Clinical Evaluation of a Rapid Polymerase Chain Reaction (PCR) Assay

for the Detection of *Chlamydia trachomatis* in Specimens from High Risk Patients

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A Rapid Polymerase Chain Reaction Assay (Amplicor®-PCR) was evaluated for the detection of *Chlamydia trachomatis* in specimens from 179 high risk patients. The results were compared to McCoy cell culture and specimens were retested with Amplicor® and primers for the Major Outer Membrane Protein (MOMP) gene when discrepancy occurred. Of 88 females enrolled in the study, 30 were infected (34%). Sensitivity, selectivity, predictive value of a positive (PVP) and a negative (PVN) on endocervical specimens were 97%, 96.5%, 96.5% and 98% respectively. Of 91 male urine specimens, 33 (36%) came from infected patients. The sensitivity and specificity of the Amplicor® assay was 94% and 74% respectively for male urine specimens and the PVP and PVN were 72% and 96% respectively. The sensitivity was low on the original run on urethral specimens but the majority of false negative specimens became positive when retested. Amplicor® performed on urine samples was the most sensitive test for detecting Chlamydial infections in males.

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Introduction

Sexually transmitted infections caused by *Chlamydia trachomatis* have been a significant health care problem in the Western world in recent years (1,2). Rapid diagnosis of Chlamydial infections is of major importance for efficient treatment of individual patients and for effective contact tracing. Culture is time consuming and in recent years a number of rapid methods have been introduced for direct detection of Chlamydial antigens in patient specimens. Although these methods lack somewhat in sensitivity compared to culture, they have come into widespread use (3–8). Recently, Rapid Polymerase Chain Reaction (PCR) Assays superior in sensitivity to older rapid methods, have been introduced (9). Molecular techniques for the detection of *Chlamydia trachomatis* require complicated techniques and competence not available in most routine laboratories. Roche Molecular Systems has recently marketed a commercial PCR, suited for use in clinical laboratories (10–12). The present study is a clinical evaluation of this PCR assay for the detection of Chlamydial infections in cervical specimens from females and urethral swabs and urine from males.

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Materials and methods

Patient population and specimen collection: The patients were high risk males and females attending the State Clinic for Sexually Transmitted Diseases (STD) in Reykjavík Iceland. Patients were enrolled in the study if they had symptoms or were contacts of patients, known to be infected with *Chlamydia trachomatis*. Patients were excluded if they had received antibiotics during the week prior to specimen collection.

If the male patients had a discharge, it was

Table I. *Performance of PCR compared with culture on cervical specimens from 88 females, 33 (38%) of whom were considered infected.*

		Results of culture		Results of PCR	
	No	No of positive	No of negative	No of positive	No of negative
Infected	33	29	4[1)]	31	2[2)]
Not infected	55	0	55	0	55
Total	88	29	59	31	57

1) Initial culture results were inconclusive on three specimens because of the cytopathogenisity of the specimen and one was a false negative.
2) One specimen was PCR negative on initial testing but was positive when the test was repeated on the same specimen. The other remained negative on repeating.

Table II. *Performance of PCR compared with culture on 91 urine specimens and urethral swabs from males, 47 (53%) of whom were considered positive.*

		Results of culture		Results of PCR on urine		Results of PCR on swabs	
	No	No positive	No negative	No positive	No negative	No positive	No negative
Infected	47	33	14	45	2	19	28[1)]
Not infected	44	0	44	3	41	0	44
Total	91	33	58	48	43	19	72

1) The results were positive in 13 instances when the test was repeated on the same specimen.

collected first on a Culturette® swab that was used for culture of *N. gonorrhoea*, *U. urealyticum* and *M. hominis*. If the patient had no discharge this specimen was taken last. Two urethral specimens were collected for the detection of *C. trachomatis*. Specimens for culture and PCR were collected in an alternating sequence. 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. A first void urine sample was collected after swab specimen collection. Specimens were delivered to the laboratory within three hours. Specimens for culture and Amplicor® were either processed right away or frozen at -70°C until processing.

Test procedures: Specimens for culture of *C. trachomatis* were agitated with glass beads and 0.6 ml of the buffer was added to two tubes with a monolayer of McCoy cells. The cells were centrifuged for one hour at 5000 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 hours and the slide from one of the tubes was examined stained with Fluorescent Antibody (Syva 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 supplied by Roche Molecular Systems for the study. The test was performed according to manufacturer's 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.

Results

Results from 179 patients were evaluated in the study, 88 females, 15 to 35 years of age with the median age of 20, and 91 males 17 to 36 years of age with the median age of 23. Of those 179 patients, 80 (45%) were considered to be infected. None of the patients had Gonorrhoea. A patient was considered infected, even if culture was negative, when two types of tests were positive, for example two tests with different primers. The prevalence of infection in the 88 females was 38% (table I). The sensitivity of culture and PCR in females was 88% and 94% respectively and specificity of both methods was 100%. Of the 91 males 48 were considered positive, a prevalence of 53% (table II). Thirty two had signs or symptoms of

urethritis but were not infected (NGU). The sensitivity of culture in males was 70% and specificity was 100%. The sensitivity of PCR in urethral specimens and urine was 40% and 96% respectively and specificity was 100% and 93% respectively. One female and 14 males were considered infected although culture was negative. All 15 patients either had symptoms or were known to have had intercourse with infected partners. PCR on urine was the only positive test in 15 instances and 12 of these patients were considered infected and three tests were considered false positive.

Discussion

Tissue culture on swabs from the cervix of female patients and the urethra of males has traditionally been the gold standard for the diagnosis of sexually transmitted infections with *Chlamydia trachomatis*. Cell culture is expensive and time consuming and requires proficiency and facilities that are not available in all clinical laboratories. A number of rapid immunological tests have been developed and come into widespread use because of low cost and ease of use, in spite of their lack of sensitivity compared to culture (13). The advent of the DNA probes with their high specificity and the introduction of very sensitive PCR technology raised hopes for new and better diagnostic tests. But the new technology proved difficult to adapt for use in routine laboratories. It was not until recently that Polymerase Chain Reaction Assays, as or more sensitive than cell culture became commercially available (11–13).

In the present study the Amplicor® PCR assay was slightly more sensitive than culture on cervical specimens (table I). In an earlier study (10,11) a prototype of the Amplicor® assay proved to be highly sensitive on cervical specimens but the sensitivity of the proposed commercial assay proved to be less. It was suggested that inadequate neutralisation of inhibitory substances caused this loss in sensitivity and some of the "false negative" tests were positive when repeated. There was one such instance in our study (table I). Whatever the reason, this problem seemed to be especially pronounced in specimens with low inclusion counts and the PCR assay was considerably less sensitive than culture on urethral specimens from males in our study. The low sensitivity of the PCR assay in these patients (table II) was surprising considering the performance of the test in endocervical and urine specimens and the performance in a previous study (12). Although our male patients were a group selected from high risk patients because of their likelihood of being infected, more than half the infected patients attended the clinic because of contact tracing and had little or no symptoms. This is a group of patients in whom the sensitivity of diagnostic tests tends to be low (5). Interestingly enough, 13 of the false negative specimens became positive when the test was repeated on the same specimen (table II).

On the other hand, whatever the technical problem that caused false negative tests on cervical and urethral specimens it did not seem to affect tests on urine specimens. The PCR assay performed on urine samples, was considerably more sensitive than cell culture performed on urethral swabs and by far the most sensitive test for detecting Chlamydial infections in males. PCR on urine was the only test positive in 10 infected patients all of whom were known to have sexual relations with infected patients.

In view of the high sensitivity and the non invasive sampling technique, the PCR assay performed on urine specimens is likely to become the test of choice for male patients.

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