

## Ability of Commercial Ligase Chain Reaction and PCR Assays To Diagnose *Chlamydia trachomatis* Infections in Men by Testing First-Void Urine

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**A total of 287 men (37.6% with symptoms of urethritis) attending a hospital-based sexually transmitted disease clinic had urethral swabs tested by culture and by direct fluorescent-antibody assay. First-void urine (FVU) was tested for *Chlamydia trachomatis* by commercially available ligase chain reaction (LCR) and PCR assays. By using an expanded reference standard, 35 men (12.2%) were found to be positive. By performing LCR and PCR, the infection prevalence was found to be approximately twice (11.5 and 12.2%, respectively) that determined by swab testing. The sensitivity values were 94.3% for LCR and 100% for PCR. One of the two positive specimens missed by LCR contained inhibitors. PCR produced five false-positive results and LCR produced one.**

Although antigen detection assays have been effective for diagnosing men who are infected with *Chlamydia trachomatis* by testing concentrated first-void urine (FVU) (3, 8, 10, 11, 14, 15), limitations in sensitivity have restricted the identification of infected individuals with few or no symptoms of urethritis. Both PCR (6, 9, 12, 13, 16) and ligase chain reaction (LCR) (4, 5) have been shown to be sensitive for identifying infected men by the testing of FVU.

This study compared LCR and PCR detection of *C. trachomatis* by using commercially available kits to test FVU from 287 men attending a sexually transmitted disease clinic. Assay performance was compared to culture and direct fluorescent-antibody (DFA) staining of a urethral swab collected at the same clinic visit. An expanded reference standard (9) was used as described below. The study was performed from September 1993 to February 1995 during which time 287 men were enrolled with consents which were approved by the McMaster University Research Ethics Committee Hospital Network. The men were attending a hospital-based sexually transmitted disease clinic and each had an FVU sample (20 ml) collected in a sterile 30-ml screw-cap plastic jar. A urethral swab specimen was collected by inserting a narrow-shafted cotton-tipped swab 2 to 3 cm into the urethra. The order of collection of swabs and FVU was reversed halfway through the study. The swabs were placed into transport tubes containing minimal essential medium with 5% sorbitol, 3% fetal bovine serum, and 2 mM L-glutamine at 4°C. The swabs were transported to the laboratory and set up in cell cultures within 24 h. FVU specimens were held at 4°C and tested by LCR or PCR within 3 days.

Urethral swabs from the men were inoculated onto McCoy cell monolayers in 96-well microculture plates, with a blind passage and iodine staining (2). Swabs were processed and tested by DFA. The urethral swabs were smeared according to the Microtrak DFA (Syva) package insert. A sample was con-

sidered positive if at least two elementary bodies were seen in the smear.

The FVU specimens were briefly vortexed before being processed into the respective assay reagents. For LCR, 1 ml was transferred to a 1.5-ml microcentrifuge tube and centrifuged at  $16,000 \times g$  (Eppendorf centrifuge model 5415C; Brinkman Instruments, Westbury, N.Y.) for 10 min at room temperature. After removal of the supernatant fluid, the pellet was resuspended in 1 ml of urine resuspension buffer provided with the LCx LCR kit (Abbott Laboratories, Abbott Park, Ill.) and heated to 95 to 100°C for 15 min. After being cooled to room temperature, processed urine samples were either tested immediately by LCR or stored at -20°C for up to 60 days and then tested. For PCR testing of FVU the starting volume was 8 ml. The FVU samples were warmed to 37°C for up to 30 min to dissolve precipitated material and then were centrifuged at  $1,500 \times g$  for 10 min. The supernatant fluid was discarded, and the pellet was resuspended in 2 ml of PCR-urine resuspension buffer and allowed to incubate for 1 h at ambient temperature. An additional 2 ml of urine diluent was added, and the mixture was incubated for 10 min. Processed specimens were stored for up to 3 days at 4°C if they were not tested immediately.

For LCR testing we followed the manufacturer's instructions as described previously (4). The package insert did not recommend retesting specimens in a gray zone. Amplicor Chlamydia (Roche Molecular Systems, Branchburg, N.J.) PCR was performed according to the manufacturer's instructions and has been described in previous reports (6, 9, 17). Specimens with absorbances of 0.5 or above were considered positive, and those with absorbances below 0.2 were considered negative. As recommended by the package insert, specimens with absorbances between 0.2 and 0.5 were retested in duplicate. If two of the three test results were below the adjusted cutoff of 0.25, the specimen was regarded as negative. If two of three were above 0.25, it was considered positive. The overall hands-on time for LCx kit testing of a batch of 20 samples was 60 to 90 min; the corresponding time for Amplicor Chlamydia PCR was 90 to 120 min. The total time to results for a batch of 20 samples was 5 h for LCx kit testing and 6 h for Amplicor Chlamydia PCR.

To prevent amplicon contamination in LCR or PCR mix-

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TABLE 1. Prevalence, sensitivity, and specificity of laboratory assays for *C. trachomatis*<sup>a</sup>

| Specimen      | Test             | % Prevalence  | % Sensitivity <sup>b</sup> | % Specificity  |
|---------------|------------------|---------------|----------------------------|----------------|
| Urethral swab | Culture          | 4.5 (13/287)  | 37.1 (13/35)               | 100 (252/252)  |
|               | DFA              | 5.2 (15/287)  | 42.9 (15/35)               | 100 (252/252)  |
| FVU           | LCR <sup>c</sup> | 11.5 (33/287) | 94.3 (33/35)               | 99.6 (251/252) |
|               | PCR <sup>d</sup> | 12.2 (35/287) | 100 (35/35)                | 98.0 (247/252) |

<sup>a</sup> Values in parentheses are the numbers of samples with positive test results divided by the numbers of samples tested. In all, samples were collected from 287 men.

<sup>b</sup> Calculations are based on 35 men determined by culture or confirmed non-culture assay to be infected with *C. trachomatis*.

<sup>c</sup> LCx kit (Abbott Laboratories).

<sup>d</sup> Amplicor Chlamydia test (Roche Molecular Systems).

tures, all work was segregated between two different areas: the sample preparation area, where sample processing and addition took place, and the thermocycling and detection area, where thermocycling and then detection of amplified product took place. Precautions were taken to prevent the transfer of material between the two areas. After detection by LCR, amplified product was destroyed by a nucleic acid inactivation reagent (copper-phenanthroline complex), which was automatically added to all specimens to destroy amplicons and thereby eliminate carryover contamination. For PCR samples, uracil-*N*-glycosylase, which catalyzed the cleavage of dUTP-containing DNA, rendering amplicons sterile and thereby preventing contamination from previous amplifications, was added (1).

Supplemental testing was used to resolve discordant results (12). All discordant and some concordant specimens were inserted, without identifiers, into a supplemental testing protocol. FVU specimens found to be negative by culture or DFA and positive by LCR or PCR were investigated further by a second LCR or PCR directed against the gene which codes for the major outer membrane protein (MOMP) of *C. trachomatis* at dilutions of 1:4 and 1:8. Abbott Laboratories performed MOMP gene LCR testing of discordant panels under code (4); a MOMP gene PCR was performed blinded in our own regional research laboratory (12).

Sensitivity, specificity, and predictive values were calculated by standard techniques (7). An expanded gold standard (9) was used for these calculations. There were 35 men considered as infected by virtue of having a urethral swab which was positive by culture or DFA or an FVU sample which was found to be positive by a plasmid amplification assay and which was confirmed by the respective MOMP gene assay.

Only 37.6% (108/287) of the men had symptoms of urethritis, and most of the positive test results were due to contacts with infected women. After the discordant specimens were tested, a total of 35 men were considered to be positive for *C. trachomatis* and 14 (40%) of them were symptomatic. Prevalences, sensitivities, and specificities for the techniques and specimen types are shown in Table 1. Both amplification assays were very sensitive with FVU specimens, and there were a few false positives (one by LCR and 5 by PCR). The sensitivities of culture and DFA were very low. This is probably due to the inability of 96-well plate cultures and iodine staining to detect low levels of the organism. The DFA test was only slightly more sensitive than culture of the urethral swabs (Table 1). The observed prevalence of infection increased twofold when FVU samples were tested by LCR or PCR as compared with the testing of urethral swabs by culture and DFA. The positive and negative predictive values were as follows: LCR, 97.1 (33/34) and 99.2% (251/253), respectively; and PCR, 87.5 (35/40)

and 100% (247/247), respectively. There were no differences in discordant results which could be attributed to the order of collection. Two FVU specimens which were only positive by the Amplicor test and which were confirmed by the MOMP gene PCR were examined for inhibitors of LCR. This was done by dilution of the specimen 1:2 in kit diluent and by spiking a tube of the undiluted and diluted specimens with control DNA, which was from a *C. trachomatis* L2 culture isolate diluted 1:60,000 in sterile water to simulate a clinical sample. One of the two specimens became positive upon dilution.

The 100% sensitivity and 98.0% specificity values for Amplicor PCR found in our study are comparable to those from three other published studies. Jaschek et al. (9) studied a group of men, 61% of whom were symptomatic and for whose specimens the Amplicor test had a sensitivity of 95.0% and a specificity of 99.8%. Bauwens and coworkers (1) reported a similar study on FVU from 365 predominantly symptomatic men showing 97% sensitivity and 99.7% specificity. The third study, reported by Domeika et al. (6), showed the Amplicor PCR to be 100% sensitive and specific on 184 asymptomatic men attending a skin- and venereal-disease clinic.

We have recently reported the ability of Abbott LCR to diagnose male urethral infections, a finding we obtained by testing FVU from men with or without symptoms (4, 5). In the multicenter clinical trial (5) involving 1,043 men from three centers in North America the overall sensitivity and specificity of the LCx assay were 93.5 and 99.8%, respectively. The results from our present "head-to-head" study are very similar (sensitivity, 94.3% and specificity, 99.6%).

This present comparative study of PCR and LCR showed no major differences from individual studies of FVU samples from men. The percentage of false positives by either technique ranged between 0 and 2.0%. A few studies did not confirm results by performing DFA or a second amplification assay which probes for a different gene target. In this study, the PCR and LCR results for 33 FVU samples showed concordance. Seventeen of these were matched with urethral swabs which were negative by both culture and DFA, and all were positive by MOMP LCR, but 11 were MOMP PCR positive.

A similar comparison of PCR and LCR performed on FVU collected from 676 asymptomatic military recruits (17) demonstrated test sensitivities of 93.1% for LCR and 62.1% for Amplicor PCR, with 3 false positives in the latter and no false positives in the former assay. After the freezing and thawing of the FVU, the sensitivity of the Amplicor test was found to be equal to that of LCR. This phenomenon was first reported by Bauwens et al. (1) for endocervical specimens. Our FVU specimens were not frozen before testing, but at least 90% were held for 72 h at 4°C. This prolonged refrigeration step may have contributed to the very high sensitivity of Amplicor with male FVU found in this study. Further studies processing FVU immediately, after 72 h at 4°C, or after freezing and thawing would contribute to our understanding and enable recommendations for processing FVU.

In this head-to-head comparison the Amplicor PCR assay identified all 35 positive specimens. The two missed by the Abbott LCR test were investigated for inhibitors by a dilution and spiking experiment, which showed one of them to be inhibitory for LCR but not PCR. More studies are needed to determine inhibitor rates and to identify and characterize urinary inhibitors of nucleic acid amplification. The ability of amplification assays to detect infected asymptomatic men by the testing of urine would lend itself to screening programs. Screening and treating infected men could impact the number of silent untreated infections in their female sexual partners and could lead to a reduction in ascending infections and

sequelae such as pelvic inflammatory disease, ectopic pregnancy, and infertility.

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