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Detection of *Chlamydia trachomatis* in Urine Samples by Nucleic Acid Tests: Comparison with Culture and Enzyme Immunoassay of Genital Swab Specimens

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Two commercially available nucleic acid-based tests, ligase chain reaction (LCR; Abbott Laboratories) and PCR (Roche Diagnostics), for the detection of *Chlamydia trachomatis* in male and female urine samples were compared with culture and enzyme immunoassay (EIA) (Microtrak; Syva) for *C. trachomatis* detection in genital samples. The samples were collected from 1,005 patients who attended a sexually transmitted disease clinic. In this study population, the prevalence of the infection was 4%. Specimens which were reactive in any of the tests were retested with a different PCR test using primers directed against the major outer membrane protein gene. With a "gold standard" of a positive culture, or any other positive test result if it was confirmed by an independent test, the Roche PCR (95% sensitive, 99.9% specific) was more sensitive than the LCR (75% sensitive, 100% specific) (χ^2, $P < 0.0001$) while both tests were more sensitive than culture (58% sensitive, 100% specific) or EIA (45% sensitive, 100% specific) (χ^2, $P < 0.001$). The Roche PCR and Abbott LCR tests of urine identified 65% and 30% more positive patients, respectively, than did testing by culture of urethral or cervical specimens. Nucleic acid testing of urine specimens for *C. trachomatis* is a more sensitive and convenient method for the detection of genital infection.

Chlamydia trachomatis is one of the more common sexually transmitted pathogens in human genital infections. In Australia, the prevalence ranges from 2.5 to 14%, with the highest rate among patients attending sexually transmitted disease clinics (6). An important feature of genital chlamydial infection is the high rate of asymptomatic infections in men (15) and women (21). An efficient screening test with a convenient specimen would facilitate patient and public health management.

Traditional laboratory diagnosis of this infection is done by cell culture of female cervical or male urethral swabs. The availability of monoclonal antibody technology led to the development of commercial immunoassays for direct detection of *C. trachomatis* in clinical samples either by immunofluorescence or enzyme immunoassay (EIA). To use a more convenient sample, urine specimens have been evaluated by a number of laboratories for rapid detection of this organism by culture and EIA with various levels of success (8, 15).

More recently, the use of nucleic acid amplification tests has enabled the detection of low copy numbers (<10 pg) of specific nucleic acids in a wide variety of genital specimens, including urine, and with claims of a sensitivity greater than that of immunoassays for detecting chlamydia lipopolysaccharide or outer membrane proteins (14, 22). However, the use of clinical samples may be associated with inhibition in nucleic acid amplification tests (1, 10, 11, 14, 19, 23) and may lead to variable sensitivity. This study compared the use of two commercially available nucleic acid amplification tests—Amplicor PCR (Roche Diagnostics) and ligase chain reaction (LCR; Abbott Laboratories) in urine samples—with culture and EIA in cervical or male urethral specimens for the detection of *C. trachomatis.*

Specimens were obtained from 1,005 consenting patients (418 females and 587 males) who attended Clinic 275, the principal sexually transmitted disease clinic in Adelaide, South Australia, from October to December 1996. Three separate cervical or urethral swabs, followed by a urine sample, were collected from each patient. The first swab collected was used to make a Gram-stained smear and then cultured for gonorrhoea, the second was used for *C. trachomatis* detection by EIA, and the third was used for *C. trachomatis* culture. Swabs for *C. trachomatis* culture were transported in liquid nitrogen and inoculated into cell cultures on the same day. The cell culture method used was essentially that described by Kuo et al. (8, 9). The cervical and urethral specimens were inoculated onto DEAE-dextran-treated Buffalo green monkey epithelial cells in a 96-well microwell plate and centrifuged at 1,000 × *g* (1 h at 35°C), and the medium was replaced before incubation at 37°C for 48 h. After washing with phosphate-buffered saline and fixation with methanol, the inoculated cell monolayers were stained with fluorescein isothiocyanate-*C. trachomatis* monoclonal antibody (Kallestad) for 30 min. The unbound antibodies were removed by washing with phosphate-buffered saline for 10 min, and 25 μl of 90% glycerol in Tris buffer (pH 8.6) was then added to each well. The microwell cell cultures were examined for specific staining by using an inverted microscope equipped with a UV light source. The presence of one or more infected cells (showing intensely fluorescent cytoplasmic inclusion bodies) per well was considered *C. trachomatis* culture positivity. The EIA used (Microtrak) was from Syva and detects chlamydial lipopolysaccharide. The specimens were heated (100°C for 15 min) in the Syva EIA diluent buffer and tested in accordance with the manufacturer's instructions. All EIA-positive results were confirmed with a blocking antibody provided by the manufacturer.

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TABLE 1. Numbers of male and female patients positive for *C. trachomatis* in each test, grouped by PCR or LCR result

Patients	No. positive by:					
	Any test	Urine PCR	Urine LCR	MOMP PCR	Swab culture	Swab EIA[a]
Group 1	2	0	0	0	2	0
Group 2	9	9	0	8	4	3
Group 3	30	30	30	27	17	12
Total positive	41	39	30	35	23	15
Total tested	1,005	1,005	1,005	41	1,005	835[b]

[a] Both group 1, 7 group 2, and 25 group 3 patients were tested by EIA.
[b] 170 patients were not tested by EIA.

Urine specimens were processed and the Abbott LCR and Roche PCR tests were performed in accordance with the manufacturers' instructions. Both the Abbott LCR and Roche PCR tests detect the chlamydial cryptic plasmid nucleic acid sequences. The LCR uses two pairs of labelled primers, in contrast to PCR tests, which use one pair of primers. All urine specimens tested with the LCR were stored at −70°C, thawed in a 37°C dry incubator, briefly vortexed, and tested in accordance with the manufacturer's instructions. Specimens for the PCR assay were stored at 4°C until tested (within 24 to 96 h) in accordance with the manufacturer's instructions.

Specimens which tested positive in any assay were confirmed by an in-house major outer membrane protein (MOMP) gene PCR test (5, 11). The MOMP gene is present as only a single copy within the organism, compared to the 10 copies of the cryptic plasmid (16). The amplified MOMP gene product (129 bp) was detected with a commercially available enzyme-linked immunosorbent assay system (Boehringer Mannheim) in microwells coated with streptavidin. The MOMP PCR incorporated digoxigenin-11-dUTP (Boehringer Mannheim) into the amplified product. The latter was then denatured and hybridized to a biotinylated probe within the MOMP gene (5). The hybrid was then detected in streptavidin-coated microwells by using peroxidase-conjugated antibodies to digoxigenin. A specimen was considered to be a true positive if it was either culture positive or positive in any two independent tests, e.g., Roche PCR plus LCR, Roche PCR plus EIA, or Roche PCR plus MOMP PCR.

To determine the detection sensitivities, in terms of specific nucleic acid copies, of the Abbott LCR, Roche PCR, and MOMP PCR tests, purified *C. trachomatis* nucleic acids were prepared from a stock culture of the K serovar strain. The K serovar strain of *C. trachomatis* was grown in Buffalo green monkey epithelial cells and purified as described by Caldwell et al. (2). Chlamydial nucleic acids were prepared from the purified organisms by two extractions with phenol-chloroform (18) and then resuspended in TE buffer (1 mM Tris, 10 mM EDTA [pH 8.0]). The endpoint titer obtained by the Abbott LCR and Roche PCR tests was 10^7, but that obtained by the MOMP PCR was 10^5, corresponding to 1 and 40 copies, respectively, of specific nucleic acids.

Table 1 summarizes the results of the study, grouping the positive patients by their LCR and PCR results. Two patients were positive by swab culture, but both were negative by PCR (including repeat testing at 1:2 and 1:10 dilutions), LCR, MOMP PCR, and EIA (group 1). In group 2, nine patients were PCR positive but LCR negative. Of these nine, one patient was negative by culture, EIA, and MOMP PCR and the PCR result was classified as a false positive. Of the remaining eight patients in this group, all were MOMP PCR positive while four of these were culture positive. Six of these eight patients were tested by EIA and three of them were EIA and culture positive. In group 3, 30 patients were PCR and LCR positive. In this group three patients were MOMP PCR negative and culture negative but one of the three was EIA positive. Of the 27 PCR-, LCR-, and MOMP PCR-positive patients, 10 were culture and EIA positive, 7 were culture positive only (4 EIA negative and 3 not tested by EIA), and 10 were culture negative (1 EIA positive, 7 EIA negative, and 2 not tested by EIA. No patient was LCR positive but Roche PCR negative. The lower sensitivity of the MOMP PCR is consistent with previous reports of the use of the MOMP PCR to test genital specimens (7, 12, 13) and with the in vitro sensitivity data described above.

Overall, samples from 41 patients were positive by at least one test, and only one of these was a Roche PCR-positive sample that was neither culture positive nor confirmed as positive by an independent test. The Roche PCR detected 65% ([38 − 23]/23) and the Abbott LCR detected 30% ([30 − 23]/23) more positive patients than did culture. In the cohort of patients also tested by EIA, the Roche PCR detected 11 and the Abbott LCR detected 5 more positives than did culture. The prevalence of *C. trachomatis* infection was 4.0% (40 of 1,005 patients) in the entire population, 3.8% (16 of 418) in females, and 4.1% (24 of 587) in male patients.

According to the resolved results, the urine PCR test showed a sensitivity of 95% (38 of 40), compared with 75% (30 of 40) for urine LCR, 58% (23 of 40) for swab culture (χ^2, $P < 0.0001$), and 45% (15 of 33) for EIA (χ^2; $P < 0.0001$). There were no significant differences in sensitivity between males and females in any of the test modalities. For PCR, LCR, culture, and EIA, respectively, the specificities were 99.9, 100, 100, and 100%; the positive predictive values were 97.4, 100, 100, and 100%; and the negative predictive values were 99.8, 99.0, 98, and 98%.

To determine the prevalence of nucleic acid amplification inhibitors in urine, urine samples from 50 patients who were negative by both the Roche PCR and LCR tests were seeded with *C. trachomatis* (K serovar) at a level 100 times greater than the detection endpoint of the PCR and LCR tests determined previously. In these seeded samples, the prevalence of inhibition was 16% (8 of 50) in the Roche PCR and 26% (13 of 50) in the Abbott LCR test but did not reach statistical significance (χ^2; $P = 0.2$).

In this study, the Abbott LCR and Roche PCR tests of urine samples identified significantly more positive patients (65 and 30%, respectively) than did culture of cervical and urethral samples. Although the LCR and Roche PCR tests of purified *C. trachomatis* nucleic acid yielded similar detection endpoint titers, the Roche PCR test showed higher sensitivity (95%) than LCR (75%). Consistent with a trend toward more frequent detection of urinary inhibitors of LCR than of PCR, the LCR repeatedly failed to detect eight patients who were both Roche PCR and MOMP PCR positive, four of whom were also swab culture positive. It is known (13, 19) that the activity of the DNA polymerase—used in both the PCR and LCR tests—may be inhibited by a variety of factors, in particular, when using clinical samples. However, it is possible that the inhibitors are different for LCR and PCR. There was insufficient urine volume to allow testing of these eight PCR-positive, LCR-negative specimens for inhibition.

While many studies have examined the sensitivity of LCR or PCR testing of urine specimens, we were able to find only four reports of direct comparisons of the LCR and Roche PCR tests in the literature. In three studies, there were no statistically significant differences between PCR and LCR analyses of

urine. In the study by de-Barbeyrac et al. (4), LCR detected 10 (16%) positive samples while PCR detected 8 (13%) positive samples among first-void urine samples of 62 men attending a sexually transmitted disease clinic (χ^2; $P = 0.6$). However, both PCR-negative, LCR-positive patients were PCR positive on retesting. Similarly, in a study by Pasternack et al. (17) of 442 women attending a sexually transmitted disease clinic, 50 of 50 (100%) urine samples were positive by the Roche PCR and 47 of 50 (94%) were positive by the Abbott LCR test (χ^2; $P = 0.2$), but only the Roche PCR detected significantly more positives than did cervical culture (44 of 50; 88%) (χ^2; $P < 0.03$). In the study by Chernesky et al. (3), the sensitivities of the Roche PCR and Abbott LCR tests were 100 and 94.3%, respectively, with urine samples from 287 male patients with urethritis. In contrast, Stary et al. (20), examining first-void urine samples from 705 asymptomatic military recruits, found the Abbott LCR test to have a sensitivity of 93% (27 of 29 positives) and to be significantly more sensitive than the Roche PCR (18 of 29 positives; 62%) (χ^2; $P < 0.005$). The reason for the differing results is not clear. Both earlier studies (3, 20) found that after freezing of the urine samples, the PCR detected additional positive samples. However, in our study, the urine samples were tested by PCR prior to freezing at −70°C until tested by LCR, yet the PCR test still performed better than the LCR.

The Roche PCR and Abbott LCR assays are similar in ease of use for *C. trachomatis* screening in a diagnostic laboratory. As these assays are highly sensitive, the potential for false positives due to inadvertent contamination exists. In the Roche PCR assay, this is minimized by the use of uracil-*N*-glycosylase, which degrades previously amplified DNA (containing dUTP) that may have contaminated the new sample as a result of carryover. The Abbott LCR assay uses a chelating metal complex and an oxidizing agent to inactivate amplicons after the detection step. However, separation of preamplification and postamplification areas with unidirectional work flow procedures needs to be done to minimize contamination. Both assays do not require viable organisms, and the collection tubes can be transported at ambient temperature. The LCx system (LCR test) is automated, apart from specimen preparation. Specimen preparation requires heating at 97°C and centrifugation at 13,000 to 18,000 × *g*. The LCx system can process 24 samples at a time; however, six controls are necessary, viz., two positive, two negative, and two for calibration, with a maximum of 18 specimens to be tested. The Roche PCR Amplicor MWP is not automated. (A semiautomated version—Cobas—of this test has since been introduced by Roche Diagnostics.) Specimen preparation requires centrifugation at 1,500 × *g* for 10 min at room temperature, and as it uses a microwell format, 92 specimens can be processed. The assay requires four controls, viz., two negatives and two positives.

In summary, in this study of a population with a low prevalence of chlamydial infection (4%), both the Roche PCR and Abbott LCR tests performed better than culture or EIA and the Roche PCR test was more sensitive than the Abbott LCR (95 versus 75%) when urine samples were tested. The use of nucleic acid tests on urine represents a more sensitive and convenient way to detect genital infection with *C. trachomatis.*

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