Comparison of a Ligase Chain Reaction-Based Assay and Cell Culture for Detection of Pharyngeal Carriage of Chlamydia trachomatis

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In 264 genitourinary medicine clinic attenders reporting recent fellatio, the prevalence of pharyngeal Chlamydia trachomatis determined by an expanded standard including cell culture and two in-house PCR tests was 1.5% in 194 women and zero in 70 men. The ligase chain reaction (Abbott LCx) had a specificity of 99.2% and a positive predictive value of 60%.

Chlamydia trachomatis is the commonest bacterial sexually transmitted pathogen in most developed countries. Infection with the genital serovars of C. trachomatis is usually acquired through unprotected vaginal or anal sex, or vertically at birth. The orogenital route has not previously been considered an important mode of transmission of C. trachomatis, and testing for pharyngeal C. trachomatis infection as part of a routine sexual infection screen is not advised in recent United States (2) or United Kingdom (3) guidelines. However, the practice of fellatio by women has increased (5), and among the sexually inexperienced, fellatio can be a substitute for vaginal intercourse, preserving “technical virginity” (6). Condom use during fellatio is rare, and so it is possible that orogenital transmission of C. trachomatis could occur in spite of “safer sex” precautions for genital sex or where the participants do not believe they have actually “had sex” (12). We now report the first evaluation of a ligase chain reaction (LCR)-based assay (Abbott LCx) compared with cell culture (including a single blind passage step) in detecting pharyngeal infection with C. trachomatis.

We recruited 264 subjects (194 women and 70 men) aged over 18 years who presented to a large urban genitourinary medicine clinic over a 9-month period with a new problem requiring a full sexual infection screen, and who reported performing fellatio in the 3 months before attendance. We excluded subjects who had received any antibiotics in the month before attendance. Pharyngeal samples were taken by rotating a swab in both fauces and then sweeping the swab firmly over the posterior pharynx. Two study samples were taken. For LCR we used the fine cotton-tipped swab provided in the LCR pack, which was then transported in LCx buffer at room temperature for analysis within 24 h. For cell culture we used a cotton-tipped aluminum-shaft swab, which was placed in sucrose phosphate transport medium (2-SP) and held at 4°C. Halfway through the study the order of swabs was reversed. After taking the study swabs, a pharyngeal swab for gonococcal isolation was taken in the same way, directly plated onto modified Thayer-Martin medium, and then incubated at 35°C in a CO₂-rich atmosphere for 48 h. All subjects then underwent a full sexually transmitted infection screen, including a cervical or (in men) urethral sample for detection of C. trachomatis by LCR, plating of cervical, urethral, and rectal swabs (as indicated) for gonococcal isolation as above, and syphilis serology.

Detection of a target sequence of C. trachomatis DNA from the cryptic plasmid by commercial LCR (Abbott Laboratories, North Chicago, Ill.) was conducted according to the manufacturer’s directions (4). C. trachomatis was cultivated in McCoy cells by standard methods, with the addition of a single blind passage step. Samples from subjects with a positive test for pharyngeal chlamydia by LCR or cell culture or both were further investigated with two PCR-based assays targeting sequences in both the major outer membrane protein gene and the cryptic plasmid. Pharyngeal swabs frozen in Chlamydia transport medium were transported on dry ice to an independent reference laboratory and were tested blind along with a matching number of pharyngeal swabs taken from subjects free of genital or pharyngeal Chlamydia. Nucleic acid was extracted using the QIAamp viral RNA kit (Qiagen) (13). PCR was performed using the LightCycler (Idaho Technology, Idaho Falls, Idaho), a glass capillary cycler with real-time fluorescent detection of PCR product and the ability to analyze reaction products by melting-point analysis. The major outer membrane protein-based PCR amplifies a 155-bp region between base pairs −83 and +51 of the sequence using primers MOMPFP2 (5’ CCA GAA AAA GAT AGC GAG CAC AAA 3’) and MOMPRI1 (5’ AGC AGA ACT CAA AGC GGC AAA T 3’). The plasmid-based PCR was adapted from Loeffelholz et al. (9) and targets a 207-bp fragment of the cryptic plasmid located 195 bp downstream of the BamHI restriction site. The detection limit of both assays is approximately 10 gene copies. Subjects with two or more positive results by different techniques (LCR, PCR, cell culture) and, in the case of LCR and PCR, with different targets (plasmid or major outer membrane protein [MOMP]) were considered true positives.

Female and male subjects had a mean age of 26.6 years (standard deviation, 7.5 years) and 33.5 years (standard deviation, 10.9 years), respectively. Unprotected fellatio was very common: no men and only two women (1%) had consistently used condoms for receptive oral sex in the 3 months before attendance. Sixty-seven women (35%) and 21 men (30%) were found to have at least one sexually transmitted infection, including 36 (19%) women and 3 (4.3%) men with genital C. trachomatis infection detected by LCR.

Three women (1.5%) had confirmed pharyngeal C. trachomatis according to the expanded standard (see Table 1). In
these women all four assays were positive and all had genital chlamydia infection, giving a prevalence of pharyngeal chlamydial infection among those with genital chlamydia of 8.3%. None of the positive samples gave a high reaction rate in the LCR assay, with the highest being 700 (S/CO, 4.0). A.

Pharyngeal swabs by: LCR, cell culture, MOMP-based PCR, and plasmid-based PCR in genital and pharyngeal swabs

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Genital swabs by LCR</th>
<th>Pharyngeal swabs by:</th>
<th>PCR</th>
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<td></td>
<td>Culture</td>
<td>LCR</td>
<td>MOMP</td>
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<tr>
<td>Women (n = 194)</td>
<td>152</td>
<td>32</td>
<td>4</td>
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<td></td>
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<td>Men (n = 70)</td>
<td>66</td>
<td>3</td>
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a Cervical swab (women) or urethral swab (men).
b ND, not done.
c Positive LightCycler plasmid-based PCR could not be confirmed. Subject not aware of any exposure to C. trachomatis.
d Positive LCR detected by plasmid-based PCR but not MOMP-based PCR.
e Patient recently exposed to C. trachomatis.

Positive LightCycler plasmid-based PCR could not be confirmed. Subject not aware of any exposure to C. trachomatis. Positive LightCycler plasmid-based PCR but not MOMP-based PCR. Patient recently exposed to C. trachomatis.

Positive LCR confirmed by plasmid-based PCR but not MOMP-based PCR. Positive LightCycler plasmid-based PCR could not be confirmed. Subject not aware of any exposure to C. trachomatis.

Positive LCR detected by plasmid-based PCR but not MOMP-based PCR.

Positive LightCycler plasmid-based PCR could not be confirmed. Subject not aware of any exposure to C. trachomatis. Positive LightCycler plasmid-based PCR but not MOMP-based PCR. Patient recently exposed to C. trachomatis.

Cervical swab (women) or urethral swab (men). ND, not done. Positive LightCycler plasmid-based PCR could not be confirmed. Subject not aware of any exposure to C. trachomatis. Positive LCR detected by plasmid-based PCR but not MOMP-based PCR. Patient recently exposed to C. trachomatis. LCR equivocal on first run and positive at low level on second run but could not be confirmed. Positive LCR detected by plasmid-based PCR but not MOMP-based PCR. Patient recently exposed to C. trachomatis.

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REFERENCES