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Genotyping of *Chlamydia trachomatis* in Urine Specimens Will Facilitate Large Epidemiological Studies

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The serovars of *Chlamydia trachomatis*-positive urine specimens ($n = 81$; as detected by PCR and ligase chain reaction) were successfully analyzed in 94% of cases by *omp1* PCR-based RFLP analysis. The use of urine specimens and this simple and sensitive typing method will greatly facilitate epidemiological studies of *C. trachomatis* serovar distribution in asymptomatic *C. trachomatis* infections in both females and males.

The species *Chlamydia trachomatis* comprises 15 serovars, serovars A to L and additional variants. Serotyping of these *C. trachomatis* isolates is performed on cell cultures of cervical and urethral specimens by using polyclonal and monoclonal antibodies (13). Recent studies showed the feasibility of typing clinical isolates (5, 14) and direct typing of cervical specimens (8, 9) by a nested *omp1* PCR-based restriction fragment length polymorphism (RFLP) assay for the differentiation of isolates into serovars and genovariants (12). By a nested PCR assay, 0.01 inclusion-forming unit (IFU) of serovar L2 DNA could be typed in reconstruction experiments (8). Nowadays, urine specimens are more frequently used for the detection of *C. trachomatis* in both males and females by nucleic acid amplification assays (LCx, COBAS, NASBA, and AMP-CT [2, 6, 7, 10, 11]). This noninvasive technique has a sensitivity higher than that of cell culture of tissue from the urethra and the cervix (6) and a sensitivity comparable to that of *C. trachomatis* detection in cervical scrape specimens by amplification assays. The use of urine specimens facilitates the screening of asymptomatically infected males and females. Typing asymptomatic *C. trachomatis* infections in urine specimens would enable the performance of large epidemiological typing studies. However, urine specimens cannot be used for serotyping purposes since cell culture using urine specimens is impossible. Also PCR-based genotyping directly on urine (e.g., 10 or 20 μl) will result in the introduction of urinary inhibitors which negatively influence *C. trachomatis* amplification. Furthermore, commercial assays (LCx [Abbott Laboratories, Chicago, Ill.] and COBAS Amplicor [Roche Diagnostic Systems, Inc., Branchburg, N.J.]) are more frequently used in routine laboratories for detection of *C. trachomatis* in clinical specimens and generate buffer sample solutions of which the composition, which is not exactly known, inhibits the PCR in the PCR-based RFLP genotyping. Therefore, the aim of this study was to develop a simple and sensitive method for the typing of *C. trachomatis* serovars in urine specimens. Firstly, the DNA isolation procedure for urine specimens had to be optimized, and secondly, *C. trachomatis* typing using the purified DNA had to be evaluated.

To optimize the genotyping of *C. trachomatis* from urine specimens, different DNA isolation methods were compared. For this, a dilution series of serovar L2 (range, 10^{1} to 10^{-3} IFU) in the background of a *C. trachomatis*-negative urine specimen (as determined by PCR and ligase chain reaction) was used to determine the sensitivities of a silica-based DNA isolation method (1) and a filter tube-based isolation method (High Pure PCR Template Preparation [HPPTP] kit; Boehringer Mannheim B.V., Almere, The Netherlands) by amplifying the plasmid target (11) and the *omp1* gene (9, 12). In addition, pellets from 1, 3, and 5 ml of five *C. trachomatis*-positive urine specimens (as determined by PCR and ligase chain reaction) were analyzed in the two different DNA isolations. Purified DNAs were subjected to a nested *omp1* PCR (1.1 kb) as previously described (9), after which the PCR product was used for RFLP analysis (12). In the comparison of the silica-based DNA isolation method with the HPPTP method, the latter not only was 10 times more sensitive (0.1 versus 0.01 IFU) for both the *C. trachomatis* plasmid target and the *omp1* target but also proved to be faster (30 versus 60 min) and easier to perform as well. For the five *C. trachomatis*-positive urine specimens, an input of 5 ml of urine gave the strongest amplification signals.

Subsequently, *C. trachomatis*-positive urine specimens ($n = 81$), obtained from asymptomatically infected men and women during a screening program in Amsterdam, The Netherlands, were used for genotyping. Urine pellets were used to isolate DNA by the HPPTP method. Serovars could be determined for 76 samples by a PCR-based RFLP method. The serovar distribution is shown in Table 1. Serovars F, E, and D (D and D^{-}) were the most prevalent *C. trachomatis* serovars in asymptomatic infected females and males. Furthermore, two *C. trachomatis* variants were found to have unknown RFLP patterns which were not due to double infections. Variants have been found in other studies, but in those studies cervical specimens were used (12). The obtained typing data are in agreement with those of other typing studies using cervical and urethral swabs (15, 16). For the urine specimens in which *omp1* amplification and typing were not possible ($n = 5$), inhibition in three eluates (urine specimens from two females and one male) was shown by means of spiking of serovar L2 DNA in the HPPTP eluates and subsequent plasmid PCR. In the two remaining urine specimens, partial inhibition could be the reason for the inability to amplify the longer *omp1* fragment. This is still a subject of further investigation.

Since nowadays commercial assays (LCx and COBAS Amplicor) are increasingly used in routine laboratories for *C. trachomatis* detection in cervical or urethral and urine speci-

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TABLE 1. Serovar distribution as found for *C. trachomatis*-positive urine specimens from asymptomatic infected patients

Serovar	No. of patients		Total no. (%)
	Female	Male	
D	2	3	5 (7)
D^-	2	3	5 (7)
E	20	13	33 (43)
F	6	6	12 (16)
G	2	5	7 (10)
H	1	1	2 (3)
I	1	2	3 (4)
J	3	1	4 (5)
K	1	2	3 (4)
Var.[a]	2	0	2 (3)

[a] Var., variant with aberrant PCR-based RFLP pattern.

mens, the HPPTP kit was also optimized for DNA isolation from the buffer sample solutions since PCR could not directly be applied to buffer sample solutions. From the LCx and COBAS Amplicor sample buffer solutions, 300 and 500 μl, respectively, were mixed with 1 volume of isopropanol and centrifuged for 10 min at 14,000 rpm. The supernatant was removed, and the obtained pellet was resuspended in 200 μl of phosphate-buffered saline. Subsequently, the HPPTP protocol was followed for DNA isolation and *C. trachomatis* serovars were determined by the PCR-based RFLP method. From the *C. trachomatis*-positive urine specimens, 80 corresponding sample buffer solutions (40 each from the LCx and Amplicor assays) were used for typing. Serovars identical to those found by DNA isolation directly from the urine specimens were found. For one LCx mixture and three Amplicor mixtures obtained from different urine specimens the subsequent *omp1* amplification was not successful and the mixtures could therefore not be typed. All samples (urines and sample buffer solutions) in which *omp1* amplification and subsequent RFLP typing were not successful were noncorresponding samples.

In this study it was shown that *C. trachomatis* typing, despite inhibition in a small number of the samples, could be successfully performed for 94% of the urines. *C. trachomatis* typing of 95% (4 of 80) of the buffer-urine solutions from the commercial assays was also possible. Preliminary results showed that identical serovars were found in urines and the corresponding cervical scrapes ($n = 24$), supporting the feasibility of urine for *C. trachomatis* typing. The typing of *C. trachomatis* serovars in routine collected material (urine specimens and buffer-resuspended clinical material) provides the opportunity to study the epidemiology of *C. trachomatis* serovars and genovariants in an asymptomatically infected population (3, 4, 15).

In conclusion, the use of urine specimens in combination with this simple and sensitive *C. trachomatis* typing method will greatly facilitate epidemiological studies of *C. trachomatis* serovars in both asymptomatically and symptomatically infected females and males.

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