

## Detection of *Chlamydia trachomatis* Infections in Women by Amplicor PCR: Comparison of Diagnostic Performance with Urine and Cervical Specimens

RAFAEL PASTERNAK,<sup>1\*</sup> PAULI VUORINEN,<sup>1</sup> AKI KUUKANKORPI,<sup>2</sup> TIMO PITKÄJÄRVI,<sup>2,3</sup>  
AND ARI MIETTINEN<sup>1</sup>

Department of Clinical Microbiology, Tampere University Hospital, FIN-33521,<sup>1</sup> Tampere Community Health Centre, FIN-33100,<sup>2</sup> and Medical School, University of Tampere, FIN-33101,<sup>3</sup> Tampere, Finland

Received 20 September 1995/Returned for modification 9 November 1995/Accepted 18 January 1996

**We used the Roche Amplicor PCR assay to compare urine and cervical swabs as sample material in the detection of *Chlamydia trachomatis* causing genital infections. The diagnostic performance of Amplicor PCR was compared with that of cell culture and the Gen-Probe PACE 2 assay with cervical specimens. If discrepant from other results, the specimens negative by PCR were diluted and reanalyzed to reveal PCR inhibitors. Of 666 patients, 39 (5.9%) were confirmed to have chlamydial infection. The respective sensitivity and specificity of Amplicor PCR were as follows: urine specimens, 82.0 and 99.7%; cervical specimens, 82.0 and 99.8%. Those for cell culture with cervical specimens were 84.6 and 100%. For the Gen-Probe PACE 2 assay, the sensitivity and specificity with cervical specimens were 79.5 and 100%, respectively. Without the effect of PCR inhibitors, the sensitivity of PCR with urine would have been 97.4%. Provided that the problems currently caused by inhibitors will be solved, the Amplicor PCR assay with urine specimens offers a tempting alternative for the diagnosis of *C. trachomatis* infection in women.**

Methods based on amplification of the genetic material have substantially increased the sensitivity of chlamydial detection. The PCR assay (Roche Amplicor PCR) has been commercially available for some time. An alternative amplification system based on ligase chain reaction has been introduced quite recently. Because of the increased sensitivity of detection, these methods seem to make possible the diagnosis of chlamydial infections by analysis of urine specimens. In males, the detection of *Chlamydia trachomatis* from first-catch urine by PCR has already been demonstrated to be more sensitive than that by cell culture of urethral swabs (2, 5, 13). In recent studies, both PCR and ligase chain reaction with urine samples from women have been found to be as sensitive as cell culture with cervical specimens (7, 11).

Most studies assessing the test performance of nucleic acid amplification methods in women have been carried out by comparing the results with those obtained by culture of cervical swab specimens. Considering the fact that a certain proportion (5 to 30%) of chlamydial infections can only be detected in the urethra (8, 9), this approach may obviously lead to misleading conclusions regarding diagnostic sensitivity. So far, few studies have been published specifically aimed at determining the ideal sample material for women with respect to the greatest sensitivity achievable by the new methods.

The use of cell culture as the "gold standard" for the detection of chlamydia has been questioned. Because of the potentially superior sensitivity obtainable by nucleic acid amplification methods, it is evident that culture-negative specimens positive by some other method should be confirmed by another reference method. Different combinations of test methods are currently used for this purpose (12). The use of such expanded gold standards is also necessary in addressing the problem caused by inhibitors of the amplification reactions. Without a

proper standard, the frequency of inhibition is likely to be underrated.

In this study, we compared the diagnostic performance of the Roche Amplicor PCR with urine and cervical swab specimens with that of cell culture and the Gen-Probe PACE 2 assay with cervical swab specimens. Our purpose was to determine the ideal sample material for PCR with reference to the frequency of PCR inhibitors in urine and cervical swab specimens.

The patients consisted of 666 women. They were examined and treated at the outpatient clinics of the Community Health Centre of the City of Tampere, Tampere, Finland, during the period November 1994 to May 1995. The study protocol had been approved by the ethical committees of Tampere University Hospital and the city of Tampere. Informed consent was obtained from all patients verbally.

Urine samples were collected in a clean cup prior to the cervical sampling and transferred to a sterile container. Endocervical swab specimens were taken in a randomly selected order with ENT-swabs (Medical Wire & Equipment Co. Ltd., Gorsham, Wiltshire, United Kingdom), chlamydia transit tubes (Labsystems Ltd., Helsinki, Finland), and the Gen-Probe specimen collection kit for cervical specimens (Gen-Probe, Inc., San Diego, Calif.). Specimens were transported to the laboratory on the day of collection. Once at the laboratory, urine specimens for PCR assay were prepared as instructed by the manufacturer, frozen at  $-70^{\circ}\text{C}$ , and analyzed within 3 weeks of sampling. Chlamydia transit tubes were thoroughly mixed, and 100  $\mu\text{l}$  was withdrawn and diluted to 1 ml with sucrose-phosphate medium. After that, 1 ml of swab specimen diluent (F. Hoffmann-La Roche Ltd., Basel, Switzerland), was added and the prepared specimens were frozen at  $-70^{\circ}\text{C}$  for PCR. The culture specimens were immediately cultured on McCoy cells or frozen at  $-70^{\circ}\text{C}$  and cultured within 1 week. Specimens for the Gen-Probe PACE 2 assay were stored at  $4^{\circ}\text{C}$  and analyzed within 3 days.

The Roche Amplicor PCR assay (F. Hoffmann-La Roche

\* Corresponding author. Mailing address: Department of Clinical Microbiology, Tampere University Hospital, Box 2000, FIN-33521, Tampere, Finland. Phone: 358-31-2475296. Fax: 358-31-2475260.

TABLE 1. Analysis of discrepant test results by Roche Amplicor PCR, cell culture, and Gen-Probe PACE 2 assay

No. of specimens	Result by:				Final result and conclusion
	PCR with:		Culture with cervix	PACE 2 with cervix	
	Urine	Cervix			
4	Positive	Negative/negative <sup>a</sup>	Negative	Negative	Positive. Infection was detectable only by PCR with urine and confirmed by MOMP PCR.
2	Positive	Negative/negative	Negative	Negative	Negative. Positive PCR result with urine could not be confirmed by MOMP PCR.
5	Negative/positive	Positive	Positive	Positive	Positive. PCR inhibitors were present in urine.
1	Negative/positive	Positive	Positive	Negative	Positive. Infection could not be found by PCR with urine.
1	Negative/negative	Positive	Positive	Positive	
1	Positive	Negative/positive	Positive	Positive	Positive. PCR inhibitors were present in cervix.
1	Negative	Positive	Negative	Negative	Negative. Positive PCR result with cervical swab specimen could not be confirmed by MOMP PCR.
1	Positive	Positive	Negative	Positive	Positive. Infection was not detected in cervix by cell culture.
1	Positive	Negative/negative	Negative	Positive	Positive. Infection was not detected in cervix by cell culture and PCR.

<sup>a</sup> Initial result/result from reanalysis of an undiluted or diluted specimen.

Ltd.) with urine and endocervical specimens for culture was performed according to the manufacturer's instructions. The PCR was accomplished with the Perkin-Elmer Thermocycler TC 9600 (Perkin-Elmer Cetus, Norwalk, Conn.). After the amplification, the amplified nucleotide sequences were detected by an enzyme immunoassay and the  $A_{450}$ s were measured with a spectrophotometer (Multiskan; Labsystems Ltd.). Specimens with an  $A_{450}$  of  $\geq 0.250$  were considered positive. Specimens that were negative by PCR but positive either by cell culture or by the PACE 2 assay were further diluted 1:10 with Roche dilution buffer (F. Hoffmann-La Roche Ltd.) and reanalyzed both diluted and undiluted to eliminate the effects of possible PCR inhibitors.

The cell culture was performed by inoculation of 500  $\mu$ l of the specimen in sucrose-phosphate medium onto a monolayer of McCoy cells in flat-bottomed, plastic shell vials with coverslips. The specimens were centrifuged (3,000  $\times$  g) at 35°C for 1 h. The medium was then changed to a medium containing 1  $\mu$ g of cycloheximide per ml (10). After an incubation of 3 days, the cells were stained with iodine and screened for inclusions. If one or more typical inclusions were seen, the specimen was interpreted as positive.

The Gen-Probe PACE 2 RNA hybridization assay was performed according to the manufacturer's instructions. After RNA-DNA hybridization, the chemiluminescence signals were recorded by a LEADER-50 luminometer (Gen-Probe, Inc.). In each series, the cutoff was set relative to the mean chemiluminescence value of the three negative controls as instructed.

Urine or cervical specimens positive by PCR from patients who were negative by cell culture on cervical swabs were subjected to a confirmatory PCR assay with major outer membrane protein (MOMP)-based primers as described previously (4). The MOMP PCR was performed by Roche Laboratories (Roche Molecular Systems, Basel, Switzerland). The probe competition assay (Gen-Probe, Inc.) was used to verify specimens giving low positive chemiluminescence values in the Gen-Probe PACE 2 test.

A specimen was considered positive if it was cell culture

positive. In addition, positive results by the PACE 2 assay for patients who were positive by PCR with urine or a cervical swab were considered to be true positive, as were those confirmed by the probe competition assay. Specimens that were positive by PCR only were considered to be true positive if they could be verified by MOMP PCR. The calculations of sensitivity and specificity were based on the total number of confirmed chlamydial infections detected.

The results of all four tests were identical for 649 (97%) of the 666 patients. Altogether 39 (5.9%) of the patients had chlamydial infection by definition. Among them, the test results were uniformly positive for 25 patients. The discrepant results from 17 patients are presented in Table 1. Of the 17 patients, 14 could be confirmed as positive by definition. The PCR with urine gave positive results for six patients who were found to be negative in all of the tests performed with cervical specimens. In four of them, the presence of chlamydial infection could be confirmed by MOMP PCR. For two patients, the positive PCR result with urine could not be confirmed by MOMP PCR. They were taken as false positive, by definition, by PCR with urine.

In contrast, there were seven patients who gave negative results by PCR with urine but were positive in the cervix by two or three test methods. When the urine specimens from these patients were reanalyzed by PCR with undiluted or diluted urine, six of the seven initially negative urine specimens turned positive, indicating the presence of PCR inhibitors. Of the six, five turned positive after freezing and thawing and one required dilution. In one patient, the negative PCR result could not be accounted for by PCR inhibition.

PCR inhibitors were also demonstrated in one cervical specimen from a patient who gave positive results by cell culture and the PACE 2 assay with cervical swab specimens and by PCR with urine. There was one PCR-positive cervical specimen that could be confirmed by neither MOMP PCR nor any of the other test methods used. It was taken as false positive, by definition, by PCR.

Cervical culture missed two cases of infection that gave

TABLE 2. Comparison of Roche Amplicor PCR with urine and cervical swab specimens with cell culture and Gen-Probe PACE 2 assay with cervical swab specimens in detection of *C. trachomatis* from 666 women

Test and result	No. of specimens		% Sensitivity	% Specificity
	Positive	Negative		
Amplicor PCR				
Urine				
Positive	32	2	82.0	99.7
Negative	7 <sup>a</sup>	625		
Endocervical swab				
Positive	32	1	82.0	99.8
Negative	7 <sup>b</sup>	626		
Cell culture (endocervical swab)				
Positive	33	0	84.6	100
Negative	6	627		
PACE 2 (endocervical swab)				
Positive	31	0	79.5	100
Negative	8	627		

<sup>a</sup> Of the seven urine specimens, six turned positive when discrepant specimens were reanalyzed diluted or undiluted, increasing the sensitivity to 97.4%.

<sup>b</sup> Of the seven cervical swab specimens, one turned positive when discrepant specimens were reanalyzed diluted or undiluted, increasing the sensitivity to 84.6%.

positive results by PCR with a cervical swab and were positive by PCR with urine. The PACE 2 assay missed one patient who was positive by culture and PCR with a cervical swab.

The respective sensitivity and specificity of the Amplicor PCR were 82.0 and 99.7% with urine specimens and 82.0 and 99.8% with cervical specimens. The respective sensitivity and specificity of cell culture were 84.6 and 100% with cervical specimens, and those of the Gen-Probe PACE 2 assay were 79.5 and 100% (Table 2). The sensitivity of the Amplicor PCR would have been 97.4% with urine specimens and 84.6% with cervical specimens if the harmful effect of PCR inhibitors had been eliminated in the first run.

Comparison of various diagnostic tests for *C. trachomatis* is currently hampered by the lack of a proper gold standard. It has become evident that the sensitivity of cell culture does not meet the demands of a reference method for nucleic acid amplification techniques. Therefore, different combinations of test methods including cell culture are currently used as a reference for comparisons. In this study, we evaluated the diagnostic performance of PCR with urine and cervical specimens in comparison with that of chlamydial culture and the PACE 2 assay with cervical swabs. The total number of confirmed chlamydial infections was used as the basis for the calculations of sensitivity and specificity. When the results of different tests were discrepant, the PCR-negative specimens were rerun diluted and undiluted to detect the specimens with PCR inhibitors.

When special precautions were taken to eliminate the effect of PCR inhibitors, 38 (97.4%) of 39 chlamydial infections in this material could be found in urine by PCR. In contrast, only 35 cervical specimens (89.7%) from 39 patients with confirmed chlamydial infection were found positive by at least one of the three methods used. Thus, there were four (10.3%) confirmed positive chlamydial infections in which the infectious agent was only demonstrable in urine. In contrast, only one (2.6%) infection that was found by cell culture from a cervical swab specimen could not be found in urine by PCR. Urine specimens thus appeared clearly superior to cervical swab speci-

mens. It is conceivable that the chlamydial material detected in the urine can originate from both the urethra and the cervix. The chlamydial material originating from cervical infection could probably be carried with the vaginal discharge to the perineum and further to the urine.

The resolved results by PCR with urine cannot, however, be applied as such to routine clinical use. In this study, inhibition of PCR was found in 6 of 39 (15.4%) patients who were confirmed positive for *C. trachomatis* infection. Because of the inhibitors, the sensitivity of PCR with primary urine specimens was actually not better than that of cell culture or the PACE 2 assay with cervical swab specimens. When first described, the inhibition of PCR was found in 4 to 10% of cervical specimens and in only 0 to 3% of male urine specimens (1, 2, 5). In later studies conducted in routine laboratories, the rate of inhibition has been higher (3, 6, 13). It is possible that more inhibitory specimens would have been found if the uniformly negative urine specimens had also been run diluted. The duplicate testing of all negative specimens (diluted and undiluted) would have been a laborious and costly procedure and was not performed.

The results of the current study clearly support the conception that PCR with urine can be used in screening programs for *C. trachomatis* in asymptomatic risk groups. The convenient sample collection will be a clear advantage, and the number of false-negative specimens due to inhibitors of PCR can be accepted, because without screening, the infections would not be found in any case. Regarding the diagnosis of symptomatic *C. trachomatis* infections, the use of nucleic acid amplification methods with urine specimens as a replacement for the methods currently used is, however, a contradictory issue. If not eliminated by specific precautions, the inhibitors of PCR in urine are likely to cause false-negative results among true-positive patients with a frequency equal to that of conventional, less-sensitive methods. The actual number of resulting false-negative cases depends on the incidence of chlamydial infections in the population involved. Thus, the problems due to inhibitors are likely to be emphasized in high-risk populations.

In conclusion, PCR with urine specimens would be clearly superior to the currently used methods if the problems due to inhibitors could be eliminated. Further evaluation of PCR and the other nucleic acid amplification methods and urine sampling under standard field conditions is needed to assess the true value of this tempting new approach to infection control.

This study was supported by a grant from the Medical Research Fund of Tampere University Hospital, Tampere, Finland.

#### REFERENCES

1. Bass, C. A., D. L. Jungkind, N. S. Silverman, and J. M. Bondi. 1993. Clinical evaluation of a new polymerase chain reaction assay for detection of *Chlamydia trachomatis* in endocervical specimens. *J. Clin. Microbiol.* **31**:2648-2653.
2. Bauwens, J. E., A. M. Clark, M. J. Loeffelholz, S. A. Herman, and W. E. Stamm. 1993. Diagnosis of *Chlamydia trachomatis* urethritis in men by polymerase chain reaction assay of first-catch urine. *J. Clin. Microbiol.* **31**:3013-3016.
3. Bauwens, J. E., A. M. Clark, and W. E. Stamm. 1993. Diagnosis of *Chlamydia trachomatis* endocervical infections by a commercial polymerase chain reaction assay. *J. Clin. Microbiol.* **31**:3023-3027.
4. Dutilh, B., C. Bebear, P. Rodriguez, A. Verkis, J. Bonnet, and M. Garret. 1989. Specific amplification of a DNA sequence common to all *Chlamydia trachomatis* serovars using a polymerase chain reaction. *Res. Microbiol.* **140**:7-16.
5. Jaschek, G., C. A. Gaydos, L. E. Welsh, and T. C. Quinn. 1993. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain reaction assay. *J. Clin. Microbiol.* **31**:1209-1212.
6. Kellogg, J. A., J. W. Seiple, J. L. Klinedinst, E. S. Stroll, and S. H. Ca-

- vanaugh. 1995. Improved PCR detection of *Chlamydia trachomatis* by using an altered method of specimen transport and high-quality endocervical specimens. *J. Clin. Microbiol.* **33**:2765–2767.
7. Lee, H. H., M. A. Chernesky, J. Schachter, J. D. Burczak, W. W. Andrews, S. Muldoon, G. Leckie, and W. E. Stamm. 1995. Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain reaction assay of urine. *Lancet* **345**:213–216.
  8. Mårdh, P. A., L. Weström, S. Colleen, and P. Wølner-Hanssen. 1981. Sampling, specimen handling, and isolation techniques in the diagnosis of chlamydial and other genital infections. *Sex. Transm. Dis.* **8**:280–285.
  9. Paavonen, J., and E. Vesterinen. 1982. *Chlamydia trachomatis* in cervicitis and urethritis in women. *Scand. J. Infect. Dis.* **32**:45–54.
  10. Ripa, K. T., and P.-A. Mårdh. 1977. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J. Clin. Microbiol.* **6**:328–331.
  11. Skulnick, M., R. Chua, A. E. Simor, D. E. Low, H. E. Khosid, S. Fraser, E. Lyons, E. A. Legere, and D. A. Kitching. 1994. Use of the polymerase chain reaction for the detection of *Chlamydia trachomatis* from endocervical and urine specimens in an asymptomatic low-prevalence population of women. *Diagn. Microbiol. Infect. Dis.* **20**:195–201.
  12. Thjels, H., J. Gnarpe, H. Gnarpe, P. G. Larsson, J. J. Platz-Christensen, L. Østergaard, and A. Victor. 1994. Expanded gold standard in the diagnosis of *Chlamydia trachomatis* in a low prevalence population: diagnostic efficacy of tissue culture, direct immunofluorescence, enzyme immunoassay, PCR and serology. *Genitourin. Med.* **70**:300–303.
  13. Wiesenfeld, H. C., M. Uhrin, B. W. Dixon, and R. L. Sweet. 1994. Diagnosis of male *Chlamydia trachomatis* urethritis by polymerase chain reaction. *Sex. Transm. Dis.* **21**:268–271.