

Diagnosis of *Chlamydia trachomatis* Infection in High-risk Females with PCR on First Void Urine

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Recently the polymerase chain reaction (PCR) has been shown to be more sensitive than older methods in detecting *Chlamydia trachomatis*, when performed on endocervical swabs.

A total of 203 high-risk females were enrolled in a comparative study of 3 methods for diagnosing *C. trachomatis* infections: McCoy cell culture and Amplicor[®] PCR on endocervical swabs, and urine. Thirty-four had positive cultures, 38 positive PCR from cervix and 37 had positive PCR on urine specimens. When discrepancy occurred, the leftover Amplicor[®] specimen was retested by Roche with Amplicor[®] and a primer for the major outer membrane protein (MOMP) gene. In all three tests, 32 were positive. The sensitivity of culture was 87%, 92% in cervical PCR and 95% in urinary PCR. The specificity was 100% in both culture and urinary PCR but 98% in cervical PCR. Amplicor[®] PCR performed on female urine is at least as sensitive and specific as cell culture. **Key word:** sexually transmitted diseases (STD).

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Culture of endocervical and urethral swab samples has been the gold standard in diagnosing chlamydial infections. It is, however, known to be lacking in sensitivity and influenced by a number of factors like the time of transportation (1, 2). A number of rapid methods for detecting *C. trachomatis* have been developed but they are less sensitive than culture (3–5). Recently the polymerase chain reaction (PCR) has been shown to be as sensitive and specific as culture when performed on swabs from cervix and male urethra (6–8), and the same applies to another DNA amplification method, the ligase chain reaction (9, 10). PCR on male urine has been used routinely in our clinic for over a year, with good results. A similar test on female urine would have a great advantage for screening and in situations where cervical specimens may be difficult to obtain. In the present study a comparison was made between Amplicor[®] PCR on urine in females and PCR and cell culture on cervical specimens.

MATERIALS AND METHODS

Patients

The study was conducted in the Sexually Transmitted Disease (STD) Clinic in Reykjavik, Iceland in November 1994 to January 1995. All women attending the clinic during a 70-day period, who would normally be tested for *C. trachomatis*, were enrolled in the study. Pelvic examination was carried out in all cases. Signs like mucopurulent cervical discharge were recorded. Symptoms like inter-menstrual bleeding, abnormal vaginal discharge or painful urination were also noted. All patients were asked about the number of sexual partners for the

last 6 months and also if condoms were used. Patients receiving antibiotics 2 weeks prior to the visit to the STD clinic were excluded.

Methods

Three different methods were used to evaluate Amplicor[®] PCR for detecting *C. trachomatis* in female urine.

Two endocervical swabs were collected for McCoy cell culture and Amplicor[®] PCR (Roche) in an alternating sequence. After the examination, 20–50 ml of first void urine were collected. The sample intended for culture was collected on a cotton swab (Medical wire Co.) and put in 1.0 ml of 0.2 M sucrose phosphate buffer, antibiotics and 10% foetal calf serum and cooled with ice. One sample was collected with collection kits supplied by Roche. All samples were transported to the laboratory within 4 h. Specimens for culture of *C. trachomatis* were agitated with glass beads, and 0.6 ml of the buffer was added to 2 tubes with a monolayer of McCoy cells. The cells were centrifuged for 1 h at 5,000 g at 35°C. The supernatant was aspirated and replaced with maintenance media containing cycloheximide. The tubes were incubated at 35°C for 48–72 h, and the slide from one of the tubes was examined stained with fluorescent antibody (Syva's MicroTrak FA). If the slide was conclusively positive or negative the slide from the second tube was stained. If the examination of the first tube was inconclusive the second was subcultured and the procedure repeated.

The Amplicor[®] assay was performed on a Perkin Elmer thermocycler. The test was performed according to the manufacturer's (Roche) instructions. When discrepancy occurred, the leftover Amplicor[®] specimen was retested by Roche with Amplicor[®] and a primer for the major outer membrane protein (MOMP) gene. This applied when either one or both PCR samples were positive and culture negative, or if culture was positive and one or both PCR samples were negative.

RESULTS

A total of 203 women were tested and enrolled in the study. The mean age was 21 and the median age was 20 years. The median and mean age of *Chlamydia*-infected patients was 20. The age distribution of the patients is shown in Fig. 1. The definition of infection was that either culture or both PCR tests and a confirmatory test with MOMP primers had to be positive. Nineteen per cent were infected, but had *Chlamydia* culture been used solely as a definition of infection only 17% would have been considered infected. The results and calculations on the performance are shown in Tables I and II. Fifteen of the 34 culture-positive patients had no symptoms and 16 of 34 PCR cx/urine positive had no symptoms. Twenty-three of those positive had white, purulent or transparent cervical discharge, but 12 were normal on examination. Only one *C. trachomatis*-positive patient had always used a condom, 5 used it often, 13 sometimes and 15 never. No difference in the use of condoms was found between those positive in the PCR only and those positive in culture. Forty per cent of those infected but only 28% of those not infected had had two or more sexual partners during the 3 months prior to the study.

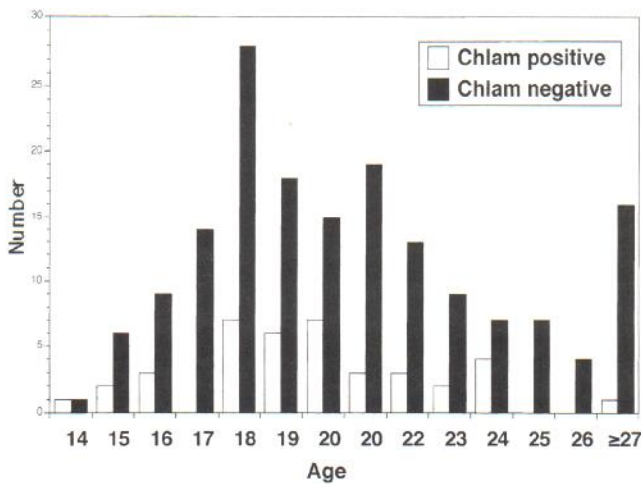


Fig. 1. The age distribution of the 203 high-risk females enrolled in the study.

Table I. Results of the three different tests from 203 patients

	Positive	True pos.	False pos.	False neg.	True neg.
Culture	34	34	0	5	164
PCR cx	38	36	2	3	162
PCR urine	37	37	0	2	164
All 3 tests	32	39			164

Table II. Calculations on the performance of the three different tests

	Culture	PCR cx swabs	PCR urine
Sensitivity	87	92	95
Specificity	100	98	100
Predictive value of positive	100	95	100
Predictive value of negative	97	98	98

DISCUSSION

Because collection of samples for *C. trachomatis* diagnostics has often caused discomfort and sometimes embarrassment for the female patients, new methods where examination is not required have definite advantages. Collecting a urine sample is in most instances considered acceptable to the patient. The PCR urinary diagnostics have been used for males in our clinic for over a year, with very satisfying results, and have been well accepted by our patients. It can be argued that omitting examination in the clinic may cause other diseases, like genital warts, to be missed. This is of some concern and examination of men and women is strongly recommended, whenever possible, regardless of which method is used for diagnosing chlamydial infections.

Amplicor[®] PCR has been shown to be more sensitive than the gold standard, cell culture (7, 8). It is difficult to resolve the issue of "false positive" PCR tests because of the lack of a standard sensitive enough. Using PCR for the MOMP gene is not quite satisfactory, since it is slightly less sensitive than the Amplicor[®] PCR. The reason for this is that Amplicor[®] PCR detects plasmid genes, of which there are usually multiple copies in each *C. trachomatis* cell. The MOMP gene, on the other hand, resides on the chromosome of which there is only

one copy per cell. Therefore the two "false positive" PCR tests were probably true positives. The results show that Amplicor[®] PCR performed on female urine is more sensitive and as specific as cell culture. The prevalence of isolated urethral asymptomatic *C. trachomatis* in the absence of cervical infection has been shown to be up to 24% (11, 12). This could partly explain the higher sensitivity of urinary PCR. This urinary test can be used to test and screen asymptomatic females, where other methods like endocervical swabs for culture and PCR can be difficult to apply. Obviously, screening of asymptomatic populations like school pupils is difficult or impossible, due to reluctance to undergo an examination, whereas a urinary test is more acceptable. This opens up new prospects in screening for *C. trachomatis* (13).

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