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# NOTES

## Diagnosis of Genitourinary *Chlamydia trachomatis* Infections by Using the Ligase Chain Reaction on Patient-Obtained Vaginal Swabs

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**We compared the ligase chain reaction (LCR) assay to cell culture for diagnosis of genitourinary chlamydial infections in women using swab specimens obtained by clinicians from the endocervix and by patients from their own vaginas. Specimens from 40 (12.9%) of 309 patients were positive for chlamydial infection by culture, while the specimens of 50 (16.2%) patients were positive by LCR. *Chlamydia trachomatis* infection was verified for 9 of 10 patients whose LCR specimens were positive but whose cultures were negative. Vaginal and cervical swab specimens were positive by LCR for 46 (93.9%) and 44 (89.8%) of 49 chlamydia-infected patients, respectively. These data suggest that LCR testing for chlamydia with vaginal swab specimens obtained by patients themselves is as sensitive as cervical LCR and more sensitive than cell culture.**

*Chlamydia trachomatis* is the most common bacterial sexually transmitted disease (STD) in the world and regularly leads to sequelae such as infertility and ectopic pregnancy (3). Amplified nucleic acid hybridization tests, such as ligase chain reaction (LCR) and PCR, have been demonstrated to accurately diagnose genitourinary chlamydial infections in women with urine as an analyte (1, 2, 6, 7). These observations suggest the potential of nucleic acid detection tests to simplify chlamydia screening and to allow testing when speculum-guided specimen collection is difficult. To potentially further expand diagnostic test options, we evaluated the LCR assay for chlamydial diagnosis of women, comparing the performance of tests using patient-obtained vaginal swab specimens with that of tests using endocervical specimens collected by an experienced clinician.

Three hundred nine women visiting the Jefferson County Department of Health STDs Clinic in Birmingham, Ala., were evaluated by using patient-obtained vaginal swab specimens and clinician-obtained endocervical swab specimens as previously described (5). The order of patient-obtained vaginal swab specimen collection was varied by using a preassigned randomization code. For clinician-obtained specimens the swab for gonorrhea culture was obtained first, followed by swabs for chlamydia cell culture and for LCR testing in that order. LCR specimens were kept at 4°C for no more than 18 h until they were transported to a laboratory, where swabs were frozen at −20°C until LCR testing was performed.

Specimens were cultured with cycloheximide-treated McCoy cells in a 96-well format as previously described (10). Following incubation, wells were stained with two different monoclonal antibody reagents (MicroTrak [Syva, Palo Alto, Calif., and Kallestad]) and then evaluated for chlamydial inclusions by immunofluorescence microscopy. Blind passage of an incubated well was performed on cultures which were negative on initial evaluation. Cultures with any inclusions noted from the first or blind pass were classified as positive for *C. trachomatis.*

The LCR procedure was performed according to the instructions of the manufacturer (Abbott Laboratories, Abbott Park, Ill.) (1, 5–8). For each DNA amplification run, 12 controls and up to 36 patient specimens were prepared. Amplicon detection was performed by a microparticle-based enzyme immunoassay and LCx instrumentation to detect a fluorescent reaction. Patient samples with values at least 0.45 the average of two calibration samples for the run were recorded as positive.

Culture-negative and LCR-positive specimens were further evaluated by direct fluorescence antibody assay (DFA) for the presence of elementary bodies. For DFA, 1.0 ml of chlamydial culture transport medium was centrifuged at 13,000 × *g* for 15 min. The sediment was then examined with fluorescent monoclonal antibody reagents (MicroTrak). Specimens with one or more elementary bodies were classified as positive. For culture-negative and LCR-positive specimens which were DFA negative, a second LCR was performed at Abbott Laboratories in a blind fashion with a second probe set for the chlamydial MOMP gene.

Following resolution of discrepant results, the sensitivity, specificity, and positive and negative predictive values for each specimen type were calculated with the results of the resolution process described above. Patients were determined to be chlamydia infected if culture was positive from either (vaginal or cervical) site. Culture-negative and LCR-test-positive specimens confirmed by either the DFA or a second positive LCR test using DNA probes which target the chlamydial MOMP gene were also considered true infections. Patients were determined to be uninfected by negative cultures and negative LCR tests from both sites or by negative cultures from both sites and positive LCR tests which were not confirmed by DFA or MOMP LCR.

Participants in this study were predominantly young (median age, 25 years; range, 14 to 56 years), African-American (270

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TABLE 1. Results of all culture and LCR assays for *C. trachomatis* performed on patient- and clinician-obtained specimens

| No. of samples (total = 309) | Result by indicated test with specimen type: | | | |
|---|---|---|---|---|
| | Vaginal | | Cervical | |
| | Culture ($n$ = 16 (5.2%)) | LCR ($n$ = 46 [14.9%]) | Culture ($n$ = 39 [12.6%]) | LCR ($n$ = 44 [14.2%]) |
| 259 | − | − | − | − |
| 14 | + | + | + | + |
| 21 | − | + | + | + |
| 3 | − | − | + | + |
| 1 | + | − | + | + |
| 1 | + | + | − | − |
| 5[a] | − | + | − | + |
| 5[b] | − | + | − | − |

[a] Four of five samples were DFA positive, one remained MOMP probe LCR positive, and all were classified as true infections.
[b] Four of five samples were DFA positive, and one remained MOMP probe LCR negative.

[87.4%]) residents of Birmingham, Ala. Self-reported primary reasons for visiting the clinic were for symptom evaluation (175) because the women had had sexual contact with partners with STDs ($n$ = 87), because they were referred from other health care providers or because they had tested positive ($n$ = 22), or for other reasons, including STD screening (25). Seventy-one percent (218) of participants reported genitourinary symptoms. Other STDs were relatively common: 44 (14.2%) participants were culture positive for *Neisseria gonorrhoeae*, and 49 (15.9%) had trichomonas visualized by wet-mount microscopy.

Forty (12.9%) participants had positive cultures for *C. trachomatis* (Table 1). Cervical cultures were positive for 39 (97.5%) women, of whom 15 also had positive vaginal cultures; for one additional participant, only the self-obtained vaginal swab was culture positive. Thus, vaginal cultures were positive for only 16 (40%) of the 40 participants with positive *C. trachomatis* cultures.

The LCR assay was positive for *C. trachomatis* more often than cell cultures. Fifty participants had positive LCR tests, of which 49 were considered to be true positives based on the confirmation algorithm described above (Table 1); 45 (92%) of the 49 infected patients had positive vaginal swab specimens, while 44 (90%) had positive cervical swab specimens. For 40 (82%) participants swabs from both sites were chlamydia positive by LCR, while for 6 (12%) only vaginal swabs were positive (including one which could not be confirmed) and for an additional 4 (8%) only cervical swab specimens were positive by the LCR assay.

When the results of LCx assays and cultures were compared, all patients with *C. trachomatis* detected by cell culture also had positive results by at least one LCx assay. For 10 (20%) of 50 participants with positive LCx assays, however, both vaginal and cervical cell cultures were negative (Table 1). For five of these, both patient-obtained vaginal swab specimens and clinician-obtained cervical specimens were positive, while for five others, only the LCx assay vaginal specimen was *C. trachomatis* positive. With culture transport media from these 10 participants, eight specimens were positive for chlamydial elementary bodies by DFA. For one additional participant, a specimen used in a repeat LCR with MOMP gene primers was positive, confirming the presence of *C. trachomatis* genetic material in the specimen. Thus, of 50 participants with specimens from either site that were reactive by the LCx assay, there was only one for whom a positive LCx test result (from a patient-obtained vaginal swab) could not be confirmed by either culture ($n$ = 40), DFA ($n$ = 8), or repeat LCR with MOMP-directed probes ($n$ = 1).

The performance of cell culture and LCR was calculated by using any positive cell culture as a standard or, for culture-negative participants, a positive LCx test result confirmed by either a positive result by DFA or a positive result by the LCx assay with MOMP gene primers (Table 2). The sensitivities of cell culture with vaginal and endocervical specimens were 32.7 and 79.6%, respectively. The sensitivities of the LCR assay with vaginal and endocervical specimens were greater than for cell culture (vaginal swab specimens, 91.8%, and endocervical swabs, 89.8%). LCR specificities were likewise high: 99.6% for vaginal swab specimens and 100% for endocervical swab specimens.

Over the past decade, the reported number of *C. trachomatis* infections has steadily increased (4), in large part due to increased screening for asymptomatic infections. Chlamydial screening has been facilitated by the availability of nonculture tests which overcome many of the logistical constraints that make cell culture difficult to offer routinely in most settings (3). Amplified nucleic acid hybridization tests have repeatedly been found to be more sensitive than chlamydial cell culture (8, 10) and offer opportunities for improved control. First, by virtue of their superior sensitivities (8, 10), amplified nucleic acid-based tests may contribute to detection of more infections. Second, screening with patient-obtained vaginal swab specimens or urine (6, 7) may permit screening at locations where testing is not typically performed, such as school-based clinics, walk-in clinics, or other sites where difficulties in conducting pelvic examinations might prevent otherwise desirable diagnostic testing. Similarly, even in settings with facilities for pelvic examination, such as family-planning clinics, pelvic examinations are not always performed even though women return regularly to receive injectable contraceptives or medication refills. The ability to accurately diagnose chlamydial infections with either urine or self-obtained vaginal swabs could substantially simplify chlamydial screening.

In this study, patient-obtained vaginal swab specimens were culture positive for less than half of infected patients. These results resemble findings of prior studies which used vaginal tampons for collection of specimens for culture (9). In contrast, the sensitivity of LCR testing of patient-obtained vaginal swabs in detecting chlamydial infections equaled the sensitivity of testing with clinician-obtained endocervical swabs, suggest-

TABLE 2. Performance of cell culture and LCR for detection of *C. trachomatis* in patient-obtained vaginal and clinician-obtained cervical specimens

| Assay and specimen source | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) |
|---|---|---|---|---|
| Culture | | | | |
| Patient-obtained vaginal swabs | 32.7 | 100 | 100 | 88.7 |
| Clinician-obtained endocervical swabs | 79.6 | 100 | 100 | 96.3 |
| LCR | | | | |
| Patient-obtained vaginal swabs | 91.8 | 99.6 | 97.8 | 98.5 |
| Clinician-obtained endocervical swabs | 89.8 | 100 | 100 | 98.1 |

ing that for LCR, the type of specimen collection has a less profound effect on test results than has been the case for cell culture or for nonamplified, nonculture tests. Thus, vaginal swab specimens, like urine specimens (1, 2, 6, 7), appear to be useful alternatives to clinician-obtained specimens for chlamydia diagnosis. In the laboratory, however, vaginal swabs have several logistical advantages over urine as specimens. Vaginal swabs are more compact than urine specimens and do not require further aliquoting or centrifugation to prepare them for processing.

In summary, this study extends previous evaluations describing the high sensitivities and specificities of the LCR tests for *C. trachomatis* detection to include testing of vaginal swab specimens obtained by patients themselves. Use of patient-obtained specimens for *C. trachomatis* screening provides the opportunity to expand chlamydial screening to individuals who might otherwise not receive chlamydial tests, thereby augmenting control efforts.

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