Diagnosis of Chlamydia trachomatis Infections in Men and Women by Testing First-Void Urine by Ligase Chain Reaction

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From April to September 1993, 305 men and 447 women in Hamilton, Canada, consented to the collection of a urethral or cervical swab, respectively, for culture and 20 ml of first-void urine (FVU) for testing by the enzyme immunoassay Chlamydiazyme and by ligase chain reaction (LCR) in the form of a kit from Abbott Laboratories called LCx Chlamydia trachomatis. Evaluation of test performance with each specimen was calculated on the basis of an expanded "gold standard" of a patient found to be positive by culture or by a confirmed nonculture test. By using this expanded standard, the prevalence of infection was determined to be 6% (27/447) for the women and 18.4% (56/305) for the men. LCR testing of FVU in both studies was the most sensitive approach (96%). The performance of Chlamydiazyme was as follows: cervical swab, 78.3% sensitivity; female FVU, 37% sensitivity; and male FVU, 67.9% sensitivity. Culture was the least sensitive approach to diagnosis: female cervix, 55.6%; and male urethra, 37.5%. LCR testing of FVU from men or women diagnosed the greatest number of genitourinary tract infections with no false positives.

The male urethra and the female cervix and urethra serve as reservoirs for Chlamydia trachomatis infection by sexual intercourse (32). If infections are present in the lower genitourinary tract, they are expressed as cervicitis or urethritis. However, infection is often asymptomatic in men or women (33). If untreated, the bacteria can ascend the female genital tract, causing endometritis, salpingitis, pelvic inflammatory disease, or tubal factor infertility in large numbers of women (27). Culture of swabs collected from the female endocervix and urethra and from the male urethra has been the approach to diagnosis for the past 20 years. More recently, concentrated first-void urine (FVU) from men has been used with considerable success to detect C. trachomatis antigens by using enzyme immunoassays (EIA) and direct fluorescent-antibody (DFA) immunoassays (1, 5, 6, 10–13, 15–17, 20, 22, 26, 28, 29, 34, 36). The same approach with female FVU has been less successful (31, 35). PCR amplification of C. trachomatis nucleic acid has also been applied to FVU for diagnosing infections in both symptomatic and asymptomatic men (2, 14, 19, 25). Our study examined the ability of a ligase chain reaction (LCR) assay (3, 8) called LCx from Abbott Laboratories to diagnose C. trachomatis infections in men and women by examining their FVU. LCR was compared with culture and EIA performed on swabs and/or FVU by using an expanded "gold standard."

MATERIALS AND METHODS

Specimens. From April to September 1993, 305 men and 447 women were enrolled in the study. The men were attending a hospital-based sexually transmitted disease clinic, and the women were attending a college student health clinic or a hospital-based therapeutic abortion clinic in Hamilton, Ontario, Canada. A consent form approved by the McMaster University Research Ethics Committee Hospital Network was administered to each patient. The men had an FVU sample (20 ml) collected in a sterile 30-ml screw-cap plastic jar. A urethral swab (US) specimen was collected from the men by inserting a narrow-shafted cotton-tipped swab 2 to 3 cm into the urethra. An FVU sample was also collected from the women. They also had two cervical swabs (CS) collected from the endocervix. If a pus exudate was present, it was cleaned with a large cotton swab before the test swabs were collected. 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 L-glutamine at 4°C; transported to the laboratory; and set up in cell cultures within 24 to 48 h.

Culture and EIA. US from the men and CS from the women were inoculated onto McCoy cell monolayers in 96-well microculture plates, with a blind passage and iodine staining (6). CS and FVU from women and FVU from men were processed and tested by Chlamydiadiyme EIA, with the confirmatory blocking reagent as described previously (6, 31). Only EIA confirmed results were included in the calculations.

LCR. FVU specimens from men or women were briefly vortexed, and then 1 ml was transferred to a 1.7-ml microcentrifuge tube and centrifuged at 16,000 × g (Eppendorf centrifuge model 5415C; Brinkman Instruments) for 10 min. After removal of the supernatant fluid, the pellet was resuspended in 1 ml of urine resuspension buffer provided with the LCx kit provided by Abbott Laboratories and was heated to 95 to 100°C for 15 min. After cooling to room temperature, processed urine samples either were tested immediately by LCR or were stored at either 2 to 8°C or −20°C for up to 60 days and then tested. For testing, 100 μl of the resuspended FVU was transferred to individual C. trachomatis unit dose tubes containing 100 μl of the LCR mixture. This mixture consisted of four C. trachomatis plasmid oligonucleotide probes, thermostable Thermus thermophilus DNA ligase and Thermus polymerase, NAD, Mg2+, dCTP, and dTTP in a buffer (pH 7.8). The DNA sequences for the plasmid target and the probe set are shown in Fig. 1. Two positive (lymphogranuloma venere-
um-infected McCoy cells in buffer) and two negative (nonspecific DNA in buffer) controls, as well as two calibrators (DNA extracted from lymphogranuloma venereum elementary bodies [EBs]), were run with each batch of processed urine samples. Reactions were amplified in a thermocycler (Model 480; Perkin-Elmer Cetus, Emeryville, Calif.) by using the following thermocycling protocol: 40 cycles of 1 s at 97°C, 1 s at 55°C, and 50 s at 62°C. After the thermocycling, the controls and specimens were pulse-centrifuged (9,000 × g for 1 s) and transferred into reaction cells. Amplified product was qualitatively detected in an LCx analyzer, which is a microparticle sandwich immunoassay based on covalent labelling of the four probes with two different hapten. The amplified product was captured onto the microparticles by immobilized antibodies directed against one of the hapten (carbazole and adaman-tane). The other end of the product contained the second hapten, which was recognized by a second antibody conjugated to a reporter enzyme (alkaline phosphatase). Only LCR-amplified product with both hapten covalently attached generated a detectable signal in the LCx analyzer. Results were expressed as counts per second per second, and a positive result was defined as one that was equal to or greater than the product of the mean of the two LCx analyzer run-specific calibrator values multiplied by 0.45.

To prevent amplicon contamination, 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 transfer of material between the two areas. After detection, amplified product was destroyed by a nucleic acid inactivation reagent (copper-phenanthroline complex), which was automatically added to all specimens to eliminate carryover contamination.

**Discordant analysis.** Supplementary testing was used to resolve discordant results. All discordant and concordant specimens were inserted, blinded and at random, into a supplementary testing protocol. Specimens found to be negative by culture and positive by LCR of either the CS or FVU were investigated further by DFA staining with the Microtrak reagent from Syva after concentration by centrifugation at 16,000 × g for 10 min. Specimens with two or more EBs were considered positive. LCR-positive specimens negative by DFA assay were subjected to a second LCR directed against the gene which codes for the major outer membrane protein (MOMP) of C. trachomatis by a protocol which used both 40 and 43 heating and cooling cycles. After discordant testing, the negative samples were diluted 1:10 and retested in the MOMP gene LCR.

**Calculations.** Sensitivity, specificity, and predictive values were calculated by standard techniques (9). The gold standard was established as a patient with at least one specimen found to be positive by culture or by a confirmed nonculture technique (13).

**RESULTS**

LCR amplification of C. trachomatis plasmid DNA in urine was 96.4% sensitive and 100% specific in the case of this group of men (Table 1). Detection of antigen in FVU by the Chlamydizyme test was 67.9% sensitive and 100% specific. Culture performed on the US was highly specific but very sensitive for culture.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample</th>
<th>% Sensitivity (% no. of positive results/no. of infected patients)</th>
<th>% Specificity (% no. of negative results/no. of uninfected patients)</th>
<th>% Positive PV (no. of infected patients found positive in test/no. of infected and uninfected patients positive in test)</th>
<th>% Negative PV (no. of uninfected patients found negative in test/no. of uninfected patients determined negative in test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>US</td>
<td>37.5 (21/56)</td>
<td>100 (249/249)</td>
<td>100 (21/21)</td>
<td>87.7 (249/284)</td>
</tr>
<tr>
<td>EIA*</td>
<td>FVU</td>
<td>67.9 (38/56)</td>
<td>100 (249/249)</td>
<td>100 (38/38)</td>
<td>93.3 (249/267)</td>
</tr>
<tr>
<td>LCR</td>
<td>FVU</td>
<td>96.4 (54/56)</td>
<td>100 (249/249)</td>
<td>100 (54/54)</td>
<td>99.2 (249/251)</td>
</tr>
</tbody>
</table>

* Calculations based on an expanded gold standard of at least one specimen from the patient determined to be positive by culture or a confirmed nonculture test.

* PV, predictive value.

* Chlamydizyme with blocking confirmation.
insensitive (37.5% sensitive). In analysis of the 33 samples with LCR-positive FVU results but culture-negative US results, 16 were confirmed positive by Chlamydiazyme testing and 32 were found to have two or more C. trachomatis EBs by DFA assay after centrifugation of the remaining culture specimen. The one remaining discordant specimen was found to be positive by LCR targeting the MOMP gene. Two patients had a positive FVU Chlamydiazyme test but a negative culture and LCR, and one of these patients had EBs present.

In the female study 14 women were found to have a positive FVU sample by LCR and a positive CS by culture. An additional 13 women were determined to be positive by LCR of FVU but negative by culture of the CS. Ten of 13 had EBs present in the centrifuged culture specimen, and the other 3 were found to be positive by the MOMP gene LCR. One woman had a positive CS culture but a negative LCR result for her FVU. Both the culture tube and the FVU from that patient became positive in the LCR assay after dilution at 1:10 or 1:100. Table 2 summarizes the performance of LCR and Chlamydiazyme testing of female FVU, which had sensitivities of 96.3% and 37.0%, respectively. Culture of the CS had a sensitivity of 55.6%, and Chlamydiazyme testing performed on CS was 78.3% sensitive. All of the assays were 100% specific.

**DISCUSSION**

LCR testing of male FVU was a very sensitive approach to diagnosis. The high rate of positivity was similar to those achieved by testing FVU by PCR (2, 14, 19, 25). Palmer et al. (25) found a PCR directed against a MOMP gene fragment to be 82% sensitive and 94% specific with FVU compared with DFA testing of US. Mahony et al. (19) used a plasmid-based PCR on FVU, which was found to be 100% sensitive and specific compared with testing of US by culture, EIA, and DFA assay. Other studies (2, 14) have used a commercially available plasmid-based PCR assay on male FVU, which showed high sensitivities (95 and 97%) and specificities (99.8 and 99.7%).

The differences in sensitivity between this group of three plasmid probe studies (2, 14, 19) and the MOMP gene C. trachomatis study (25) may be attributed to several factors, one of which is an inherent greater sensitivity of PCR directed against the plasmid as opposed to the MOMP gene (18).

Evaluations using newer, more sensitive techniques, such as LCR or PCR, accentuate the need for multiple testing of more than one specimen to enable the determination of performance on the basis of an expanded gold standard (13). By doing this, we were able to show the relative ability of each technique performed on a specific type of specimen to diagnose an infected person. The low culture sensitivity in the present study enabled assessment of the collection and transportation protocols and uncovered less than optimal performance caused by less than optimal specimen collection and transportation as well as a degree of resistance to infection demonstrated by cell cultures of high passage and older growth.

Over the past 5 years there have been a number of publications concerning the use of antigen detection assays with male FVU to diagnose urethral infection with C. trachomatis. The original studies from our laboratory (6) and from England (5, 26) showed that concentration of the FVU was necessary to enable EIA testing of the resuspended sediment to diagnose as many infections in men as could be detected by urethral swabbing. Most subsequent studies (10, 12, 13, 15, 17, 20, 36) have confirmed and extended these findings. However, at least three studies (1, 16, 29) have reported poor sensitivity for the use of EIA with FVU. These differences could be influenced by urine volume, the use of FVU versus midstream urine, or the time since the last urination (30). The EIA in use and how the high level of comparison was calculated may have affected the results. Although original studies focused on men with symptoms, it appears that detection of antigen in FVU may be successful in the case of asymptomatic men or those being investigated as contacts of positive partners (17). The results of the performance of Chlamydiazyme testing on men reported here are similar to those of our previous studies and those of the majority of reports in the literature concerning this assay.

The high sensitivity rates attained in our study by testing urine in the LCR assay were achieved with FVU without concentration. The use of nucleic acid amplification technology to detect infected females by examination of their FVU is a newly recognized investigative maneuver. We have previously tested female FVU by EIA and found the approach only 40% sensitive (31), which is similar to the Chlamydiazyme testing data obtained with the women in the present study (37%). Studies of the frequency of C. trachomatis infection in cervical or urethral sites using culture have shown that approximately 50% of all infected women are infected at both sites, 30% are infected in the cervix only, and 5 to 30% are infected in the urethra only (4, 21, 23, 24, 31, 37). Thus, FVU testing should detect 80% or more of all infected women, and the increase in sensitivity for LCR over EIA is probably due to the high sensitivity for detecting the C. trachomatis plasmid or MOMP gene DNA as opposed to antigens in the urine.

In summary, an FVU sample is an excellent specimen for diagnosing C. trachomatis infection of the lower genitourinary tracts of men or women by LCR. Nearly all (96.4%) of the infected patients were detected, with few, if any, false positives. More studies will need to be done to determine whether inhibitory factors may be present in some patient specimens and whether concentrating urine, collecting it as the first void, and varying the length of time since the last micturition will be factors in diagnostic accuracy with LCR.

**TABLE 2. Ability of culture, EIA antigen detection, and LCR performed on CS or FVU to diagnose C. trachomatis infection in 447 women**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>% Positive PV (no. of infected patients found positive in test/total no. of infected and uninfected patients positive in test)</th>
<th>% Negative PV (no. of uninfected patients determined negative in test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>CS</td>
<td>55.6 (15/27)</td>
<td>100 (420/420)</td>
<td>100 (15/15)</td>
<td>97.2 (420/432)</td>
</tr>
<tr>
<td>EIAa</td>
<td>CS</td>
<td>78.3 (18/23)</td>
<td>100 (420/420)</td>
<td>100 (18/18)</td>
<td>98.8 (420/425)</td>
</tr>
<tr>
<td>EIA</td>
<td>FVU</td>
<td>37.0 (10/27)</td>
<td>100 (420/420)</td>
<td>100 (10/10)</td>
<td>96.1 (420/437)</td>
</tr>
<tr>
<td>LCR</td>
<td>FVU</td>
<td>96.3 (26/27)</td>
<td>100 (420/420)</td>
<td>100 (26/26)</td>
<td>99.8 (420/421)</td>
</tr>
</tbody>
</table>

a Calculations based on an expanded gold standard of at least one specimen from the patient determined to be positive by culture or a confirmed nonculture test.
b PV, predictive value.
c Chlamydiazyme with blocking confirmation.
ACKNOWLEDGMENTS

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REFERENCES