Acta Derm Venereol (Stockh) 1998; 78: 63–66

Potential Public Health Benefits from Testing with *Chlamydia trachomatis* PCR Technique on First Void Urine in Men

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Urine samples from 467 men living in the Stockholm area were tested with the polymerase chain reaction (PCR), Roche Amplicor, and with an enzyme-linked immunosorbent assay, Syva MicroTrak EIA, for detection of *Chlamydia trachomatis*. The predictive value of urine versus urethral samples was subsequently compared on a second urethral sample from 25 *C. trachomatis*-positive cases. The urethral samples were in addition cultured for *C. trachomatis*.

***C. trachomatis* was found more often in urine by Roche Amplicor than by Syva MicroTrak, 9.9% and 7.9%, respectively. Nine urine samples, positive only by Amplicor, could be confirmed as true positives by complementary testing. *C. trachomatis* was detected with the same frequency in urine and urethral samples. The sensitivity was highest for PCR, 88% and 92%, and lowest for EIA, 76% and 80%, on urethral and urine samples, respectively.**

Urine sampling, offering a non-invasive procedure, was found suitable for the diagnosis of *C. trachomatis* in men, with the use of Roche Amplicor. *Key words: urethra; Momp; EIA; culture.*

(Accepted August 4, 1997.)

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Over the last two decades, *Chlamydia trachomatis* has been recognised as the most prevalent bacterial sexually transmitted infection. The WHO has estimated that 50–70 million cases occur every year, world-wide (1). *C. trachomatis* causes a wide range of genital tract problems. Symptoms and signs vary from asymptomatic, low-level symptomatic to frank illness. In both sexes symptomatic infection appears to be the exception rather than the rule, and up to 70% remain asymptomatic for a varying time (2–6).

Although the cell culture method for identification of *C. trachomatis* (7) is considered the diagnostic golden standard, several problems exist. Sensitivity ranges within 70–90% even in laboratories where one has a lot of experience of this method (8). Also, culture is labour-intensive, relatively slow and requires stringent transport conditions. Therefore, alternative methods have been widely used, including enzyme-linked immunosorbent assays (EIA) and direct immunofluorescence antigen (DFA) detection, which are independent of viable bacteria in the samples. The initial enthusiasm for EIA, which is relatively simple and possible to automate, has to a large extent been misplaced because of insufficient sensitivity to detect small numbers of elementary bodies. DFA, on the other hand, is sensitive and specific but requires experience and is unsuitable for screening of large sample numbers (9–11).

The introduction of specific nucleic acid amplification methods, such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR) and transcription-mediated amplification (TMA), provides new ways to implement laboratory diagnosis, since these methods are the most sensitive ones so far for the detection of *C. trachomatis* (10–16). Gene amplification methods, as tested for in first void urine (FVU) from men, represent sensitive and specific screening assays that may detect all of the common urogenital *C. trachomatis* serovars, even when they are present at a low titre (17–21).

We have evaluated the potential usefulness for screening purposes of a PCR technique, based on amplification of *C. trachomatis* plasmid DNA (Roche Amplicor® PCR), versus that of an EIA method (Syva MicroTrak®) on FVU in three different male population groups, i.e. patients attending an STD clinic, asymptomatic men consulting a public youth clinic (PYC), and presumptively healthy military conscripts. In addition, urine and urethral swabs as well as culture, PCR and EIA have been compared in a number of the *C. trachomatis*-positive men with respect to sensitivity and specificity for the diagnosis of *C. trachomatis*.

MATERIAL AND METHODS

The study comprised 467 men, who had given their consent to participate in agreement with a local Ethical Medical Committee decision and who had not received antibiotic treatment immediately prior to enrolment. Three study groups were investigated. Group A comprised 206 men who attended the STD clinic in the Karolinska Hospital due to either contact with a female with diagnosed or suspected chlamydial infection, urethral symptoms such as dysuria and discharge, or other penile conditions including condylomas and balanoposthitis, as well as men who sought for a general STD check-up due to change of partner(s). None of these men had a concurrent gonorrhoeal infection. Two additional groups of asymptomatic healthy young men were recruited outside of the hospital setting: group B, comprising 109 men attending a PYC, and group C, including 152 men currently doing their military service. The age of the total study group was on average 27 years (range 13–54). The corresponding age distribution in the various study groups was 28 (range 17–54) for group A, 19 (13–32) for group B and 20 (18–27) for group C, respectively.

Clinical specimens

All men were instructed not to micturate 2 h prior to attendance. An FVU sample of 15–20 ml was collected in a cone-tipped vial and stored at +4°C until further laboratory handling. Following micturation the men in Group A were investigated twice with cotton-tipped ENT swabs (Swedish Hospital Supply, Mölndal, Sweden). Swab investigation was performed according to standard procedures, aiming at sampling epithelial cells from the distal 3–4 cm of the urethra. The first swab, used for culture assay, was placed immediately in a transport buffer, 2 SP-medium (20 mM phosphate buffer, pH 7.0; 6.48 g/l sucrose; 10 mg/l gentamicin; 2.5 mg/l amphotericin B; 100 mg/l vancomycin/10% foetal calf serum). This sample was in addition used for retrospective PCR analysis in order to dissolve discordant PCR/EIA results and to compare urine and urethral sampling in *C. trachomatis*-infected men. The second swab was placed in a commercial transport buffer for EIA analysis. Men in Group B

 ISSN 0001-5555

and C were, as a routine, investigated only by FVU. However, whenever a urine test was found to be EIA- and/or PCR-positive, patients were also offered to attend the STD clinic in the Karolinska Hospital, where two ENT swabs were inserted 3–4 cm into the urethra in the case of those who gave their consent and tested according to the procedures outlined above.

Chlamydia diagnostics

Urine samples. Fifteen to twenty millilitres of urine was collected from each patient and divided into two aliquots, each of which was centrifuged at 1,600 x g for 15 min. The supernatant was decanted and one of the pellets was used for Syva MicroTrak® EIA (Palo Alto, CA) and the other pellet for Roche Amplicor® PCR (Hoffman-LaRoche Molecular Systems, Basel, Switzerland).

Syva MicroTrak EIA. The urine pellet and the urethral sample were suspended in 1 ml specimen treatment solution. The samples were preheated for 15 min in a preheating block (95–100°C) and were cooled for 10 min. The EIA assay was performed according to the instructions of the manufacturer. Positive EIA specimens were confirmed by Syva MicroTrak Direct fluorescent antigen (DFA) assay. For DFA the specimens were centrifuged at 1,600 × g for 10 min, and 30 µl of the pellet was transferred onto a glass slide, which was left to dry. The slides were fixed in 100% methanol and stained with MicroTrak fluorescein conjugated monoclonal antibody against *C. trachomatis* and were examined under a fluorescence microscope at 400× magnification. The finding of one elementary body was considered confirmatory.

Roche Amplicor PCR. The urine pellet was suspended in 2 ml resuspension buffer. The tube was kept for 1 h at room temperature; 2 ml urine diluent was then added and was left at room temperature for 1 h. The amplification assay was performed according to the instructions of the manufacturers. In the Amplicor PCR uracil-N-glucosidase is included to destroy possible amplification products from previous Amplicor runs, which all incorporate dUTP instead of dTTP. The amplified product was detected by EIA using a specific probe in the microtitre plate.

From the urethral culture sample (2 SP-medium) 100 µl was suspended in 900 µl transport media diluent. The tube was kept for 1 h at room temperature, and 1 ml STD swab specimen preparation diluent was then added and left at room temperature for 1 h. The amplification assay was performed according to the instructions of the manufacturers.

Cell culture. Isolation of *C. trachomatis* was performed on cyclohexiimide-treated McCoy cells in 24-well plates (7). Forty-eight hours after inoculation the cells were fixed in 100% ethanol and stained with Syva MicroTrak fluorescein conjugated monoclonal antibody against *C. trachomatis* and were examined under fluorescence microscope at 100× magnification.

Definitions. C. trachomatis infection was defined as positive culture, and/or positive results in EIA that were confirmed positive by DFA and/or positive reaction in Amplicor Roche. A sample positive by only PCR had to be confirmed by Momp PCR to be considered as true positive.

Statistical analysis

Yates' corrected chi-square test was used for analysis of differences between the test methods regarding sensitivity of the assays applied by us for *C. trachomatis* detection.

RESULTS

PCR versus EIA on urine samples

In Table I the results of PCR and EIA on 467 urine samples are shown. *C. trachomatis* were found more often in urine samples by PCR (9.9%) than by EIA (7.9%), although the difference was not statistically significant ($p=0.3007$). *C. trachomatis* was significantly more prevalent among the men who attended the STD clinic (group A), compared to the asymptomatic men (groups B and C) in youth clinics and among military recruits, 28/206 (13.6%) versus 18/261 (6.9%), ($p=0.0159$).

Table I. *Roche Amplicor PCR versus Syva MicroTrak EIA for detection of C. trachomatis in urine samples*

	MicroTrak EIA		
Amplicor PCR	Positive	Negative	Total
Positive	37	9	46
Negative	0	421	421
	37[a]	430	467

[a]Confirmed by direct immunofluorescence antigen.

Nine of the urine samples were positive only by PCR. The discrepant PCR/EIA results were further resolved by complementary testing. This was performed by Hoffman La-Roche in Basel using alternative primers from the Momp gene as well as doing a repeat Amplicor PCR. Urethral samples were available from only 5 of the 9 patients, as 4 men did not give their consent to this type of sampling. Yet, all 9 discrepant PCR/EIA results could be confirmed as *C. trachomatis*-positive, since all samples were positive by PCR using Momp primers as well as on repeat testing by Amplicor PCR. In addition, culture as well as PCR on urethral specimens were positive in 3 out of the 5 patients who had partners with *C. trachomatis* infection.

The sensitivity, specificity, positive and negative predictive values for Roche Amplicor PCR were found to be 100% in the present study. In comparison, the sensitivity of EIA was only 80% but the specificity and positive predictive value 100%, since only confirmed positive EIA results were included. The negative predictive value for EIA was 98%.

Urethral versus urine samples and comparison between culture, PCR and EIA

Samples were available from both urethra and urine in 25 of 46 *C. trachomatis*-infected males, enabling a direct comparison of efficiency between these two types of specimen as well as between culture, EIA and PCR. The PCR test detected *C. trachomatis* in 22/25 (88%) and 23/25 (92%), respectively, of urethral and urine samples, while EIA only detected 19/25 (76%) and 20/25 (80%), respectively. Culture had a sensitivity of 23/25 (92%) on urethral swabs. The differences were, however, not statistically significant, probably due to the small number of patients. These results demonstrate that urine specimens are as efficient as urethral samples for diagnosis of *C. trachomatis* infections in males and indicate that PCR is more sensitive than EIA.

DISCUSSION

The high frequency of serious complications to *C. trachomatis* infection represents a significant threat to public health, and the potential cost-benefit aspects of implementing more efficient diagnostic methods and optimising screening programmes have been emphasised (2, 16, 20, 22, 23).

The sensitivity of the amplification assays is increased com-

pared with non-amplification procedures like EIA and cell culture, and the discrepancy of the non-amplification assays is especially high for specimens with a low number of chlamydial particles, as is often the case in asymptomatic persons (16). This aspect is of key importance when implementing screening strategies for the prevention of chlamydial infections and their sequelae (2, 16, 23).

For the PCR assay concerns have been raised about false positivity, and stringent precautions must be taken to avoid exogenous contamination (10). In the present study this did not appear to be a problem. All those results of PCR that were in discordance with either EIA or culture were subsequently shown to be PCR-positive using Momp-specific primers, clearly indicating that in the present study the PCR assay was 100% specific. Although no statistically significant differences were detected in the present study, PCR and culture yielded a higher positivity rate than EIA. By PCR 88% and 92% of urethral and urine samples, respectively, were positive, as was 92% of swabbed urethral material that hade been cultured, compared to 76% and 80% for the EIA assay when performed on urethral swab material and on FVU, respectively.

The clinical significance of using PCR for screening purposes is supported by the present study, showing that 5 of the 9 men found true positive in FVU using PCR but negative using EIA were partners of *Chlamydia*-positive female patients. This supports the concept that application of amplification assays for screening outrivals immunoassays. The differences obtained in sensitivity between PCR and EIA might have been statistically significant if the study groups had been larger.

The difference in the mean ages of the population groups in the present study, which was 28 years for the STD clinic compared to 19 and 20 years for the PYC and military conscript groups, respectively, represents a marker for differences in past or current sexual behaviour. Also, while the STD patients self-attended due to either symptoms or a history of risk for *C. trachomatis* infection, the men in the two younger population groups were asymptomatic. It may be assumed that the conscripts rather represent a relatively unselected group, comprising both males who have experienced their first intercourse and males who have not, while a larger proportion of the PYC males are already sexually experienced. As the purpose of the present study was to investigate the usefulness of PCR as a screening method on FVU, we chose to combine the PYC and military conscript groups for analysis of the results.

There are several reasons to screen for *C. trachomatis* (23). First, at least half of those infected are asymptomatic carriers; screening and identifying such cases allow detection of additional positive cases through partner notification. The latter seems highly rewarding, as more than half of all partners of *Chlamydia*-infected index cases are likely to be infected, and breaking the transmission chain by detection and treatment of carriers appears to be of crucial importance for an epidemiologically oriented strategy. It has been calculated that it is cost-beneficial to screen for *C. trachomatis* when the prevalence is approximately 6% , and a prevalence of 3% has been suggested to be a lower limit for screening to be cost-beneficial. However, even at prevalence rates below this threshold it might still be cost-beneficial when applying a strategy based on selective screening, including high-risk target groups such as sexually active persons attending STD and PYC clinics, as well as symptomatic patients in the age group 15–35 years. In clinical settings where screening is performed of subjectively healthy men the sensitivity and reliability of the test are of crucial importance.

Sweden was the first country to establish a national laboratory service for detecting chlamydial infections. In 1988 *C. trachomatis* was included in legislative regulations (24), including examination free of charge to the patients and mandatory obligation of the physicians to trace contacts of infected patients. Results from multivariate logistic regression analysis from the county of Uppsala, north of Stockholm, on samples analysed during the period 1985–1993 (25), recently demonstrated a decline in the detection rate from 107.2 per 1 000 to 32.3 in women, and from 183.3 to 70.7 in men. Positivity rates were highest in STD and youth clinics and lowest in private practises. Although the chlamydial infection rate generally declined, it appeared that the legislation change reduced the probability of a positive test in men but not in women. Furthermore, in recent years an increase appears to have occurred in male youths and the prevalence to have levelled off among female youths. Quite evidently, young asymptomatic males appear to represent an infectious reservoir for the potential maintenance of *C. trachomatis* at a steady-state level in the population. Accordingly, the epidemic may possibly continue to persist until highly sensitive *C. trachomatis* detection methods have been developed and are used routinely for screening.

In contrast to in women, where *C. trachomatis* testing is currently implemented as part of the public health screening services in youth clinics, family planning settings and in antenatal care units, men generally have few opportunities to be tested routinely. In men the diagnosis rather depends on their active attendance due to symptomatic illness, or due to secondary prevention measurements as part of assiduous contact tracing. The discomfort of urethral swabbing appears to be common knowledge that often causes refusal for screening testing by asymptomatic young men. Accordingly, it appears obvious that if satisfactory epidemiological screening and case finding strategies are to be implemented, the introduction of non-invasive diagnostic methods for males is also highly warranted, particularly in PYC settings. We find PCR testing on FVU ideal for this purpose. Additional gains, not the least when focusing on primary prevention of HIV infection, may also be made when test strategies are combined with active information about other STDs as well, with an emphasis on targeted risk-reduction intervention and condom promotion strategies (26, 27).

ACKNOWLEDGEMENT

We wish to express our sincere gratitude to Per Lundbergh, M.D., Assoc. Prof. Head of the Department of Environmental, Health and Infectious Diseases Control, for encouragement and generous financial support.

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