Comparison of Performances of Two Commercially Available Tests, a PCR Assay and a Ligase Chain Reaction Test, in Detection of Urogenital Chlamydia trachomatis Infection

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The diagnostic performance of a PCR test (Roche Cobas Amplicor CT/NG Test) and that of a ligase chain reaction (LCR) test (Abbott LCx Chlamydia trachomatis assay) were compared by using endocervical and urethral swab specimen culture as a reference test. First-void urine (FVU) and endocervical and urethral swab specimens were collected from 1,015 unselected patients attending a sexually transmitted disease clinic and a clinic for adolescents in Helsinki, Finland. Chlamydia trachomatis was cultured from samples from the endocervix or urethra. PCR was performed with fresh and frozen urine and the culture transport medium. LCR was performed with fresh and frozen urine and LCx swab transport medium. Diagnostic consistency and diagnostic accuracy were statistically tested. The test results were identical for 984 patients (97%). Discrepant results were observed for 31 patients. Overall, LCR and PCR showed excellent kappa coefficients of consistency for both swab and FVU specimens (0.93 and 0.95, respectively). Sixty-one patients (6%) were culture positive. Testing of FVU by LCR or PCR increased the overall positivity rates to 7.0 and 7.7%, respectively. While PCR of FVU detected the greatest number of C. trachomatis infections (sensitivity, 96.1%), for some PCR-positive FVU specimens the results could not be confirmed (specificity, 99.6%). PCR and LCR were more sensitive than culture (sensitivities, 92 and 93% versus 79% for culture) in the diagnosis of genital C. trachomatis infection. In conclusion, both tests can be recommended for use in the clinical laboratory and for the screening of asymptomatic C. trachomatis infections.

Chlamydia trachomatis is one of the most common causes of treatable sexually transmitted diseases (28). Serious sequelae, including pelvic inflammatory disease, tubal factor infertility, ectopic pregnancy, chronic abdominal pain, and reactive arthritis are associated with C. trachomatis infection (4, 14). Non-specific clinical signs and symptoms of C. trachomatis infection and the frequent occurrence of asymptomatic infections render the clinical diagnosis of C. trachomatis infections difficult (24). Since asymptomatic individuals serve as a reservoir of infection and since complications also occur after silent infection, detection of infected individuals is a major challenge to the preventive health care system.

With advances in DNA technology, laboratory methods for the amplification and detection of the multicopy plasmid DNA present in all C. trachomatis serovars have been introduced for the diagnosis of C. trachomatis infection (19). Theoretically, this approach offers high sensitivity (multicopy target) and high specificity (the target is present only in C. trachomatis). The PCR or ligase chain reaction (LCR) technology is demanding with respect to laboratory facilities and equipment, but standardized easy-to-use kits are available. Furthermore, partially automated assays (e.g., the LCx Chlamydia trachomatis assay; Abbott Laboratories, North Chicago, Ill.) and fully automated assays (Cobas Amplicor Chlamydia trachomatis [CT/NG] test; Roche Diagnostic Systems Inc., Branchburg, N.J.) have been developed and are already available for routine use. Assays based on PCR (Cobas Amplicor) and LCR (LCx) tests have been applied in the detection of C. trachomatis infection worldwide. Testing of first-void urine (FVU) specimens has been a major breakthrough in the detection of chlamydial infection in both symptomatic and asymptomatic males and females (1–3, 5, 11, 12, 18, 20–22, 26). Both PCR and LCR tests have proven to be superior in the diagnosis of C. trachomatis infection when compared to the previously used methods in terms of both sensitivity and specificity. However, reports of few studies that have compared the two most widely used amplification methods have been published (6, 17).

Our objective was to compare the performance of an automated PCR test (Roche Cobas Amplicor Chlamydia trachomatis [CT/NG] Test) and an LCR test (Abbott LCx Chlamydia trachomatis assay) with endocervical and urethral swab specimen culture as a reference test. We also wanted to assess the rate of inhibition of the amplification reaction in our material using the PCR test, which includes an internal control (IC). The effect of freezing on the performance of PCR and LCR testing of urine was also studied. In addition, the clinical utility of testing urine samples instead of swab specimens was studied.

MATERIALS AND METHODS

Patients. From May 1996 through October 1996, a total of 1,030 patients were recruited into the study. Informed consent was obtained from all patients, and a questionnaire regarding symptoms, sexual behavior, and contraception was completed. Altogether, 912 patients attending a sexually transmitted disease (STD) clinic and 118 females attending a clinic for adolescents in Helsinki were included in the study. Both symptomatic and asymptomatic patients were included. None of the patients had received antibiotic treatment 1 week prior to the visit.

Specimen collection and aliquoting. One endocervical swab specimen obtained from each woman or a urethral swab specimen obtained from each man was placed into C. trachomatis transport medium (2SP). Another endocervical or...
urethral swab (LCx STD swab; Abbott) specimen was placed into an LCx STD swab Transport Tube. The order of collection of the swabs was alternated weekly. FVU (10 to 50 ml) was collected after swabbing of the urethra for males and before swabbing of the endocervix for females and was transferred to a 15-ml polypropylene tube. Forty-two percent of the FVU specimens were stored at 4°C during transport to the laboratory, and processed within 24 h; the specimens collected on Friday afternoons, however, were stored at 4°C over the weekend and were processed on the following Monday morning. This is a standard practice in our laboratory. The specimens in 2SP were aliquoted for culture, PCR, and direct fluorescent-antibody (DFA) testing. The FVU specimens were stored at 4°C and transported to the laboratory within 24 h (except the specimens collected on Friday afternoons, which were stored at 4°C over the weekend and transported on the following Monday morning). Specimens were then aliquoted at 4°C for up to 4 [LCR] or 7 [PCR] days and frozen (−20°C) and to be used in discrepant analyses.

**Specimen testing.** Culture for C. trachomatis. Specimens in 2SP medium were vortexed vigorously. Aliquots of 250 μl were inoculated onto McCoy cell monolayers growing on glass coverslips in 24-well plates. After the plates were centrifuged at 3,400 × g for 1 h at 32°C, the inoculum was removed and was replaced with culture medium (BHI supplemented with 10% fetal calf serum, 2 mM glutamine, gentamicin, vancomycin, and nystatin) containing 0.5 μg of cycloheximide per ml. The cultures were incubated at 37°C in a 5% CO2 atmosphere. After 48 h, the cultures were fixed with methanol, stained with fluorescein isothiocyanate-labeled MicroTrak Chlamydia trachomatis Direct Specimen Reactant (Syva, Palo Alto, Calif.), and examined microscopically for the presence of chlamydial inclusions.

**DFA testing.** An aliquot of 2SP medium (100 μl) was centrifuged at 10,500 × g for 10 min, and the pellet was suspended in phosphate-buffered saline (PBS) and smeared on a glass slide. The slide was fixed in methanol at −20°C for 10 min and stained with fluorescein isothiocyanate-labeled MicroTrak Chlamydia trachomatis Direct Specimen Reactant (Syva), and examined microscopically for the presence of C. trachomatis elementary bodies (EBs).

**Cobas Amplicor CT/NG test.** The Cobas Amplicor CT/NG PCR test was performed according to the manufacturer's instructions. Fresh urine samples were vortexed vigorously. Frozen urine samples were completely thawed and were vortexed vigorously. A 500-μl aliquot of urine was transferred to a polypropylene tube containing 500 μl of CT/NG Urine Wash Buffer, and the contents were mixed well by vortexing. The tubes were incubated at 37°C for 15 min and centrifuged at 125,000 × g for 5 min. The supernatant was discarded and the remnants of the supernatant in each tube were blotted on a separate sheet of absorbent paper. A total of 250 μl of CT/NG Lysis Buffer was added to each tube, and the contents were mixed well by vortexing. After incubation for 15 min at room temperature, 250 μl of CT/NG Specimen Diluent was added to each tube and the contents were mixed by vortexing. The tubes were centrifuged at 12,500 × g for 10 min, and a 50-μl aliquot of each supernatant was used for testing.

The endocervical or urethral swab specimens collected in 2SP medium were processed as follows: 100 μl of CT/NG Lysis Buffer was mixed with 100 μl of the 2SP sample and the mixture was incubated at room temperature for 30 min. A total of 200 μl of CT/NG Specimen Diluent was added to each tube, and the tubes were incubated at room temperature for 10 min. Aerosol-barrier pipette tips were used throughout the procedure. Of 555 processed specimens, an aliquot of 50 μl was transferred to A-tubes containing the Working Master Mix, and the tubes were placed into the thermal cycler segments of the Cobas Amplific. Amplification and detection of C. trachomatis and IC DNA were automatically performed by the Cobas Amplific. System. Results were expressed as A600 and were regarded as positive (C. trachomatis A600 >0.8), as negative (C. trachomatis A600 <0.2; IC A600 =0.2), or in a gray zone (C. trachomatis A600 =0.2 but <0.8). For inhibitory specimens the A600 of the IC was <0.2 and that of C. trachomatis was <0.2. For specimens containing inhibitory substances, another aliquot was tested at full strength and following 1:10 dilution in CT/NG Specimen Diluent.

**Abbott LCx Chlamydia trachomatis assay.** The LCx test was performed according to the manufacturer's instructions. A 1-ml aliquot of the FVU was centrifuged at 13,000 × g for 15 min and the supernatant was discarded. A pellet was resuspended in 1 ml of LCx Urine Specimen Resuspension Buffer. The treated FVU samples and swab specimens in the transport tubes were heated at 97°C for 15 min. For amplification of Chlamydia by LCx, 100 μl of the treated specimens was used. An LCx Analyzer (microparticle enzyme immunoassay) was used to detect the amplicons. The LCx Analyzer calculated the cutoff value (0.45 × mean of LCx Chlamydia Calibrator Values), and specimens with an assay value equal to or greater than the cutoff value were considered positive. Direct and Conventional Results for the Set of Patient Specimens With or Without Discrepant Results for Both Specimen Types in all Tests were considered Discrepant. The specimens were retested as follows. To check for the presence of nonviable C. trachomatis EBs in culture-negative specimens with discrepant results, the DNA assay was performed with the 2SP processed specimen. If either the 2SP processed specimen or the swab tested in the LCx assay had discrepant results, the specimen was retested both undiluted and following 1:10 dilution in LCx Urine Specimen Buffer. The LCx assay was repeated with the processed swab specimen. One of the frozen aliquots of urine was also processed and tested. Testing of the specimens with discrepant results by the Cobas Amplific CT/NG test and conventional PCR was performed with either specimen type (2SP or processed LCx swab specimens, whereas FVU aliquots from both the two patients tested were also positive for C. trachomatis MOMP DNA.

Testing of FVU by PCR failed to detect C. trachomatis in one male patient, and testing of FVU by LCR failed to detect
C. trachomatis in one female and two males. However, discrepant analysis revealed C. trachomatis DNA in another aliquot of FVU from these three patients.

Eleven patients had positive results only by PCR. FVU gave a positive signal for four patients and 2SP gave a positive signal for eight patients (both the swab and the urine were positive or one patient). Two of these 11 patients were confirmed to be positive by PCR for MOMP and plasmid-based PCR with another aliquot of FVU, 2SP, or a processed LCx swab specimen. These patients were not counted as true positives when calculating the performance of the tests, since an expanded "gold standard" was not created. However, 9 of these 11 patients most likely initially had false-positive PCR results because they remained negative when retested by PCR for MOMP and plasmid-based PCR. For one female, only tests performed with the 2SP medium (culture, PCR, and the DFA assay) remained negative, although PCR of FVU and LCR of swabs as well as LCR of FVU gave positive results, suggesting technical failure in swabbing.

Detection of the IC by PCR. Specimens from 10 (1%) patients were found to be inhibitory when tested for the IC in the Cobas Amplicor Chlamydia trachomatis (CT/NG) Test. These were eight urine samples and three endocervical swab specimens (from one female, both the FVU and the swab specimen turned out to be inhibitory). All samples were from females (mean age, 34 years; age range, 20 to 51 years). Six were symptomatic and four were asymptomatic. One used birth control pills, and none was taking antibiotics. In addition to these

<table>
<thead>
<tr>
<th>Patient sex and test (specimen)</th>
<th>No. of specimens positive/ no. of specimens tested (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<td><strong>Women</strong></td>
<td></td>
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<tr>
<td>Culture (swab)</td>
<td>19/449 (4.2)</td>
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<td>100 (421/421)</td>
<td>100 (19/19)</td>
<td>97.9 (421/430)</td>
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<td>PCR (swab)</td>
<td>27/447 (6.0)</td>
<td>78.6 (22/28)</td>
<td>98.8 (414/419)</td>
<td>81.5 (22/27)</td>
<td>98.6 (414/420)</td>
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<td>22/448 (4.9)</td>
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<td>100 (421/421)</td>
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<td>PCR (FVU)</td>
<td>28/442 (6.3)</td>
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<td>99.8 (413/414)</td>
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<td>LCR (FVU)</td>
<td>25/443 (5.6)</td>
<td>92.6 (25/27)</td>
<td>100 (406/406)</td>
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<td>99.5 (406/408)</td>
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<td>94.0 (47/50)</td>
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<td>94.9 (74/78)</td>
<td>99.7 (926/929)</td>
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<td>100 (918/918)</td>
<td>100 (70/70)</td>
<td>99.5 (918/923)</td>
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</table>

- PPV, positive predictive value.
- NPV, negative predictive value.
- Swab, endocervical swab from females and urethral swab from males.
- Values in parentheses are numbers used to calculate sensitivity, specificity, PPV, and NPV (9).
11 specimens, the amplification of the IC was negative for another 11 specimens that were positive for *C. trachomatis*.

**Testing of frozen FVU.** Aliquots of FVU were stored at −20°C for 2 to 6 months before retesting by PCR and LCR. Fewer positive results were obtained by both PCR and LCR when frozen aliquots of FVU were thawed and analyzed. Ten of the 78 initially PCR-positive FVU specimens became negative. Six of these were from patients with other positive markers for *C. trachomatis* infection, and four were most likely initially false positive. Four of the 10 aliquots of FVU were also initially LCR positive and all also became negative by LCR. Seven of the 70 initially PCR-positive FVU specimens became negative by LCR. Six of these seven were from patients with true *C. trachomatis* infection, and one was most likely an initially false-positive result. Four of the seven aliquots of FVU were also initially PCR positive and all also became negative by PCR.

**DISCUSSION**

Most urogenital *C. trachomatis* infections are initially asymptomatic but may subsequently cause considerable long-term morbidity. Consequently, accurate diagnosis of *C. trachomatis* infection requires the use of specific laboratory techniques. Performance of these methods varies considerably. Culture was earlier considered the gold standard, but PCR and LCR studies suggests that the sensitivity of the culture even in expert laboratories is as low as 75 to 85% (5, 21). It is universally accepted that culture can no longer serve as a reference method in the evaluation of diagnostic tests for *C. trachomatis*.

Recently introduced PCR and LCR techniques have been shown to be very sensitive in the diagnosis of chlamydial infection of the male urethra and in detecting chlamydial DNA in male urine specimens. Lower sensitivity has usually been observed when female specimens have been tested, possibly due to the more frequent occurrence of inhibitors in the specimens. For the first time, we compared the diagnostic performance of commercially available PCR and LCR with both swabs and FVU using cell culture as the reference. In earlier studies evaluating PCR and LCR, FVU had been tested by the amplification methods and swabs had been tested by culture (17) or by culture and the DFA assay (6). In our hands, testing of FVU from females and urethral swabs from males by nucleic acid amplification methods detected most *C. trachomatis*-positive patients. Culture was less sensitive for female endocervical swabs than for male urethral swabs (67.9 versus 85.7%). Thus, culture missed every third infected female and 14% of the infected males. The site of infection might, however, well explain why swab PCR and LCR failed to detect *C. trachomatis* infection in six patients, because all these women had negative cervical specimen culture results and chlamydia DNA was found in the FVU only. This study showed that PCR and LCR showed excellent consistency by kappa coefficients and performed better than culture when compared to our standard (positive culture result or positive PCR and LCR results with either specimen type).

Use of discrepant analysis is one attempt to improve the performance characteristics of the reference method. Discrepant analysis aims to identify by an alternative (confirmatory) method true-positive specimens originally missed by the reference method. However, if only samples with discrepant results are tested by confirmatory tests, which is usually the case, a bias is introduced (10). The selective testing includes only specimens initially giving discrepant results and could theoretically favor the performance of the tests being evaluated. In our material, the criteria for proven infection were culture positivity or both PCR and LCR positivity. Discrepant analysis was done, but the results were used only to clarify the initial results. An expanded reference standard was not created, although this was recommended recently by the STD Diagnostic Initiative (9). Our retesting showed that 60% of the swab specimens that were obviously culture failures did contain *C. trachomatis* EBs when the specimens were analyzed by DFA staining. This is as expected, since culture and DFA assay are considered less sensitive than PCR.

Clinical specimens are known to contain several factors that inhibit DNA polymerase. Roche has developed a system that permits the identification of such polymerase reaction-inhibiting factors: detection of amplification of an IC in included in the Cobas Amplicor test, thus ensuring the integrity of the result. In our material, the occurrence of such inhibitors was low (less than 1%). Others have noted higher inhibition rates detectable with the IC system with clinical specimens (16) or spiked clinical specimens (27). It is possible that in this study obvious false-negative LCR results were due to the presence of inhibitors of the LCR, but this could not be confirmed in our study, because the LCx does not incorporate an internal control. Our earlier experience with the PCR, LCR, and transcription-mediated amplification (TMA) techniques in routine clinical microbiology laboratory use suggests that some specimens contain factors that inhibit the amplification reactions, but the factors are not identical for different techniques.

Specificity is another concern with the DNA amplification techniques. Theoretically, the tests are highly specific, but because the potential of the amplification reaction is enormous, even a slight contamination can result in erroneous results. In published studies, PCR is usually found to be more sensitive and LCR is found to be more specific when compared to the sensitivity and specificity of a created standard (6, 17). This was also the case in our study when FVU specimens were analyzed: The sensitivities of PCR and LCR were 96.1 versus 93.3%, respectively, and the specificities of the tests were 99.6 versus 100%, respectively. To increase the specificities of the amplification techniques, retesting of the initially positive specimens or confirmation of the results by another amplification method can be done. However, because freezing and thawing of the specimen can have an effect on test performance, the results may not be comparable. Freezing and thawing can eliminate inhibitory factors (25), but as our results suggest, the DNA in the specimens can also be degraded.

The most attractive aspects of the DNA amplification methods are their excellent sensitivities and good performance with FVU samples. In our study, 96 and 93% of the infections could be detected by testing FVU by PCR or LCR, respectively, when the results for symptomatic and asymptomatic patients were analyzed together. In the detection of both symptomatic and asymptomatic infections, testing of FVU by PCR or LCR is thus a preferred alternative to testing of swab specimens for both females and males. We have shown earlier that screening for chlamydial infections by the PCR test with FVU is cost-beneficial in a low-prevalence population if the prevalence of infection exceeds 3.9% (15). Because recent studies suggest that *C. trachomatis* infection is also a risk factor for adverse pregnancy outcomes other than ectopic pregnancy (8) and is possibly also a risk factor for the development of cervical neoplasia (13), the cost-effectiveness of screening of the sexually active population by DNA amplification methods needs to be reevaluated.

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REFERENCES


