

Diagnosis of Genital *Chlamydia trachomatis* Infections in Asymptomatic Males by Testing Urine by PCR

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An enzyme-linked immunosorbent assay (EIA) (MikroTrak; Syva) was compared with PCR (Amplicor; Roche) for detection of *Chlamydia trachomatis* in first-void urine (FVU) from 184 men attending a skin and venereal disease clinic. The prevalence of *C. trachomatis* in the population studied was 18.5%. Discrepant results between Syva EIA and Roche PCR were retested by using major outer membrane protein primer-based PCR. After retesting, the sensitivity, the specificity, and the positive and negative predictive values for the Syva EIA were 85.3, 100, 100, and 77.5%, respectively, and those for the Roche PCR 100, 100, 100, and 100%, respectively. It was concluded that PCR provides a highly sensitive and specific noninvasive screening method for genital chlamydial infection in asymptomatic men.

Detection of chlamydial antigen in sediments of first-void urine (FVU) from men is a feasible and accurate test for chlamydial urethritis (5, 13, 20). The use of FVU, especially for asymptomatic carriers, is more acceptable to males than urethral swabbing (4). The clinical and epidemiological implications associated with undetected genital chlamydial infections demand a rapid and accurate laboratory screening test (12, 21, 23).

Asymptomatic male carriers of *Chlamydia trachomatis* are of particular concern because the infection may be passed to female partners, resulting in pelvic inflammatory disease and its sequelae, i.e., ectopic pregnancy and infertility.

Our study was aimed at evaluating a PCR test for the detection of *C. trachomatis* in male FVU and comparing it with an enzyme immunoassay (EIA) test.

MATERIALS AND METHODS

Study population. FVU from asymptomatic males ($n = 184$) presenting at a skin and venereal disease clinic, for reasons other than venereal disease, was collected. Patients were supplied with 20-ml urine tubes and were asked to return after having collected their early-morning urine. The median age of the patients was 32 years.

Specimen collection and preparation. Fresh urine was divided into two aliquots of 10 ml each. One aliquot of urine was immediately centrifuged at $1,500 \times g$ for 15 min, and the pellet was resuspended in 1 ml of Syva MicroTrak specimen treatment solution. The samples were kept at 4°C for ≤ 2 weeks before being investigated.

The other aliquot of urine was frozen at -20°C and kept for about 2 weeks until the PCR was done. Before the investigation, urine was thawed at room temperature and then heated for 30 min at 37°C to dissolve any precipitate. Then the specimens were vortexed (≈ 1 min) and centrifuged at $1,500 \times g$ for 10 min at room temperature. The supernatants were replaced with 2 ml of Roche Amplicor urine resuspension buffer and kept for 1 h at room temperature. After 2 ml of urine diluent had been added, the sample was left for 10 min at room temperature before amplification.

Testing of urine. EIA with the MicroTrak Chlamydia EIA

was done by following the instructions of the manufacturer (Syva).

PCR (Amplicor; Roche) based on plasmid primers was performed according to the manufacturer's instructions. The result was read with a Syva MicroTrak EIA autoreader at a wavelength of 450 nm.

Samples considered PCR positive were those having optical densities higher than $2.0 A_{450}$ units. Optical densities for the negative samples were below $0.25 A_{450}$ units.

Analysis of discrepant results. All samples giving discordant results were retested twice, by both plasmid-based PCR and EIA. Samples remaining positive only by plasmid-based PCR were sent to Roche Laboratories (Switzerland) to be retested with major outer membrane protein (MOMP) primer-based PCR.

RESULTS

One hundred eighty-four FVU samples from males were obtained for the EIA and PCR assay. Of the 184, 29 (15.8%) were positive by both EIA and PCR. Five FVU samples (2.7%) were positive for *C. trachomatis* by the plasmid-based PCR but negative by the Syva EIA. One hundred fifty EIA-negative FVU samples were also PCR negative.

Compared with EIA, plasmid primer-based PCR had a sensitivity, specificity, and positive and negative predictive values of 100, 98.6, 85.3, and 100%, respectively (Table 1). In light of these test results for the five samples, they were thought to have false-positive PCR results. In repeated plasmid primer-based PCR and in MOMP primer-based PCR, the five samples remained positive. No samples were PCR negative and EIA positive. The sensitivity, specificity, and positive and negative predictive values for the EIA compared with PCR were 85.3, 100, 100, and 77.5%, respectively. Table 1 shows that after retesting with MOMP primer, the plasmid primer-based PCR and MOMP primer-based PCR had detected equal numbers of chlamydia-positive samples. The sensitivity and specificity of PCR after retesting with MOMP primers were 100%.

DISCUSSION

To detect genital chlamydial infection in males, culture tests require urethral sampling, which asymptomatic persons are

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TABLE 1. Sensitivities of PCR and EIA for detection of *C. trachomatis* infection in FVU from males

Method ^a	Result for indicated no. of samples		
	29	5	150
Plasmid primer-based PCR	+	+	-
MOMP primer-based PCR	+	+	-
EIA	+	-	-

^a The sensitivity, specificity, and positive and negative predictive values for PCR were 100%, and those for EIA were 85.3, 100, 100, and 77.5%, respectively.

reluctant to undergo as urethral sampling is often painful in asymptomatic males with no discharge.

Genital infections caused by *C. trachomatis* have traditionally been detected by cell culture, but for urine, culture is a highly insensitive method to detect a genital chlamydial infection (5).

For this reason, noninvasive antigen detection tests, such as DIF (11) and EIA (5, 9, 14), have been proposed as alternative diagnostic means. Two DNA amplification techniques for the detection of urogenital infections due to *C. trachomatis* have been developed, i.e., PCR and ligase chain reaction (1, 6, 12, 24).

In general, PCR has been shown to be highly sensitive for detection of *C. trachomatis* in clinical specimens. Several PCR-based assays that have used plasmid primers (12, 17, 19), the MOMP gene (2, 3), or rRNA (8, 25) for detection of *C. trachomatis* infection have been described.

However, when dealing with PCR, a few possible difficulties need to be overcome.

One problem associated with PCR is sample contamination. Concerns about false positives due to amplimer contamination of specimens have urged poststerilization procedures for the improvement of specificity (7).

Some samples, e.g., urine, may possess inhibitory substances interfering with PCR results. The major factor affecting the sensitivity of this assay seems to be the activity of *Taq* polymerase, which can be blocked by the presence of *Taq* polymerase inhibitors (18).

The evaluation of the results of nucleic acid detection and amplification techniques, such as PCR and ligase chain reaction, which in most cases are more sensitive than other methods used for the diagnosis of genital chlamydial infections, e.g., cell culture and EIA, has often met with difficulties. Therefore, a general strategy to use at least two other independent test systems when evaluating new tests has been proposed (22). One of those systems in the case of PCR is the use of different sets of primers which are equally sensitive and able to confirm positive findings (15, 16). In our study, discrepancies between plasmid primer-based PCR and EIA were resolved by MOMP primer-based PCR.

The high sensitivity of PCR over EIA was demonstrated in our study. Being more sensitive than EIA, PCR detected five more positive samples than EIA. Because their results were not confirmed by a common evaluation strategy (22), these samples should be considered false positives; this reduces the sensitivity of PCR to 96.8%. When confirmatory testing by the equally sensitive MOMP PCR primers was applied, PCR became 100% sensitive and specific, reducing the sensitivity of EIA to 85.3%.

In most of the studies whose results have been published, comparison of PCR of FVU and cultures of cells from urethral swabs was done. In these cases, PCR was found to have a sensitivity of 95 to 97% and a specificity of 99.7% (1, 12).

PCR has been shown to be cost-effective for screening of adolescent males for *C. trachomatis* (10). Our study indicates that PCR is a suitable method for the detection of genital chlamydial infections in FVU samples from asymptomatic males.

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