Comparison of the ligase chain reaction with cell culture for the diagnosis of *Chlamydia trachomatis* infection in women

G L Ridgway, G Mumtaz, A J Robinson, M Franchini, C Carder, J Burczak, H Lee

Abstract

**Objective**—To determine the sensitivity and specificity of ligase chain reaction (LCR) analysis of cervical and urine specimens from women compared with cell culture of cervical and urethral specimens for the diagnosis of genitourinary chlamydial infection.

**Methods**—Women (n = 624) attending the Genitourinary Medicine Clinic at University College London Hospitals, were enrolled. Patients who had received antibiotics within the previous two weeks were excluded. Specimens were obtained from the urethra and cervix for chlamydial culture, and from the cervix for LCR. A specimen of first void urine was also obtained for LCR. Discrepancies were resolved by direct immunofluorescence or a major outer membrane protein targeted LCR, or both.

**Results**—The prevalence of *Chlamydia trachomatis* in 600 patients, using an expanded standard of a positive cell culture or two confirmed positive non-culture tests, was 13.2% (79/600). Cervical culture detected 68.4% and urethral culture 62% of all positive results compared with 81% detected by cervical LCR and 69% by urine LCR. Cervical and urethral culture combined detected 87.3% whereas cervical and urine LCR combined detected 91.1% of positive cases. Specificity of LCR was 100% in the cervix and 99.8% in urine.

**Conclusion**—This study demonstrates that LCR analysis of cervical and urine specimens is a reliable method for the diagnosis of chlamydial genital infection in women. However, the study also demonstrates that no single test will detect all chlamydial infections. Conventional non-culture tests and cell culture may grossly underestimate the prevalence of chlamydial infection. LCR analysis of a cervical specimen was superior to conventional cell culture without blind passage as a single test for diagnosing chlamydial infection in women, followed by LCR of a urine specimen.


Keywords: *Chlamydia trachomatis*, genital infection in women, ligase chain reaction.

The role of nucleic acid amplification technology in the routine diagnosis of infection is rapidly being established. Techniques include not only the polymerase chain reaction, but also the ligase chain reaction (LCR), self-sustained sequence replication, Ampilprobe, and Qβ replicase. Birkenmeyer and Armstrong reported preliminary studies on the use of LCR for the diagnosis of *Neisseria gonorrhoeae* infection, and Dille et al reported on the detection of type strains of *Chlamydia trachomatis* with LCR, specifically targeting the cryptic plasmid.

One of the many problems in diagnosing genital infections is obtaining the sample. Testing urine for genital pathogens isvery attractive as invasive sampling is not required, but urine is unsuitable for chlamydial culture. The advent of non-culture detection systems for chlamydiae has resulted in a reappraisal of the use of urine, and this policy is finding increasing favour for the investigation of genital infection in men. Urine testing for chlamydial genital infection in women is more controversial, because infection may involve either the cervix or the urethra, or both sites. Initial reports showing that urine can be used in a LCR assay to detect chlamydial infection reliably in women as well as men are encouraging. Urine LCR can also be used to detect gonococcal genital infection reliably in women.

One criticism of the chlamydial studies with LCR on women reported to date is that urine LCR was compared with cervical culture only. Cervical culture underestimates genital tract prevalence, whereas urine LCR potentially favours the detection of single site urethral infection. Here, we report a study comparing LCR analysis of cervical and urine specimens with cell culture of cervical and urethral specimens for the detection of chlamydial infection in women.

**Methods**

Women attending the Genitourinary Medicine Clinic at University College London Hospitals, who had not received antibiotics in the previous two weeks and who agreed to participate in the study, underwent a speculum examination of the vagina. The cervix was wiped with sterile gauze cotton-wool, and an endocervical swab taken and placed in 2SP transport medium for cell culture. A second endocervical swab was placed in LCR transport medium as supplied by the manufacturer. The order of obtaining cervical specimens was determined by a random number table. A third swab was gently introduced into the urethra and placed in 2SP medium as for the cervical specimen. Finally, 15–20 ml of first void urine was obtained. Specimens for culture were either processed the same day having been held at 4°C, or were...
stored at −70°C for up to 48 hours. Urine and cervical swabs for LCR were stored at −70°C until tested. The study was approved by the University College London Hospitals’ Ethics Committee.

Cell culture was carried out as described previously, using cycloheximide treated McCoy cells.11 A blind passage was performed on all samples. If the first pass culture was negative on iodine staining, the second pass was stained with a major outer membrane protein (MOMP) specific immunofluorescent monoclonal antibody (Syva Corp, Palo Alto, California, USA).

LCR was performed according to the manufacturer’s instructions (Abbott Laboratories, Abbott Park, Illinois, USA). Urine samples were thawed, shaken to resuspend any solids, and 1 ml transferred to a microcentrifuge tube. After centrifuging at 13 000 × g for 10 minutes, the supernatant fluid was removed and the deposit resuspended in 1 ml urine resuspension buffer. The sample was then heated at 95°C for 15 minutes. Cervical specimens were heated at 95°C for 15 minutes and then on cooling, the swab was expressed against the side of the transport tube and discarded. A 100 μl aliquot of the prepared specimen (cervical or urine) was added to a unit dose tube. This latter contains all of the ingredients for the amplification step, including thermostable DNA ligase, thermostable DNA polymerase, NAD, magnesium, 2′-deoxycytidine 5′-triphosphate, 2′-deoxythymidine 5′-triphosphate, and an oligonucleotide probe set specific for the cryptic plasmid of C trachomatis.3 Negative, positive and calibrator controls were similarly prepared.

The unit dose tubes were subjected to 40 cycles of separation, annealing and ligation (97°C for one second, 55°C for one second, and 62°C for 50 seconds in each cycle) in a Perkin Elmer Cetus DNA Thermal Cycler. The product of the amplification step was assayed by microparticle enzyme immunoassay using the Abbott LCX analyser. Preparation of samples was carried out in an area separate from the thermocycler and LCX analyser. LCR inactivation reagent is automatically added to specimens after reading to prevent further contamination of subsequent samples.

Discrepancies were resolved by direct immunofluorescence (Microtrak, Syva) analysis of the relevant specimens. Samples found to be positive on plasmid LCR, but negative by cell culture and direct immunofluorescence were tested further using a MOMP based LCR.

A confirmed positive was defined as either positive on cell culture or positive by two of the other tests (plasmid LCR and direct immunofluorescence, or plasmid LCR and MOMP LCR) at any site.

Results
Six hundred and twenty four women were entered into the study. Twenty four patients were excluded from further analysis for the following reasons. Two patients (one positive on urine and cervical LCR, and the other positive on cervical LCR only) had been positive for C trachomatis elsewhere and had been treated recently. One patient had recently received antibiotics for another condition. Seven patients were inadvertently admitted to the study twice (the second episode is excluded).

Eight patients did not have a urine specimen submitted for assay, one patient’s urine assay tube broke, preventing testing, and another did not have a urethral specimen taken. Plasmid LCR specimens from the final 213 patients were analysed by Abbott Laboratories and four of the cervical specimens sent leaked in transit. Thus, complete results from 600 patients were available for analysis.

The prevalence of C trachomatis on cell culture or LCR plus direct immunofluorescence, or both, was 12-7% (76 of 600). A further three cervical specimens positive on plasmid LCR only were confirmed by MOMP LCR, and are considered true positive results, giving an overall prevalence of 13-2% (79 of 600).

The sensitivities for the different tests and sample combinations are shown in table 1. Thus, culture of cervical and urethral specimens combined detected 87-3% of all confirmed positive cases. However, it must be stressed that few laboratories perform culture with passage as a routine method to identify genital C trachomatis, let alone take both a cervical and urethral specimen. The effect of only recording the results of first pass cell culture is also demonstrated in table 1. Whilst the results of LCR are unchanged, first pass culture of cervical and urethral specimens now detected only 72-2% of all positive cases. Were only a single passage cervical culture to have been carried out, only 55-7% of all positive cases would have been detected. LCR analysis of cervical or urine specimens would have detected 81% and 69-6% of all confirmed positive cases, respectively. When the results of LCR analysis of urine and cervical specimens were combined, 91-1% of all confirmed positive cases were identified.

Table 2 compares the LCR results with the confirmed positive results. As expected, the specificity of the LCR test is very high. There were 25 confirmed positive results where the LCR analysis of cervical and urine specimens was discordant. The urine LCR was negative in 19 cases. In six of these a high negative was reported, which became a low positive on retesting three. In one case, where the cervical LCR was positive and all other tests negative, enzyme immunoassay and direct immunofluorescence on a cervical specimen obtained

<table>
<thead>
<tr>
<th>Cell culture with blind passage</th>
<th>Cell culture without blind passage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases detected (total = 79)</td>
<td>Percentage of total positives</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Cervical culture</td>
<td>54</td>
</tr>
<tr>
<td>Urethral culture</td>
<td>49</td>
</tr>
<tr>
<td>Cervical and urethral culture</td>
<td>69</td>
</tr>
<tr>
<td>Cervical LCR</td>
<td>64</td>
</tr>
<tr>
<td>Urine LCR</td>
<td>55</td>
</tr>
<tr>
<td>Cervical and urine LCR</td>
<td>72</td>
</tr>
</tbody>
</table>

* Includes three specimens confirmed by MOMP LCR.
Table 2 Comparison of cervical and urine LCR results with confirmed C trachomatis positive results

<table>
<thead>
<tr>
<th></th>
<th>Cervical LCR</th>
<th>Urine LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Confirmed positive for Chlamydia*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>64</td>
<td>15</td>
</tr>
<tr>
<td>negative</td>
<td>0</td>
<td>521</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>81-0%</td>
<td>69-6%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>98-8%</td>
</tr>
<tr>
<td>Predictive value of positive</td>
<td>97-2%</td>
<td>95-6%</td>
</tr>
</tbody>
</table>

* Extended gold standard, defined as culture positive, or LCR plus direct immunofluorescence positive, or LCR confirmed by MOMP LCR at any site.

Discussion

This study set out to answer two important questions. Firstly, to determine the overall sensitivity of LCR analysis of urine or cervical specimens when compared with a cell culture system that included a single blind passage, backed up by a direct monoclonal antibody immunofluorescence test; and secondly, to compare how LCR tests on urine and cervical specimens compared with a routine cell culture diagnostic system without passage. We cultured specimens from the urethra and cervix in all cases to ensure maximum yield of positive isolates.

It is well recognised that cell culture is far from being the gold standard for chlamydial diagnosis, and that in women investigation of specimens from only one site will underestimate the prevalence of chlamydial genital infection. The use of urine samples from women has not been considered feasible previously because of the predominately cervical site of chlamydial infection. Hay et al recently demonstrated that direct immunofluorescence analysis of the centrifuged deposit of a first pass urine from women was 80% sensitive compared with a cervical smear sensitivity of 70% for diagnosing chlamydial genital infection, using the combined results of centrifuged urine deposit, urethral smear, cervical smear and centrifuged cervical smear deposit as the standard. Chernesky et al studied 447 women, and found the sensitivity of LCR on first void urine to be 96-3%, compared with 55-6% for cervical culture (with single blind passage) and 78-3% for cervical enzyme immunoassay (confirmed Chlamydialzyme test (Abbott Laboratories)). A four centre study extended these findings, analysing cervical specimens and first void urine samples from 1937 women by culture and LCR assay, respectively. Urine LCR was 93-8% sensitive compared with a cell culture sensitivity of 65-0%. Bassiri et al compared urine LCR with cervical cell culture and enzyme immunoassay of urine (IDEA III, Novonordisk Diagnostics, Berkshire, UK). They studied 447 women, and found sensitivities of 56-3% on cell culture, 18-8% on urine enzyme immunoassay and 87-5% on urine LCR. Thus, all three studies have found that urine LCR was much superior to cervical cell culture. For urine LCR specificity ranged between 99-9 and 100%. However, these studies did not investigate culture specimens from the urethra, nor was LCR carried out on cervical specimens. Cervical culture would therefore underestimate total genitourinary tract infection, and may favourably bias the urinary LCR results. Comparison of urethral and cervical culture with urine or cervical LCR has demonstrated that LCR analysis of urine or cervical specimens is more sensitive than a single cervical culture without passage, such as would be carried out routinely in a centre using cell culture for diagnosis.

Of concern is that the quoted sensitivity of commercially available enzyme immunoassays is within the range 70-90% compared with cell culture (often without blind passage). The sensitivity of cervical cell culture in this and the other reviewed studies was between 55-6
and 68.4%. It is therefore apparent that current routine procedures in the field are liable to be grossly underestimating the prevalence of chlamydial genital infection in women.

We have demonstrated that LCR is consistently more reliable than cervical or urethral cell culture alone, either with or without blind passage. Analysis of urine or cervical specimens by LCR assay was of equivalent sensitivity to combined cervical and urethral cell culture without passage. Indeed, for a cervical culture without passage LCR was superior (72.2% and 81.0%, respectively). The most reliable tests in this study were either a combined cervical and urethral multiple passage cell culture, or a combined LCR analysis of cervical and urine samples. Neither of these latter options is feasible in the routine diagnostic setting, and it should be noted that 12.7% and 8.9%, respectively, of confirmed positive results were missed by the combined tests.

We conclude that detection of genital C. trachomatis infection in women by use of the LCR test on cervical or urine specimens is a convenient and more reliable substitute for cell culture in a diagnostic setting.

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