation. Statistical signifi- 
cance was accepted at P ≤ 0.05 (two-tailed).

### RESULTS

The results of cell culture compared to those of the AMP-CT test are presented in Table 1. The prevalence of chlamydial infection for the entire group (n = 1,000), as measured by a positive culture result, was 7.7%. For males (n = 544) and females (n = 456) the prevalences were 6.8 and 8.8%, respectively.

In a comparison of the AMP-CT test results with those of cell 
culture, the sensitivity, specificity, and positive and negative 
predictive values were 77.9, 94.2, 46.9, and 98.1%, respectively. 

AMP-CT test and cell culture results were discordant for 70 
(7%) of the samples. Table 2 presents the results of the anal- 
ysis of samples with discordant results. In total, results for eight 
samples were discordant due to sample-to-sample variation. 
More than half of the samples with discordant results (37 
of 70), were considered to be false negative by culture. Of the 
eight samples that were false negative by the AMP-CT test, all 
were from females.

After resolution of discordant results by PCR, the sensitivity, 
specificity, and predictive values were determined for both the 
AMP-CT test and cell culture (Table 3). The sensitivity of the AMP-CT test 
appears to be much higher than that of cell culture (92.4 versus 64.8%). The specificities of both tech- 
niques were quite similar. To determine whether the sensitiv- 
ity of the AMP-CT test appears to be much higher than that of cell 
culture compared to the AMP-CT test results, 12.5% of the samples. Table 2 presents the results of the anal- 
ysis of samples with discordant results. In total, results for eight 
samples were discordant due to sample-to-sample variation. 
More than half of the samples with discordant results (37 
of 70), were considered to be false negative by culture. Of the 
eight samples that were false negative by the AMP-CT test, all 
were from females.

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TABLE 2. Number of patients whose samples had shown discrepant cell culture and AMP-CT test results and interpretation after resolution by PCR analysis

<table>
<thead>
<tr>
<th>Discordance</th>
<th>PCR result</th>
<th>No. of samples</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP-CT test result</td>
<td>Culture result</td>
<td>Urine</td>
<td>CS/US</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
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<td>+</td>
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</table>

* These two samples (both from females) were PCR positive in the culture wells.

DISCUSSION

In this study, we evaluated the value of a new technique, the AMP-CT test, for detecting C. trachomatis in urine samples. One of the remarkable findings in this study was the number of false-negative culture results. More than half of the samples with discordant results had false-negative culture results. Other amplification techniques, such as the commercially available AMPLICOR PCR and the LCx, have provided similar results (4, 5, 10, 13). There are several explanations for this. Theoretically, one inclusion would be enough to show a positive result in a PCR-based test, while culture has been shown to need multiple elementary bodies to be positive. Another argument which is often used is that culture techniques need viable elementary bodies to become positive, while amplification techniques only show the presence of DNA (or RNA). Patients who have been successfully treated with anti-chlamydial agents can remain positive for some time. Since the AMP-CT test is based on RNA amplification, in contrast to the AMPLICOR and the LCx, it could be expected that this issue is of less importance for this technique. RNA is much less stable than DNA in the environment and is degraded quickly by naturally occurring RNases (19). On the other hand, the AMP-CT test is based on amplification of rRNA, making use of the thousands of copies of the sequence to be detected, while only 10 copies per inclusion are available for the DNA amplification techniques. Which of the two foregoing mechanisms is going to prevail remains to be investigated. Alternatively, it has been shown that C. trachomatis can persist in mononuclear phagocytes, which can be responsible for the prolonged positive amplification reaction. It is then the longevity of the phagocytes rather than the persistence of DNA or RNA in situ that determines the duration of positivity.

Therefore, although the amplification techniques are probably more sensitive overall than cell culture, the sensitivity is still not 100%. After discrepancy analysis, eight samples yielded false-negative AMP-CT test results, and these were all from females. One explanation may be that inhibitory substances were present in the sample. Inhibitory substances have been shown to influence PCR-based results considerably (25). This finding warrants further study.

In this study, we chose to analyze the results after analysis of samples with discordant results but not including those samples with discordant results due to sample-to-sample variation (a total of eight samples). This could be debated, since it could be argued that the purpose of testing is to find whether a person is infected, irrespective of the method used. In that case the figures in this study would have provided slightly different results. Seven of the eight sample-to-sample variations occurred with samples from the AMP-CT test-positive and culture-negative group, mostly with samples from males (Table 2). The sensitivity of the AMP-CT test would therefore increase. Among the samples from the AMP-CT test-negative and culture-positive group, results for only one of the samples varied among the tests. The sensitivity for samples from males would be slightly lower if this sample is taken into consideration as a false-negative AMP-CT test result and would decrease from 100 to 98.4%.

The overall prevalence found by cell culture was somewhat lower than that found in earlier studies in our hospital. In a study performed in 1991, we found prevalences of 8.7 and 13.5% for females and males, respectively, compared to prevalences of 8.8 and 6.8%, respectively, in this study. It appears that the lower prevalence is due to a shift in the prevalence in males only. Although this figure increases after resolution of the results for samples with discordant results between the tests, we have no explanation for this. It seems, however, that the prevalence of Chlamydia in the male population is decreasing. This may be explained by the difference in clinical symptoms between males and females. Most male C. trachomatis infections are asymptomatic, while 75% of the female C. trachomatis infections remain asymptomatic (9). Successful prevention strategies and the availability of C. trachomatis diagnostic tools may also contribute to this decline.

Both PCR with the original sample and PCR with material obtained from the culture wells were negative for eight subjects, whose samples were initially culture positive and AMP-CT negative, yielding a specificity of less than 100% for

TABLE 3. Sensitivity, specificity, and positive and negative predictive values for the AMP-CT test and cell culture after resolution of discordant results

<table>
<thead>
<tr>
<th>Group and test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMP-CT</td>
<td>84.3</td>
<td>98.8</td>
<td>89.6</td>
</tr>
<tr>
<td>Cell culture</td>
<td>72.5</td>
<td>99.2</td>
<td>92.5</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMP-CT</td>
<td>100</td>
<td>99.2</td>
<td>93.1</td>
</tr>
<tr>
<td>Cell culture</td>
<td>57.4</td>
<td>99.0</td>
<td>86.1</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMP-CT</td>
<td>92.4</td>
<td>99.0</td>
<td>91.5</td>
</tr>
<tr>
<td>Cell culture</td>
<td>64.8</td>
<td>99.1</td>
<td>89.5</td>
</tr>
</tbody>
</table>
cell culture. There are several explanations for this. One possibility would be that only one *C. trachomatis* elementary body was present in the whole sample. However, in that case the PCR with material from the culture wells should have been positive for at least some of the samples and is further mainly due to PCR with material from the culture wells should have been present in the whole sample. However, in that case the sensitivity would be that only one cell culture. There are several explanations for this. One possibility would be that there is aspecific binding of the *C. trachomatis*-specific monoclonal antibodies or other fluorescent artifacts. We are evaluating the sensitivity and specificity of several other assays, including the PACE-2 assay (Gen-Probe), for the detection of *C. trachoma-

tis*, and although more assays are used and thus the overall sensitivity increases, preliminary results indicate there are still some false-positive culture results, probably on the basis of the last of the two foregoing explanations (data not shown).

We conclude that the AMP-CT test is a fast and reliable test for the detection of *C. trachomatis* in urine specimens from females and, in particular, males.

**REFERENCES**