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CLINICAL REPORT

# Cervical, Urine and Vaginal Specimens for Detection of *Chlamydia trachomatis* by Ligase Chain Reaction in Women: A Comparison

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**Screening for *Chlamydia trachomatis* in women is generally done using only one specimen from each patient in order to minimize costs. In this study the aim was to compare the performances of vaginal, cervical and urinary specimens in a population of young women with sparse symptoms. During 1998, specimens from 1,001 women at the Departments of Venereology and Youth Health Care at the University Hospital of Uppsala, Sweden were examined by both ligase chain reaction and cell culture for detection of *C. trachomatis*. The samples from the cervix, vagina and urine were tested by ligase chain reaction, while specimens for cell culture were collected from the cervix and urethra. The prevalence of genital *C. trachomatis* infections was 5.1%. A single urine specimen had a sensitivity of 80.0%, while the sensitivity of a single vaginal specimen was 96.0%. The specificity was 100% for the urine specimens and 99.4% for the vaginal specimens. The sensitivity and specificity of a single cervical specimen was 92.0% and 99.6%, respectively. Although the urine ligase chain reaction seemed to have the lowest sensitivity of the compared specimens for testing of *C. trachomatis* infections in this population, the differences in sensitivity between urine, cervical and vaginal specimens were not statistically significant.** ***Key words: Chlamydia trachomatis; urine; cervix; vagina; ligase chain reaction.***

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For many years cell culture has been used as the "golden standard" for detection of *Chlamydia trachomatis* in genital specimens. The introduction of enzyme immunoassays (EIAs) made it possible to detect the bacteria in urine, which facilitated sampling from men (1, 2). Several trials using EIAs on women's urine failed, as the sensitivity reported was too low (3). The development of nucleic acid amplification techniques has, however, increased the sensitivity of detection levels for *C. trachomatis* (4, 5), with male urine samples becoming attractive targets for many investigators. Both polymerase chain reaction (PCR) and ligase chain reaction (LCR) have shown high sensitivity when urine samples were used (6, 7). Many studies have shown an excellent performance of these techniques also on female urine (4, 5, 8, 9), although results in pregnant women have been somewhat conflicting (10, 11).

Lately, sampling from the vagina, as an alternative to urine or cervical samples, has been introduced and has been shown to be highly sensitive for detection of *C. trachomatis* in women (12–16). This approach represents a very simple sampling technique and will probably be highly acceptable to women since, as for urine, the specimen can be obtained by the patient herself.

This study was designed to compare the sensitivity and specificity of different sampling techniques in young Swedish women living in an urban area. All the samples in this study were collected by a clinician.

## MATERIAL AND METHODS

### *Study population*

Specimens during speculum examination were collected from 1,001 women (aged 15–53 years, mean 22.9 years, median 21 years) consulting either the Venereology Department or the Youth Health Clinic at the University Hospital of Uppsala, Sweden, during May–October 1998. The study includes patients presenting consecutively at the clinics during that period. All the women presenting at the Venereology Department were sexually active. Most of them were involved in a steady relationship with one man and did not have a history of any sexually transmitted disease (STD). About 50% presented for a normal checkup and did not have any symptoms.

The patients at the Youth Health Clinic were all below 20 years of age and most of them were sexually active. Most were free from symptoms and came for a routine checkup and/or a prescription for contraceptive pills. None of the patients had a history of any STDs. There were no prostitutes visiting either clinic.

### *Specimen collection*

*Chlamydial culture.* Specimens from the urethra and cervix were sampled using alginate *C. trachomatis* culture swabs (Biohospital AB, Kopparberg, Sweden). The swabs were immediately placed in 2-SP medium and transported to the laboratory on the same day. The samples were stored for a maximum of 2 days at +4°C before cultivation.

*Ligase chain reaction (LCR).* Samples from the cervix and vagina were taken with the LC × sampling kit (Abbott Laboratories, Chicago, IL, USA). A first-catch urine specimen was collected in a 10 ml sterile plastic tube. All LCR specimens were stored at +4°C and processed the same day or on the day following sampling. The order of sampling was randomized by taking the specimens for cell culture first in the first 500 women and the specimens for LCR first in the remaining patients.

The cervical swab was inserted about 1 cm into the cervix and rotated for about 15 s. The vaginal specimen was obtained by rotating the swab through at least the lower half of the fornix.

### *Laboratory procedures*

*Cell culture.* The cervical and urethral samples were inoculated into McCoy cells grown in 24-well plates, as described by Ripa & Mårdh (17). After 48-h incubation the wells were stained with FITC-conjugated monoclonal antibodies to *C. trachomatis* (MikroTrak *Chlamydia trachomatis* Culture Confirmation Test, Trinity Biotech plc., IDA

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Business Park, Bray, Co. Wicklow, Ireland) and examined microscopically in an inverted luminescent microscope (Zeiss Axiovert, Germany) at a magnification of ×400. Specimens producing at least one inclusion per well were regarded as positive.

*Ligase chain reaction assay.* The LCR procedure was performed as recommended by the manufacturer (Abbott Laboratories, Chicago, IL, USA). Values within 10% from the cutoff value were considered as equivocal. Positive and equivocal results were consistently confirmed by a second run. Equivocal results that were positive in the confirmation run were considered as positive results.

### *Confirmation of discrepant results*

All specimens found positive in cell culture were considered as true positive. All specimens that were equivocal or positive in at least one LCR run giving a negative cell culture result were sent to the Abbott Laboratories, Chicago, IL, USA, to be retested using *C. trachomatis* major outer membrane protein (MOMP)-based LCR. Specimens testing positive with MOMP-LCR were considered to be true positive.

Since LCR has a higher sensitivity than both cell culture and MOMP-based amplification techniques, specimens found repeatedly as positive by LCR were also considered true positive if the patient had a repeatedly positive LCR result in at least one other sampling site, irrespective of the culture or MOMP-LCR result.

To summarize, a positive LCR result was considered true positive if:

(a) the patient had a positive cell culture; or
(b) the specimen was positive with MOMP-LCR; or
(c) the patient repeatedly had positive LCR results from at least one other sampling site.

A patient who had a confirmed positive result in any sample, was considered to be a true case of *Chlamydia* infection.

### *Statistical analyses*

Sensitivity, specificity, positive predictive values and negative predictive values were calculated using the JMP computer program (SAS Institute Inc., NC, USA). The equivocal results were regarded as positive in the calculation of these values; $\chi^2$ analysis of independent proportions was used for studies of significance of difference.

## RESULTS

A total of 1,001 women gave their consent to participate in the study. For culture of *C. trachomatis*, cervical samples were obtained from all the women and urethral samples from 991. Altogether, 1,000 cervical, 1,000 vaginal and 980 urine samples were subjected to LCR testing. All five specimens were obtained from 978 women, who finally constituted our study population.

In total, 50 patients had at least one sample confirmed as true positive, so that the total prevalence among the population tested was 5.1%.

Among these 50 women, 22 had a mild cervicitis and/or urethritis with sparse discharge. The other 28 patients had no clinical signs of genital infection.

The cervical cultures were found to be positive in 35 and the urethral cultures, in 34 patients. In total, *C. trachomatis* was isolated from the cervix, urethra, or both, in 41 patients, which gives a sensitivity of 82%. Using cell culture, the prevalence of *C. trachomatis* was therefore estimated to be 4.2%. All culture-positive patients were also found positive by LCR at at least one site.

After completion of all the confirmatory steps, 45 cervical, 39 urine and 47 vaginal specimens were confirmed as true positive LCR results (Table I). The LCR assay on cervical specimens alone missed 4 patients; on urine samples alone 10 patients and on vaginal samples alone 2 patients were missed, who were confirmed to be true *Chlamydia* cases. Three patients were positive only in cervical LCR while 5 patients were positive only in vaginal LCR. One cervical, one urine and one vaginal sample, which yielded equivocal results when tested by LCR, became positive when retested by MOMP-LCR. One cervical and one vaginal sample gave equivocal results with both LCR methods. Of the 13 specimens found positive by LCR in only one run, only one (a urine specimen) was confirmed as positive.

A combination of a cervical and a vaginal sample, as well as of a urine and a vaginal sample found 49 (98%) of the 50 true-positive patients. The combination of a urine and a cervical sample found 48 (96%) of the true-positive patients.

The sensitivity, specificity and positive and negative predictive values for the different sampling sites and methods are shown in Table I. The highest sensitivity after the resolution was noted when cervical and vaginal samples were examined (92.0% and 96.0%, respectively). The urine samples showed the lowest sensitivity (80.0%), even below that of cell culture (82%). Although the differences between the calculated sensitivities for urine specimens and cervical/vaginal specimens seem to be large, they do not reach statistical significance (urine versus vaginal specimen, $p > 0.5$). All test methods had high specificities and positive and negative predictive values at all the sites tested, although the positive predictive values of urine LCR were greater than those of LCR on cervical and vaginal samples. The vaginal LCR had the lowest positive predictive value.

## DISCUSSION

The results of this study show a very high sensitivity and specificity for both cervical and vaginal specimens for the detection of *C. trachomatis* in women. The sensitivity for urine specimens was somewhat lower, even lower than that for culture from the urethra and cervix, although the specificity was very high. It is, however, important to note that positive specimens should be repeated to achieve a high specificity, since only 1 out of 13 specimens positive in only one run could be confirmed. This is of great importance when testing is done in a low-prevalence population, as discussed by Östergaard (18).

Many studies have shown an excellent performance of the urine specimens in detecting chlamydial infection, especially in males (see, e.g. 6, 7). Other studies, however, have found a lower sensitivity for urine than for other specimens from women (10, 14, 16). It is important to use the same technique when comparing the usefulness of different sampling sites. Studies that compare urine LCR with cell culture from the cervix and/or urethra usually show a superior performance of the LCR (6, 19) as the DNA amplification methods are more sensitive than the cell culture, while studies that use the same detection method have reported different results (9, 10, 12, 14, 16).

In this study we compared different specimen sampling locations, using the same technique. Cell culture and MOMP-LCR are used only as confirmation tests. The majority of the women seeking medical care at the Venereology Department and Youth Health Clinic had no signs of cervical inflammation. Moderate inflammation, where present, resulted in a small amount of discharge into the urine. We may assume that this could explain why the urine samples were not so sensitive in

Table I. *The ligase chain reaction results obtained from testing genital samples from 978 women for* Chlamydia trachomatis

| | No. of specimens | No. of confirmed positive patients[a] | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) |
|---|---|---|---|---|---|---|
| LCR cervix | | | 92.0 | 99.6 | 92.0 | 99.6 |
| Positive[b] | 48 | 45 | | | | |
| Negative | 928 | 4 | | | | |
| Equivocal | 2 | 1 | | | | |
| LCR vagina | | | 96.0 | 99.4 | 88.9 | 99.8 |
| Positive | 52 | 47 | | | | |
| Negative | 924 | 2 | | | | |
| Equivocal | 2 | 1 | | | | |
| LCR urine | | | 80.0 | 100 | 100 | 98.9 |
| Positive | 39 | 39 | | | | |
| Negative | 938 | 10 | | | | |
| Equivocal | 1 | 1 | | | | |

[a]The figures show the number of patients in each group that had been confirmed by any sample to be a true case of *Chlamydia* infection. See Material and Methods for determination of true results.
[b]Only results that were repeatedly positive in ligase chain reaction (LCR) or positive in one run but confirmed by another method are included.

this study. Urine may also contain inhibitors of the DNA amplification process, which could affect the sensitivity (11). The difference was, however, not statistically significant, which makes it impossible to rule out the urine specimen as useful for screening.

The advantage of urine and vaginal specimens over cervical specimens is that the former are easily collected, even by the patient herself (15, 16, 20, 21), and in asymptomatic patients this could be encouraged. It should be pointed out, however, that women with symptoms should be gynaecologically examined, as the symptoms experienced by the patient may have many other causes than a chlamydial infection.

A combination of two samples increased the sensitivity to at most 98% but none of the combinations of two samples reached 100% sensitivity. It is doubtful whether an increase in sensitivity from 96%, which was obtained with vaginal LCR only, to 98% with a combination of specimens motivates the increase of the costs for multiple sampling.

One of the patients who was tested as positive by LCR in both the vagina and the cervix, but for whom the results were not confirmed by cell culture or MOMP-LCR, had had a chlamydial infection one month previously and was then treated with doxycycline. The present positive LCR result could be due either to a small amount of DNA still present in the patient's tissue despite treatment, or to a new infection. As the patient was involved in a steady relationship and had no clinical signs of infection, it is probable that the former applies. Gaydos et al. (22) performed a follow-up study after treatment of chlamydial infection in 33 women and found that none of the patients had positive PCR or LCR 15 days after treatment. These authors used only a urine specimen for the follow-up.

To conclude, we could not find any statistically significant difference in performance between urine, cervical and vaginal samples for *C. trachomatis* testing in a low-prevalence population.

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## REFERENCES

1. Domeika M, Bassiri M, Mårdh P-A. Enzyme immunoassay and direct immunofluorescence for detection of *Chlamydia trachomatis* antigen in male first-void urine. Acta Derm Venereol 1994; 74: 323–326.
2. Matthews RS, Pandit PG, Bonigal SD, Wise R, Radcliffe KD. Evaluation of an enzyme-linked immunoassay and confirmatory test for the detection of *Chlamydia trachomatis* in male urine samples. Genitourine Med 1993; 69: 47–50.
3. Chernesky M, Gastriciano S, Sellors J, Stewart I, Landis S, Seidelman W, et al. Detection of *Chlamydia trachomatis* antigen in urine as an alternative to swabs and culture. J Infect Dis 1990; 161: 124–126.
4. Loeffelholz M, Lewinsky C, Silver S, Purohit A, Herman S, Buanagurio D, et al. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. J Clin Microbiol 1992; 30: 2847–2851.
5. Bassiri M, Hu H, Domeika M, Burzak J, Svensson L, Lee H. Detection of *Chlamydia trachomatis* in urine specimens from women by ligase chain reaction. J Clin Bact 1995; 33: 898–900.
6. Chernesky M, Lang D, Lee H, Burczak J, Hu H, Sellors J, et al. Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. J Clin Microbiol 1994; 32: 2682–2685.
7. Jaschek G, Gaydos C, Welsh L, Quinn T. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain assay. J. Clin Microbiol 1993; 31: 1209–1212.
8. Pasternak R, Vuorinen P, Pitkäjärvi T, Koskela M, Miettinen A. Comparison of manual Amplicor PCR, Cobas Amplicor PCR and LC× assays for detection of *Chlamydia trachomatis* infection in women by using urine specimens. J Clin Microbiol 1997; 35: 402–405.
9. Doornum G, Buimer M, Prins M, Henquet C, Coutinho A, Plier P, et al. Detection of *Chlamydia trachomatis* infection in urine samples from men and women by ligase chain reaction. J Clin Microbiol 1995; 33: 2042–2047.
10. Jensen I, Thorsen P, Moller B. Sensitivity of ligase chain reaction assay of urine from pregnant women for *Chlamydia trachomatis*. Lancet 1997; 349: 329–330.
11. Gaydos C, Rene H, Quinn T, Gaydos J, McKee Jr K. Use of ligase chain reaction with urine versus cervical culture for detection of *Chlamydia trachomatis* in an asympotomatic military population of pregnant and non-pregnent females attending Papanicolaou smear clinics. J. Clin Microbiol 1998; 36: 1300–1304.

12. Thomas B, Pierpoint T, Taylor-Robinson D, Renton A. Sensitivity of the ligase chain reaction assay for detecting *Chlamydia trachomatis* in vaginal swabs from women who are infected at other sites. Sex Transm Inf 1998; 74: 140–141.
13. Witkin S, Inglis S, Polaneczky M. Detection of *Chlamydia trachomatis* and *Trichomonas vaginalis* by polymerase chain reaction in introital specimens from pregnant women. Am J Obstet Gynecol 1996; 175: 165–167.
14. Wiesenfeld H, Heine R, Macio I, DiBiasi F, Sweet R. Vaginal introitus: a novel site for *Chlamydia trachomatis* testing in women. Am J Obstet Gynecol 1996; 174: 1542–1546.
15. Östergaard L, Möller J, Andersen B, Olesen F. Diagnosis of urogenital *Chlamydia trachomatis* infection in women based on mailed samples obtained at home: multipractice. BMJ 1996; 313: 1186–1189.
16. Domeika M, Bassiri M, Butrimiene I, Venalis A, Ranceva J, Vasjanova V. Evaluation of vaginal introital sampling as an alternative approach for the detection of genital *Chlamydia trachomatis* infection in women. Acta Obstet Gynecol Scand 1999; 78: 131–136.
17. Ripa T, Mårdh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. Clin Microbiol 1977; 6: 328–331.
18. Östergaard L. Detection of *Chlamydia trachomatis* in a low prevalence population. Eur J Clin Microbiol Infect Dis 1995; 14: 471–472.
19. Schachter J, Moncada J, Whidden R, Shaw H, Bolan G, Burzak J, et al. Noninvasive tests for diagnosis of *Chlamydia trachomatis* infection: application of ligase chain reaction to first-catch urine specimens of women. J Infect Dis 1995; 172: 1411–1414.
20. Polaneczky M, Quigley C, Pollock L, Dulko D, Witkin S. Use of self-collected vaginal specimens for detection of *Chlamydia trachomatis* infection. Obstet Gynecol 1998; 91: 375–378.
21. Domeika M, Drulyte O. Use of PCR for the detection of genital *Chlamydia trachomatis* infection on self-obtained mailed vaginal samples. Acta Obstet Gynecol Scand 2000; 79: 570–575.
22. Gaydos C, Crotchfelt K, Rene H, Kralia S, Hauptman P, Quinn T. Molecular amplification assays to detect chlamydial infections in urine specimens from high school female students and to monitor the persistence of chlamydial DNA after therapy. J Infect Dis 1998; 177: 417–424.