Detection of Chlamydia trachomatis by ligase chain reaction compared with polymerase chain reaction and cell culture in urogenital specimens

B de Barbeyrac, P Rodriguez, B Dutilh, P Le Roux, C Bébéar

Abstract

Objective—The aim of this study was to evaluate the newly developed ligase chain reaction (LCR) assay for the detection of Chlamydia trachomatis in urogenital specimens using cell culture and Amplicor™ PCR for comparison.

Subjects—Two hundred and eighty patients attending hospital or urban STD clinics (high-risk population, 62 men and 84 women) and obstetric/gynaecology clinics (low-risk population, 134 women) in Bordeaux, France.

Methods—Specimens from men were tested with LCR on urethral swabs and urine, with Amplicor™ on urine, with cell culture on urethral swabs. Specimens from women were tested with LCR, Amplicor™ and cell culture on endocervical swabs and with LCR on urine. When the three methods generated different results, the LCR and Amplicor™ tests were repeated on the remaining samples. Samples with discordant LCR and Amplicor™ results and a negative culture were further analysed by major outer membrane protein gene omp1-PCR.

Results—After analysis of discrepant results, the overall prevalence was 7.5% (21/280) calculated on the basis of an expanded “gold standard” defined as culture positive or LCR plus Amplicor™ positive or omp1-PCR positive for discrepant results between LCR and Amplicor™ tests. Of the 21, 20 were detected by LCR, 17 by Amplicor™ and culture. The specificity of LCR and Amplicor™ was 99.6%.

Conclusion—The LCR Chlamydia trachomatis test is a highly sensitive nonculture technique and a good alternative test for the detection of chlamydial infections.

Despite a sensitivity of less than 100%, cell culture is still considered to be the reference method for chlamydia detection. This technique is time-consuming, labour-intensive and requires trained personnel and optimal conditions of transport and storage of clinical specimens to ensure the chlamydia are alive and infectious. Alternative test methods have been developed in the last decade. However, these non-culture methods, including direct staining using immunofluorescence, enzyme immunoassays and DNA probe techniques, sometimes fail because they lack sensitivity and specificity. In the last five years, DNA amplification by PCR has been proposed for the diagnosis of C trachomatis urogenital infections, and the Amplicor C trachomatis test has been commercially developed by Roche Molecular Systems (RMS, Branchburg, New Jersey, USA) as a rapid and sensitive test. It combines the PCR technique applied to the C trachomatis cryptic plasmid and a colourimetric micowell DNA hybridisation detection method. The ligase chain reaction (LCR) test, developed more recently by Abbott Laboratories (Chicago, Illinois, USA), amplifies a double set of primers specific to the cryptic plasmid of C trachomatis, the amplification being detected by a microparticle-based sandwich immunoassay. The purpose of this study was to compare the LCR test, the Amplicor test, and cell culture for the detection of C trachomatis in urogenital specimens collected from men and women.

Patients and methods

PATIENT POPULATION AND SPECIMEN COLLECTION

From November 1993 to May 1994 218 women and 62 men were enrolled. Patients attended the obstetrics/gynaecology clinics (low-risk population, 134 women) at the Pellegrin Hospital, Bordeaux or were seen in consultation in hospital or urban STD clinics (high-risk population, 62 men and 84 women). Two urethral swabs were collected from male patients, one swab was placed into a transport medium for culture (consisting of sucrose-phosphate buffer (2SP) supplemented with 5% fetal bovine serum), and the other was placed in the Chlamydia LCR transport medium. Next, a first void urine (FVU) (15 to 20 ml) was collected in sterile collection cups and aliquoted into two parts for LCR and Amplicor™. An FVU for LCR and three endocervical swabs were collected from female patients. One swab was placed in 2SP for
culture, one placed in the Chlamydia LCR transport medium, and one into the Ampli
cor™ specimen transport medium. For both men and women, all swabs were ran
domly collected. Urine samples could not be obtained from 121 women, and genital speci
mens were tested alone. No information about previous antibiotic therapy could be ob

tained.

CHLAMYDIA CELL CULTURE
Female endocervical and male urethral swabs, in 2SP transport medium, were stored at 
−70°C until inoculation on to McCoy cell monolayers. Culture was performed on 280 
clinical samples, following a previously described method.16 C trachomatis inclusions were 
detected by using a fluorescein-conju
gated monoclonal antibody raised against the 
major outer membrane protein (Syva Microtrak, Palo Alto, California, USA). Specimens 
were positive when at least one inclusion could be detected on either the ini

tial or blind pass cultures. The number of inclusions per coverslip was counted.

CHLAMYDIA LCR ASSAY
Female endocervical (n = 218), male urethral swabs (n = 62) in LCR transport medium, 
and uncentrifuged FVU (n = 153) from men and women were stored at −70°C until the 
LCR test was performed. The test was 
processed following the manufacturer’s 
instructions. FVU specimens were vortexed 
and 1 ml was centrifuged for 10 minutes at 
13,000 g. After removing the supernatant, the 
pellet was resuspended in 1 ml of the urine 
resuspension buffer. Treated urine samples 
and genital swabs in their transport medium 
were then heated at 95-100°C for 15 minutes. 
After cooling to room temperature, samples 
were tested immediately by transferring 100 µl 
to a C trachomatis LCR unit dose tube contain

ing 100 µl of the LCR reaction mixture. Two 
positive and two negative controls, as well as 
two calibrators, were run with each batch of 
processed samples. The unit dose tubes were 
subjected to 40 cycles in a Perkin Elmer ther

mocycler 480. The amplified products were 
placed manually in reaction cells and revealed 
automatically in a modified Abbott IMx analyser. Results were expressed as counts per 
second per second (c/s/s) with a positive result 
being defined as equal to or greater than the 
product of the mean of the two calibrator val

tues times 0.45.

AMPLICOR™
The Ampli
cor™ C trachomatis PCR test was performed on the 218 endocervical swabs, the 
56 male urine samples and six male urethral 
swabs. Endocervical swabs in Ampli
cor specimen transport medium were stored at 4°C. 
FVU were centrifuged at 2,500 g for 10 min
utes at room temperature, the supernatants 
were discarded and the pellets stored at 
−70°C until the Ampli
cor™ was performed as 
previously described.12 Urine pellets were 
processed following the manufacturer’s 
instructions. Specimens were considered posi

tive when the optical density (OD) values at 
450 nm were greater than 0.5, and negative 
when the OD values were less than 0.25. 
Specimens with OD values between 0.25 and 
0.5 (grey zone) were tested again.

ANALYSIS OF DISCREPANT RESULTS
Discrepant results were analysed by doing the 
different tests a second time from the remain

ing clinical specimens stored at −70°C. The 
specimens were retested by LCR without 
modification of the initial protocol and by 
Ampli
cor™ after ten-fold dilution in Ampli
cor™ 
specimen transport medium for swabs or in 
urine dilution buffer for FVU. Samples with 
discordant Ampli
cor™ and LCR results and a 
negative culture, were submitted to PCR by 
using primers CT1/CT2 directed against the 
onpl gene (outer membrane protein) on the 
2SP culture transport medium.

STATISTICAL ANALYSIS
A clinical specimen was considered to be truly 
positive if either the cell culture was positive or 
both LCR and Ampli
cor™ results were positive 
or ampl-PCR positive when LCR and 
Ampli
cor™ gave discordant results. A clinical 
 specimen was considered to be truly nega

tive when the cell culture and at least one of 
the two other test results were both negative.

Results
A total of 280 patients were tested by using the 
three detection methods, LCR, Ampli
cor™ and 
cell culture. There was a good correlation 
among the three techniques, even without 
 arbitration of discrepant results, since 269 of 
the 280 patients (96%) had totally concordant 
results (table 1). Of these 269 patients, 14 
were positive (six men and eight women) and 
255 were negative by all the techniques used. 
Of the 14 patients with concordant positive 
results, six men had LCR-positive results on 
both urethral and urine specimens, positive-

Ampli
cor™ on urine and positive-culture 
results on urethral swabs. Eight women 
detected as positive for C trachomatis infection 
had positive LCR, Ampli
cor™ and culture on 
endocervical swabs, and four of them had pos

Table 1 Initial results of LCR, Ampli
cor™ and cell culture (no resolution of 
discrepant)

| LCR     | Ampli
cor™ | Cell Culture | Number of patients (case no)* |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervix</td>
<td>Urine</td>
<td>Cervix</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>114</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>−</td>
<td>89</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>1 (case 5)</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>−</td>
<td>1 (case 10)</td>
</tr>
<tr>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>2 (cases 3, 4)</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>grey zone</td>
<td>1 (case 11)</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urethra</td>
<td>Urine</td>
<td>Urethra</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>+</td>
<td>52</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>1 (case 1)</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>+</td>
<td>1 (case 2)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>1 (case 6)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>1 (case 7)</td>
</tr>
</tbody>
</table>

* case no detailed in table 2
ND = not done.
The number of inclusions detected on coverslips in the culture positive specimens varied from between one and more than 500.

Table 2 shows the detailed results obtained for the 11 patients (four men and seven women) presenting discrepant results among the three techniques used or among results obtained on different samples from the same patient. Of these, four were considered to be truly positive according to our criteria, before arbitration of discrepant results (cases 2, 5, 6, 7). Of these four, three were confirmed after a duplicate LCR (case 6) or a duplicate AmplicorTM (cases 2 and 5). In case 3, the positive LCR result was confirmed by the duplicate AmplicorTM result and by omp1-PCR on 2SP culture medium contrary to case 4. This latter case is considered to be false LCR-positive, and the case 8 as false AmplicorTM-positive according to our criteria. In case 9, the duplicate LCR result was positive on the endocervical specimen, but was still negative on the FVU. Interestingly, in case 10, positive results were only obtained on the FVU by LCR while the three techniques gave negative results on the endocervical swabs. The urine data were excluded from the statistical analysis and a woman was considered infected or not based on the results of cervical specimens. In case 11, the first AmplicorTM result gave an OD value in the grey zone (0-409). This specimen was considered to be truly negative after dilution and retesting. False negative culture results were obtained in cases 1, 3, 7 and 9. In order to resolve culture discrepancies, omp1-PCR was performed on the 2SP transport medium. In cases 1 and 3, the positive omp1-PCR results confirmed the results of AmplicorTM and LCR contrary to cases 7 and 9.

After analysis of discrepant results, seven patients were finally considered as true positive (cases 1, 2, 3, 5, 6, 7 and 9), and four as true negative cases (cases 4, 8, 10 and 11). The overall prevalence was 7.5% (21/280) and detailed prevalences in the different populations tested were 2.2% in women with low risk of infection, 12.3% for patients with high-risk of infection (16.1% in men and 9.5% in women). Of these 21 patients, 20 were detected by LCR, 17 by AmplicorTM and culture. When considering only the initial results, LCR, cell culture and AmplicorTM had sensitivities of 95.2%, 80.9%, and 80-90% respectively (table 2). When the duplicate tests were considered, LCR and PCR tests gave equivalent results with a sensitivity of 100% and a specificity of 99.6%.

### Discussion

New diagnostic assays using molecular techniques have been developed, especially to diagnose and control STDs. Because of the better sensitivity of these tests, DNA amplification has been used on organisms that are difficult or impossible to culture.1 PCR and LCR techniques have been applied on C trachomatis and are now (AmplicorTM) or will soon (L) be commercially available. The new LCR test has been evaluated in our laboratory, on genital and urine specimens from both men and women. The LCR results have been compared with those obtained using AmplicorTM and cell culture. The overall LCR sensitivity was 95.2%, and its specificity was 99.6%. Previous data on LCR performed on urogenital specimens13–15 reported sensitivities from 81 to 100%, and specificities from 93-5 to 100% depending on the specimen. The sensitivity and specificity values and the differences between the tests observed in our study, cannot be generalised because there were too few positive specimens. Differences in sensitivity are usually even more pronounced in low-risk than in high-risk population and the overall sensitivity is lower in specimens from males than from females.4

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**Table 2** Arbitration of discrepant C trachomatis results

<table>
<thead>
<tr>
<th>Case no./sex</th>
<th>Initial LCR*</th>
<th>Duplicate LCR*</th>
<th>Initial Amplicor™</th>
<th>Duplicate Amplicor™</th>
<th>Culture (number of inclusions per slip)</th>
<th>omp1-PCR on 2SP medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M+</td>
<td>+/+</td>
<td>ND</td>
<td>–</td>
<td>+</td>
<td>– (10)</td>
<td>ND</td>
</tr>
<tr>
<td>2/M+</td>
<td>+/+</td>
<td>ND</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>3/F-</td>
<td>+/ND</td>
<td>+/ND</td>
<td>–</td>
<td>+</td>
<td>+ (5)</td>
<td>ND</td>
</tr>
<tr>
<td>4/F+</td>
<td>+/ND</td>
<td>–/ND</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>5/F-</td>
<td>+/ND</td>
<td>–/ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>6/F+</td>
<td>–/+</td>
<td>+/+</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>7/M+</td>
<td>–/+</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>8/F+</td>
<td>–/–</td>
<td>–/–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>9/F+</td>
<td>–/+</td>
<td>+/+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>10/F-</td>
<td>–/+</td>
<td>–/–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>11/F-</td>
<td>–/ND</td>
<td>–/ND</td>
<td>–</td>
<td>ND</td>
<td>grey zone</td>
<td>–</td>
</tr>
</tbody>
</table>

*Male urethral swab/FVU or endocervical swab/FVU.
†Considered to be true-positive infected patients.
‡Considered to be true-negative patients.
ND, not done.

---

**Table 3** Comparison of LCR, Amplicor™ and culture according to initial test results after resolution of discrepancies

<table>
<thead>
<tr>
<th>Tests and results</th>
<th>No of resolved specimens</th>
<th>Positive</th>
<th>Negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCR</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>95-2%</td>
<td>99-6%</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>258</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td>1</td>
<td>258</td>
<td>80-9%</td>
<td>99-6%</td>
</tr>
<tr>
<td>Amplicor™</td>
<td>17</td>
<td>1</td>
<td>258</td>
<td>80-9%</td>
<td>99-6%</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>258</td>
<td>0</td>
<td>80-9%</td>
<td>100%</td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td>0</td>
<td>259</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Most of the initial discrepancies of LCR and Amplicor\textsuperscript{TM} tests were resolved by repeating the tests. Six initially false negative results (four Amplicor\textsuperscript{TM} and two LCR tests) were modified by diluting the samples or after freezing at -70°C. It has been shown that the polymerase and ligase enzymes are more or less sensitive to inhibitors that decrease after a few days of storage or dilution of clinical samples.\textsuperscript{12} The problem of inhibitors is of utmost importance in comparison with those of sensitivity. Indeed, a sensitivity evaluation test of LCR and Amplicor\textsuperscript{TM}, performed in our laboratory on a diluted C trachomatis strain, showed that LCR and Amplicor\textsuperscript{TM} were at least as sensitive as culture, detecting one inclusion forming unit. Moreover, no correlation between the results of the molecular techniques and the quantitative results of culture was found. Furthermore, when the duplicate tests were considered, LCR and Amplicor\textsuperscript{TM} gave equivalent results with a sensitivity of 100%. Future improvements in molecular techniques should strive to eliminate polymerase inhibition.

In case 4, a positive LCR result was initially obtained that was negative by duplicating the tests. The specimen was negative by all three techniques. The reason why this first LCR test was positive is unclear. As the evaluation of the C trachomatis LCR technique was performed using the prototype version, samples were manually dispensed into the IMX reaction cells for the final detection. During this step, cross-contamination of the samples was possible and could explain an initial positive and a duplicate negative test result.

Concerning the four false negative culture results, two (cases 7 and 9) may be explained by sampling variability due to the number of samples performed on the same patient. For cases 1 and 3, the omp1-PCR control performed on the culture transport medium was found to be positive. This may have resulted from an infected patient who had been treated, or an infected patient with extremely low numbers of organisms not detectable in culture because of conditions of specimen collection or transport.\textsuperscript{1}

Furthermore, Lin et al\textsuperscript{17} have shown that culture results are highly dependent on procedures used to store specimens (time, temperature) and also the technique used for inoculation (vortexing, sonication, blind passage). In our laboratory, C trachomatis cultures are performed twice a week. Clinical specimens are kept at -70°C until inoculation on to McCoy cells. Frozen storage has been shown to decrease 11% of the positive cultures and 68% of the isolation rate for specimens with less than 50 inclusions forming units per swab.\textsuperscript{17}

Only a small number of techniques have been demonstrated to be suitable for the detection of C trachomatis in urine. Urine culture as well as enzyme immunoassays performed on female FUV is very insensitive.\textsuperscript{14,18} Amplicor\textsuperscript{TM} has proved to be convenient for the detection of C trachomatis in males.\textsuperscript{19,20} In our study, LCR results on male urine samples were almost identical to those obtained with urethral swabs by the same technique (except case 6). With Amplicor\textsuperscript{TM}, a duplicate test was necessary for cases 1 and 2. After resolution of discrepancies, the results obtained on male urethral and urine samples were in complete agreement. In another study,\textsuperscript{21} LCR assay of male FUV was much more sensitive than culture of a urethral swab. Of the 97 cases where LCR was performed on female urines and endocervical swabs, the results were in agreement except for two cases (cases 9,10). These cases may be explained by there being only urethral or cervical C trachomatis localisations. Infections localised only in the urethra have been reported in a range of 5 to 30% of infected women and cervical infections alone in about 30%.\textsuperscript{15} It would be possible to resolve the cases with positive LCR results only from urines by a confirmatory LCR, using probes targeting another sequence on the plasmid or directed to the major outer membrane protein gene, as described by Schachter et al.\textsuperscript{24} In a recent study,\textsuperscript{22} LCR assay performed on urine samples showed a detection rate almost 30% greater than that of endocervical swab culture.

In conclusion, both LCR and PCR tests appear to be promising for the diagnosis of C trachomatis infections in the urogenital tract from men and women. The LCR is a simple, rapid and easy to perform test. However, both LCR and Amplicor\textsuperscript{TM} tests will become truly reliable if they are entirely automated, avoiding any potential contamination of the clinical samples. Similarly, to avoid false-negative results due to inhibitors present in the samples, an internal control monitoring the effectiveness of the reaction (LCR or PCR) will be necessary.


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