Vulval Swabs as Alternative Specimens for Ligase Chain Reaction Detection of Genital Chlamydial Infection in Women

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A ligase chain reaction (LCR)-based assay was recently shown to be highly sensitive and specific for the detection of *Chlamydia trachomatis* not only in cervical specimens but also in first-void urine (FVU) specimens from women. The suitability of using vulval swabs as an alternative specimen that can be obtained by noninvasive means for the diagnosis of genital chlamydial infection by LCR was investigated. In a first study of 169 women, vulval, endocervical, and urethral swabs were tested by LCR, culture, and a combination of enzyme immunoassay (EIA) followed by confirmation by direct fluorescent-antibody assay (DFA), and the results were compared with those obtained by testing FVU specimens by LCR and EIA-DFA by using a specimen from an infected patient as a reference standard. Of the 169 women tested, 27 (16%) were shown to be infected. Whereas LCR showed high sensitivities with all specimen types (85.2% for vulval, urine, and endocervical specimens; 92.6% for urethral swabs), the sensitivities of culture and EIA-DFA were high only with endocervical swabs (74.1 and 70.4%, respectively), being 22.2 and 40.7%, respectively, with vulval swabs. In addition, urine testing by EIA-DFA also showed a poor sensitivity (48.1%). In order to further compare LCR performance with vulval specimens to that with FVU specimens, a second study was carried out with specimens from 312 women, of whom 26 were infected. Comparable sensitivity was obtained by LCR with vulval swabs (88.5%; 23 of 26) and FVU specimens (92.3%; 24 of 26). The results indicate that vulval swabs may serve as suitable alternative to specimens that can be obtained by noninvasive means for the detection of *C. trachomatis* by LCR.

Nucleic acid amplification by the ligase chain reaction (LCR) represents a highly sensitive and specific approach, compared with cell culture, for the detection of *Chlamydia trachomatis* in genital specimens from men and women (2–4, 6). LCR assay of first-void urine (FVU) specimens is highly effective in identifying urethral infections in men with or without symptoms of urethritis (3, 4, 9). Furthermore, LCR testing of FVU specimens detected up to 30% more infected women than culture of endocervical specimens, indicating that FVU is a suitable noninvasively obtained specimen for screening high-risk populations, especially populations of asymptomatic individuals, (1, 5, 7).

We have compared the performance of LCR with those of other assays for the detection of *C. trachomatis* infection with various specimen types, including vulval swabs, from women. Vulval swabs as well as swabs from the endocervical canal and the urethra, the sites of chlamydia infection and the traditional sources of specimens, were tested by LCR, culture, and enzyme immunoassay (EIA) confirmed by direct fluorescent-antibody assay (DFA); FVU specimens were tested by LCR and EIA-DFA, but not by culture because of the low sensitivity of the combination of the culture technique and FVU samples. Our primary goal was to determine whether the vulval region can serve as a site for noninvasive sampling so that vulval specimens could be substituted for FVU specimens in the detection of *C. trachomatis* infection in women.

MATERIALS AND METHODS

Study population. In the first study, specimens were collected from a selected high-risk group of 169 women attending an outpatient center for sexually transmitted diseases in Vienna, Austria, from April to November 1994. In a further study, 312 women were examined between January and September 1996. Persons enrolled in the study underwent a standard examination, including examinations for the diagnosis of genital chlamydial and gonococcal infections, as well as for other sexually transmitted pathogens.

Specimen collection and processing. In the first study, endocervical and urethral specimens were collected from each of the 169 women in random order with three different swabs for cell culture, EIA-DFA, and LCR. For consistency, specimen collection was restricted to two physicians. Excess mucus was removed with a cotton swab before collection of endocervical specimens. In addition, three separate swab specimens were collected by the physician from the vulval region and were placed into transport tubes containing sucrose buffer (2-SP) for cell culture or in plastic tubes, provided by the manufacturers, containing sample extraction buffer for EIA and LCR. The women were also instructed to collect ~20 to 30 ml of FVU in a sterile screw-cap plastic jar at the clinic after urogenital specimens had been taken. Urine specimens were divided into three portions, one each of which was processed for LCR and EIA-DFA and one of which was stored at −20°C for analysis in case of discrepant results. In the second study, one swab each was collected by the physician from the endocervical, urethral, and vulval sites from 312 women. FVU was collected by the women themselves. All four sample types were tested by LCR. In addition, the endocervical swabs were also tested by culture.

Cell culture. The cervical, urethral, and vulval specimens either were processed for cell culture within 24 h (78% of specimens) or were stored at −70°C (22% of specimens) for up to 1 week prior to culture. Chlamydiae were cultured for 48 to 72 h with monolayers of cycloheximide-treated McCoy cells in shell vials after a 1-h centrifugation at $3,000 \times g$. After fixing with 95% ethanol, the cells were stained with fluorescein-labelled monoclonal antibodies to *C. trachomatis* (MicroTrak; Syva, Palo Alto, Calif.). Specimens were considered positive if one or more inclusions were present. No blind passage was performed in the case of a negative culture.

LCR assay. The LCR assay was developed for commercial use by Abbott Laboratories (Abbott Park, Ill.) and has been described previously (3, 4, 9). Genital specimens were collected in LCR specimen collection tubes and were transported to the laboratory within 3 h of collection. Samples were stored at 4°C and were tested within 1 to 4 days or were stored at −70°C in transport buffer for up to 1 week before testing. FVU was vortexed, and a 1-ml portion was centrifuged at $11,000 \times g$ in a microcentrifuge for 15 min. The supernatant was

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discarded, and the pellet was resuspended in 1 ml of urine resuspension buffer. DNA was released from cells by heating the sample at 95°C for 15 min. A portion (100 µl) of the resulting lysate was added to the amplification reagent mixture, which was then placed in a thermocycler (model 480; Perkin-Elmer) and subjected to amplification for 39 cycles. Amplicons were detected by a microparticle-based EIA with an automated detection instrument (LCx; Abbott Laboratories).

EIA-DFA. Endocervical, urethral, and vulval specimens, as well as urine sediments, were tested for the presence of chlamydial antigens with the MicroTrak EIA (Syva) and the automatic MicroTrak XL processor. Swab and urine specimens were processed according to the manufacturer's instructions. The presence of elementary bodies in all specimens that gave positive or borderline EIA results was confirmed by the MicroTrak DFA with the pellet from the EIA tube after centrifugation (3,000 × g for 20 min at 4°C). For consistency, DFA results were assessed by only one technician. Only EIA-positive specimens with one or more elementary bodies confirmed to be positive by DFA were considered true positives.

Resolution of discrepant results and use of a sample from an infected patient as the expanded "gold standard". Diagnosis of urogenital chlamydial infection was based on the results for a sample from an infected patient, which was used as the standard, and was defined as a positive result for a sample from any one sampling site: at least one specimen must be positive by culture or, for culture-negative samples, positive by EIA (confirmed by DFA) or by LCR (confirmed either by DFA or by LCR with a different target sequence). The LCR assay used in the initial screening targeted a DNA sequence within the chlamydial cryptic plasmid. For confirmation testing, specimens were blinded and sent to Abbott Laboratories for assay by LCR with a target sequence located in the major outer membrane protein gene. The data obtained by all test methods for each sampling site were analyzed based on data for an infected patient as the reference standard.

RESULTS

Identification of women infected with *C. trachomatis*. In the first study, a total of 169 women were tested for *C. trachomatis* infection by using four specimens and three different techniques, with the exception that FVU specimens were not assayed by cell culture. Thus, 11 results were obtained for each subject. Examinations of the women for genital chlamydial infection were performed because of vaginal discharge (47.9%), contact tracing (23.2%), pelvic inflammatory disease (11.3%), treatment control (7%), or various other reasons (10.6%). Of the 169 women, all specimens from 142 subjects tested negative by all assays and the subjects were therefore considered not infected. On the basis of the expanded gold standard, 27 (16%) of the 169 women were shown to be infected with *C. trachomatis*. Of these 27 women, only from 1 woman were all specimens positive by all techniques, from 24 women at least one specimen type was positive by both LCR and culture, and 2 women were positive only by LCR. Of these last two women, both the urethral and urine specimens were positive by LCR for one woman and for one woman the endocervical and urethral specimens were low-level positive and the FVU specimen was borderline positive; the second of these two women was asymptomatic and was tested as a result of contact tracing, being the sexual partner of a chlamydia-infected individual. For both women, the positive results of the LCR assay were confirmed by DFA performed with the sediment of urethral swabs as well as by LCR with the major outer membrane protein target sequence.

Of the 27 women with a genital chlamydial infection, 17 (63%) were clinically asymptomatic and were screened for *C. trachomatis* because of contact tracing triggered by a chlamydia-positive sexual partner. Ten women (37%) had symptomatic chlamydial infections with either vaginal discharge ($n = 8$) or pelvic inflammatory disease ($n = 2$).

Variation of detection level of *C. trachomatis* infection by specimen type. The performance of LCR, culture, and EIA-DFA with the various specimen types is summarized in Table 1. Among the endocervical swabs from the 27 infected women, 23 were positive by LCR, 20 were positive by culture, and 19 were positive by EIA-DFA. Whereas a high concordance (18 of 27) between the three techniques was observed with endo-

cervical specimens, urethral and vulval swabs from only 10 and 4 women, respectively, tested positive by all three assays. With urethral swabs, LCR detected 25 of 27 infected women, whereas culture or EIA-DFA gave a positive result for only 14 women. Of the 169 vulval specimens tested, 146 were negative and 4 were positive by all test procedures; 23 (85.2%) of the vulval swabs from the 27 women were positive by LCR, 11 (40.7%) were positive by EIA-DFA, but only 6 (22.2%) were positive by culture. Whereas the number of women with infections detected by LCR of FVU specimens was the same as the number with infections detected by LCR of endocervical swabs (23 of 27), urine testing by EIA-DFA had a low detection rate (13 of 27).

As indicated in Table 2, the rate of detection of chlamydia-infected women, or the sensitivity, by the LCR assay was high for all specimen types, ranging from 85 to 93%. In contrast, large differences in the detection rate were observed for culture of the samples from the three sites, ranging from 74% for those from the endocervix to 52% for those from the urethra and 22% for those from the vulva. For EIA-DFA, the highest detection rate was obtained with endocervical swabs (70%), followed by urethral (52%) and urine (48%) specimens; as with culture, the lowest sensitivity of EIA-DFA was apparent with vulval specimens (41%). The specificity for all tests or test combinations was 100%.

Performance of LCR with vulval swabs compared to that with urine or endocervical swabs. In order to further assess the suitability of vulva as an alternative site from which to obtain samples for LCR, a second study involving 312 women was carried out. Endocervical swabs were tested by both LCR and culture. In addition, vulval swabs and FVU specimens were tested by LCR only. Of the 312 women tested, 286 were negative by both methods with all sample types (Table 3). A total

TABLE 1. Detection of *C. trachomatis* infection in 169 women by LCR, cell culture, and EIA of various urogenital swab and urine specimens

Test result for urogenital specimens			No. of samples from the following urogenital sampling site:			Test result for urine specimens		No. of FVU specimens[a]
LCR	Culture	EIA	Endocervix	Urethra	Vulva	LCR	EIA	
−	−	−	146	144	146	−	−	145
+	+	+	18	10	4	+	+	12
+	+	−	2	4	2	+	−	11
+	−	+	1	4	7	−	+	1
+	−	−	2	7	10			

[a] Culture testing of FVU not done.

TABLE 2. Sensitivity and negative predictive value of LCR, culture, and EIA-DFA for the detection of *C. trachomatis* in urogenital specimens[a]

Specimen	Sensitivity (%)			Negative predictive value (%)		
	LCR	Culture	EIA-DFA[b]	LCR	Culture	EIA-DFA
Endocervical	85.2	74.1	70.4	97.3	95.3	94.7
Urethral	92.6	51.9	51.9	98.6	91.6	91.6
Vulvar	85.2	22.2	40.7	97.3	87.1	89.9
Urine	85.2	ND[c]	48.1	97.3	ND	91.0

[a] For all test methods, specificity and positive predictive value were 100%.
[b] Initial EIA results were confirmed by DFA. Results were calculated on the basis of results for samples which were both EIA and DFA positive.
[c] ND, not done.

TABLE 3. Detection of *C. trachomatis* infection in 312 women by LCR and cell culture of urogenital specimens and urine[a]

No. of specimens	Culture result with endocervical specimen	LCR result with the following specimens:		
		Endocervical	Vulvar	Urine
286	−	−	−	−
13	+	+	+	+
9	−	+	+	+
1	−	+	+	−
2	−	−	−	+
1	−	+	−	−

[a] Of the 312 specimens tested, 13 endocervical specimens were positive by culture and 24 endocervical specimens, 23 vulvar specimens, and 24 urine specimens were positive by LCR.

of 26 women were found to be infected; all samples from 13 of these women were positive by both methods. Culture of endocervical specimens detected 13 of 26 infected women, whereas LCR testing identified a greater number of positive individuals and showed sensitivities similar to those obtained with FVU specimens (24 of 26) and with samples taken from the endocervix (24 of 26) and vulva (23 of 26).

DISCUSSION

Our data confirm the results of previous studies showing that testing of urine by LCR detects more women infected with *C. trachomatis* than does culture of endocervical swabs (1, 3, 5, 7). Comparison of LCR, culture, and EIA-DFA with four different specimen types has revealed that LCR has a high sensitivity, irrespective of specimen type, whereas culture and EIA-DFA detected a relatively high percentage of infected women only when endocervical swabs were tested. Furthermore, LCR detected of vulval swabs as many infected women as LCR of endocervical swabs or FVU specimens. Thus, it appears that the vulval region of women infected with *C. trachomatis* is contaminated with infected cellular material in the same manner as urine is. Recently, PCR analysis of vaginal introitus specimens was shown to be more sensitive than culture of endocervical swabs for the detection of *C. trachomatis* infections, demonstrating that the vaginal introitus may also represent an alternative sampling site from which samples can be obtained by noninvasive means for the detection of *C. trachomatis* (10). The low sensitivity of culture with vulval swabs may be attributable to the fact that no blind passage was performed, with 20% of the samples frozen at −70°C prior to culture. However, it is also likely to be due to the presence of only a low number of viable organisms, given that the vulva does not represent the site of infection. As with FVU, vulval specimens therefore do not appear suitable for use in the diagnosis of chlamydial infection by culture (8). Although antigen testing does not require viable organisms, the low sensitivity (41%) of EIA-DFA with vulval swabs presumably reflects the poor performance of this detection method with specimens that contain a small number of elementary bodies. Although with endocervical specimens it showed a performance similar to that of culture, EIA-DFA of all other specimen types failed to detect about 50% of infected women. In contrast to culture and an-

tigen detection, the sensitivity of LCR is not influenced by either the viability of the organism or the presence of a low number of infectious particles as a result of the ability of this technology to amplify even a small amount of chlamydial DNA. However, despite the high concordance of LCR-positive or -negative results with all specimen types, testing of only a single specimen type by using an amplification technology such as LCR nevertheless failed to detect all infected women in our study. Indeed, by using the results for an infected patient as the expanded gold standard, the sensitivity of LCR with any single sample type (FVU specimens or urogenital swabs) was found to be in the range of 87 to 92%.

In summary, our results indicate that vulval swabs may be suitable specimens that can be obtained by noninvasive means for the detection of *C. trachomatis* by nucleic acid amplification-based methods such as LCR, but not by culture or EIA-DFA. Indeed, vulval swabs may be more suitable than FVU specimens for screening high-risk individuals who are not willing to undergo a urine examination by LCR, because testing of urine requires a centrifugation step to prepare the sediments in FVU specimens for assay. As with urine collection, sampling of the vulva may be performed by the women themselves, an important factor for screening of at-risk young women for genital chlamydial infection. By avoiding a speculum examination, swabbing of the vulva may increase a patient's willingness to undergo a screening examination and may represent an accurate and cost-effective approach to the diagnosis of chlamydial infection, particularly in asymptomatic women.

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