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Chlamydia trachomatis Infection in a High-Risk Population: Comparison of Polymerase Chain Reaction and Cell Culture for Diagnosis and Follow-Up

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A study to compare the polymerase chain reaction (PCR) test with the cell culture method in diagnosing urogenital *Chlamydia trachomatis* infections was performed. From 497 patients (212 women, 285 men) attending an outpatient clinic for sexually transmitted diseases, a total of 814 samples (female patients, cervix and urethra; male patients, urethra) were collected. This total included follow-up samples from 35 women and 35 men positive for *C. trachomatis* by cell culture and/or PCR test, which were collected 2 weeks after treatment with doxycycline (two 100-mg doses per day for 7 days). The PCR test was performed directly on clinical samples without performing phenol-chloroform extraction and ethanol precipitation of DNA. The prevalence of *C. trachomatis* as measured by positive cell culture was 64 of 497 (12.9%) for all patients, 31 of 212 (14.6%) for women, and 33 of 285 (11.6%) for men. The prevalences as measured by positive PCR test were 71 of 497 (14.3%), 36 of 212 (17.0%), and 35 of 285 (12.3%), respectively. The sensitivities of the cell culture and the PCR test compared with that of true-positive samples were 77.5 to 78.4% and 99.0 to 100.0%, respectively. Discrepancies between cell culture and the PCR test were found for 23 of 497 patients (4.9%), 19 of 212 females (9.0%), and 4 of 285 males (1.4%). Nineteen pretreatment samples from 19 patients (4 female endocervical, 13 female urethral, and 2 male urethral samples) were cell culture negative and PCR test positive, while 1 pretreatment female endocervical sample was cell culture positive and PCR test negative. The posttreatment samples from all patients were cell culture negative, but the PCR test remained positive for 3 of 70 patients (1 female endocervical and 2 male urethral samples). One of these samples became spontaneously negative in three more weeks. The medical history of the individual patient and the negative PCR tests after treatment for nearly all patients support our hypothesis that the positive PCR test results were clinically relevant for the cell culture-negative but PCR test-positive patients of the population studied.

Chlamydia trachomatis is the most frequent cause of sexually transmitted disease in the Western world. Every year, an estimated 4 million cases occur in the United States (4). Asymptomatic infections frequently occur (17, 18). Clinical manifestations in the urogenital tract of women are urethritis, cervicitis, and pelvic inflammatory disease with infertility as a possible consequence (3, 20). In the urogenital tract of men, the symptoms are urethritis and, rarely, epididymitis (20). Given the frequent absence of symptoms, rapid spread, high prevalence, and the seriousness of the complications, especially in women, the quality of a diagnostic test for the detection of this microorganism is of great importance. The cell culture method has always been regarded as the "gold standard." However, factors such as collection, transport time, and storage of the sample as well as toxicity of the swab can negatively influence the sensitivity of cell culture (1, 12).

Therefore, other methods were developed in recent years. Tests using direct immunofluorescence, enzyme immunoassays (2, 9, 21, 22) and DNA probe techniques (10, 16) have been described. Generally, the specificity of these techniques was satisfactory, but they lacked good sensitivity compared with cell culture, except for the latest generation of enzyme immunoassays and enzyme-linked immunosorbent assays (22). In the past few years, a new technique which appears to exceed the sensitivity of cell culture has been developed, the polymerase chain reaction (PCR) (5, 6, 13, 14, 22).

In this study, the value of the PCR test was compared with that of cell culture for diagnosis of *C. trachomatis* infections in cervical and urethral samples from 497 patients (212 women, 285 men) attending a clinic for sexually transmitted diseases. In addition, a follow-up study was performed with 70 patients in order to collect data on the effect of treatment.

MATERIALS AND METHODS

Patient population. A total of 814 samples was collected from 212 women and 285 men attending the outpatient clinic for sexually transmitted diseases of the University Hospital in Groningen, The Netherlands, from October 1990 to October 1991. This total includes follow-up samples which were collected from 35 women and 35 men who were positive for *C. trachomatis* by cell culture and/or PCR test. Follow-up samples were taken 14 days after arrest of treatment (doxycycline, two 100-mg doses per day for 7 days). Duplicate endocervical samples were taken with a swab after cleaning the cervix, and duplicate urethral samples were taken at a depth of at least 1 cm after rotating the swab a few times. The samples were immediately placed into 2 ml of transport medium (2-sucrose phosphate buffer [pH 7.0] supplemented with 5% fetal bovine serum, 50 μg of streptomycin per ml, 100 μg of vancomycin per ml, and 12.5 μg of amphotericin B

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[Fungizone] per ml). Samples were stored at 4°C until processing and were tested within 5 h after collection.

Cell culture. Chlamydial cell culture was performed on McCoy cells on glass coverslips (Menzel, Darmstadt, Germany) in flat-bottom tubes (Greiner, Nürtingen, Germany). After removing the glucose medium from the flat-bottom tube, 0.3 ml of the vortexed sample was added and centrifuged at 3,000 × *g* for 60 min. The supernatant was then replaced by 1 ml of complete growth medium (RPMI 1640 medium; GIBCO BRL, Grand Island, N.Y.) supplemented with 10% fetal bovine serum, 1% 200 mM L-glutamine (GIBCO BRL), 50 μg of gentamicin per ml, 12.5 μg of amphotericin B per ml, and 2 μg of cycloheximide per ml. The coverslips were incubated at 37°C for 48 h. The medium was then removed, and the coverslips were fixed with 96% alcohol at room temperature for 10 min. After removing the coverslips from the flat-bottom tubes, they were dried and attached with nail polish to a microscope slide and stained with a fluorescent monoclonal anti-*C. trachomatis* antibody (MicroTrak; Syva Co., Palo Alto, Calif.). Coverslips were then examined for inclusions.

PCR test. In order to prevent contamination of the samples, the different steps took place at different sites in the laboratory. Samples were prepared by centrifuging 1 ml of a vortexed sample for 30 min at 5,000 × *g*. After removing the supernatant, the pellet was dissolved in 50 μl of lysis buffer which contained 1 mM EDTA, 1% Triton X-100 (Sigma, St. Louis, Mo.), and 50 mM Tris hydrochloride (pH 7.5). Two microliters of proteinase K in a final concentration of 400 μg/ml was also added. The sample was incubated for 60 min at 37°C and then heated for 10 min at 95°C to inactivate the proteinase K. For PCR analysis, 1 μl of the remaining sample was used. This method of sample preparation proved satisfactory compared with phenol-chloroform extraction and ethanol precipitation of DNA used by others (5) (unpublished data). One set of oligonucleotide primers derived from sequences of the common endogenous plasmid of *C. trachomatis* (19) and generating a species-specific, 200-bp amplified product with all known *C. trachomatis* serovars (T1, CTAGGCGTTTGTACTCCGTCA; T2, TCCTCAGAAGTT TATGCACT) was used. The oligonucleotide primers were synthesized on a Gene Assembler (Pharmacia LKB, Woerden, The Netherlands). The amplification product has a specific cleavage site for the restriction enzyme *Hpa*II. Positive controls (strong positive patient sample and weak positive cultured sample) and negative controls (negative patient samples and water) were included in each PCR experiment. The negative controls were placed after every four patient samples. The amplification reaction was performed in a volume of 15 μl containing 1 μl of the sample DNA; 50 mM Tris hydrochloride (pH 8.5); 50 mM NaCl; 6 mM $MgCl_2$; 2 mM dithiothreitol, 250 μM (each) dATP, dTTP, dGTP, and dCTP; 0.1 μg of each primer; and 0.66 U of *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). Three drops of mineral oil (Sigma) was added to prevent evaporation. The amplification was performed in a PCR processor (Biomed, Ditfurth, Germany), and each cycle contained a denaturation step at 94°C for 1 min, a primer annealing step at 52°C for 2 min, and an elongation step at 74°C for 3 min. After 40 cycles, a total of 8 μl of each amplified sample was electrophoresed on a 2% agarose gel at 150 V for 45 min and stained with ethidium bromide.

Cleavage with restriction enzymes. For confirmation of the PCR product, a restriction enzyme analysis was used. The cleavage reaction was performed in a volume of 9 μl containing 7 μl of the amplified sample and 2 μl of a buffer consisting of 1 μl of 50 mM $MgCl_2$, 0.5 μl of distilled H_2O, and 0.5 μl (5 U) of the restriction enzyme *Hpa*II (Boehringer GmbH, Mannheim, Germany). The DNA fragments (74 and 126 bp) were separated by electrophoresis on a 2% agarose gel that was run for 45 min at 150 V.

TABLE 1. Comparison of cell culture and PCR test results[a] for *C. trachomatis* in samples from males and females

PCR test	No. of samples in cell culture					
	Males (n = 326)			Females (n = 488)		
	+	−	Total	+	−	Total
+	33	4	37	47	18	65
−	0	289	289	1	422	423
Total	33	293	326	48	440	488

[a] +, positive for *C. trachomatis*; −, negative for *C. trachomatis*.

Statistical analysis. A sample was considered true positive if (i) both cell culture and PCR test were positive; (ii) in female samples, when there was a discrepancy between cell culture and PCR test in one of the samples (urethral or cervical), the other sample (cervical or urethral, respectively) was positive in both tests; (iii) in the remainder of the discrepancies, there was evidence from the medical history of the patient that the sample could be positive. A true-negative sample was a sample for which both the cell culture and the PCR test were negative. If samples could not be categorized according to the above criteria, calculations were performed for situations in which these samples were considered positive or negative, thus resulting in some cases in a range for the calculated parameter. In determining the true prevalence, a patient was considered positive if at least one sample from that patient was true positive. All other patients were considered negative. Sensitivity was calculated as the percentage of positive cell cultures or PCR tests among the true-positive samples. Specificity was calculated as the percentage of negative cell cultures or PCR tests among the true-negative samples. Positive and negative predictive values were calculated according to Bayes' rules (7) by using the true prevalences calculated according to the criteria mentioned above.

RESULTS

A comparison between the results of cell culture and the PCR test for all samples (n = 814) by sex is shown in Table 1. The prevalences of *C. trachomatis* as measured by positive cell culture were 64 of 497 (12.9%) for all patients, 31 of 212 (14.6%) for women, and 33 of 285 (11.6%) for men. The prevalences as measured by positive PCR test were 71 of 497 (14.3%), 36 of 212 (17.0%), and 35 of 285 (12.3%), respectively. The true prevalences as measured by patients with at least one true-positive sample were 70 to 72 of 497 (14.1 to 14.5%), 35 to 37 of 212 (16.5 to 17.4%), and 35 of 285 (12.3%), respectively (Table 2). Table 3 shows the sensitivity, specificity, and positive and negative predictive values for the PCR test and cell culture as calculated for all samples from all patients and calculated for the samples taken from different sites (female urethra and cervix, male urethra). Data from patients with discrepancies between the results of cell culture and the PCR test (discrepancies occurring for 23 of 497 [4.9%] patients) are shown in Table 4. The original PCR test samples from these patients were all retested, but the outcome remained unchanged. For only two patients with discrepant results (Table 4, patients 17 and 23) could the

TABLE 2. Prevalence of *C. trachomatis* infections in 497 patients as measured by positive cell culture, positive PCR test, or true-positive sample

Population (*n*)	No. (%) of samples		
	Cell culture positive	PCR test positive	True positive[a]
All patients (497)	64 (12.9)	71 (14.3)	70–72 (14.1–14.5)
Females (212)	31 (14.6)	36 (17.0)	35–37 (16.5–17.4)
Males (285)	33 (11.6)	35 (12.3)	35 (12.3)

[a] For definition, see the "Statistical analysis" section in Materials and Methods.

samples not be categorized according to the criteria mentioned in "Statistical analysis."

For the patients shown in Table 4, more details are given regarding their medical history and the results of follow-up tests. Patients 1 to 4 were males. Patient 1, asymptomatic with a positive contact, did not receive therapy and was lost to follow-up. Patient 2, symptomatic, had a history of several chlamydial infections, received therapy, and was cell culture and PCR test negative at follow-up after treatment. Patients 3 and 4, both symptomatic and cell culture and PCR test positive, were cell culture negative but remained PCR test positive 2 weeks after the treatment course.

Patients 5 to 23 were females. The infections in patients 5 to 14 were all from contacts with men with a proven chlamydial infection or a symptomatic urethritis of unknown cause. They were asymptomatic and were all cell culture and PCR test negative in cervical and urethral samples at follow-up after treatment. Patient 15 was symptomatic after contact with a man with gonorrhea and was cell culture and PCR test negative at follow-up after treatment. Patient 16 was symptomatic and had a positive *C. trachomatis* contact. Patient 17 was asymptomatic and had no known *C. trachomatis*-positive or symptomatic contact. Patient 18 had a positive *C. trachomatis* contact and was PCR test negative at follow-up after treatment. Patient 19 was symptomatic, had a history of chlamydial infections, and was PCR test negative at follow-up after treatment. Patients 20 and 21 are the same person. At her first visit, as patient 20, she went untreated. At her second visit, as patient 21, 4 months later, she received treatment and became PCR test negative at follow-up. Patient 22 was treated for a chlamydial infection

TABLE 3. Sensitivity, specificity, and positive and negative predictive values of cell culture and PCR test compared with true-positive samples

Sample and test	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
All samples				
PCR	99.0–100.0	99.9–100.0	99.4–100.0	99.8–100.0
Cell culture	77.5–78.4	99.9–100.0	99.2–100.0	96.3–96.6
Female urethra				
PCR	100.0	100.0	100.0	100.0
Cell culture	57.7	100.0	100.0	91.8
Cervix				
PCR	96.9–100.0	99.5–100.0	97.5–100.0	99.3–100.0
Cell culture	84.4–87.5	99.5–100.0	97.1–100.0	96.8–97.6
Male urethra				
PCR	100.0	100.0	100.0	100.0
Cell culture	89.2	100.0	100.0	98.5

TABLE 4. Patients with discrepancies between cell culture and PCR test results[a]

Patient(s)	Sex	Result by:			
		Cell culture		PCR	
		Cervix	Urethra	Cervix	Urethra
1, 2, 3[b], 4[b]	M		−		+
5[c]	F	−	−	+	+
6–14	F	+	−	+	+
15	F	−	+	+	+
16, 17	F	−	−	+	−
18–21[d]	F	−	−	−	+
22[b]	F	−	NA[e]	+	NA
23	F	+	−	−	−

[a] +, positive for *C. trachomatis*; −, negative for *C. trachomatis*.
[b] Follow-up sample 2 weeks after the treatment course (cell culture and PCR test were positive before treatment).
[c] Patient 5, who remained untreated, was seen 5 months later as patient 10.
[d] Patient 20, who remained untreated, was seen 4 months later as patient 21.
[e] NA, not available for testing.

(urethral and cervical cell cultures and PCR test positive), but at follow-up the cell-culture of the cervix was negative while the PCR test remained positive. Patient 23 was the only patient with a positive cell culture and a negative PCR test (cervix). She was asymptomatic. After treatment, the cervical cell culture was negative.

In our follow-up study, all cell cultures were negative 2 weeks after the treatment course, but 3 of 70 patients (Table 4, patients 3, 4, and 22) still yielded a positive PCR test. Patient 3 received a second course of treatment (erythromycin, three 500-mg doses per day for 7 days), while patient 4 did not. Both were cell culture and PCR test negative at the next follow-up visit 3 weeks later. From patient 22, we could not collect further follow-up samples.

DISCUSSION

Isolation of *C. trachomatis* by cell culture was thought to be the most sensitive and specific test available for diagnosing this infection. The choice of the most appropriate laboratory test depends on the local situation. In our setting, where there is a central laboratory for the region, transportation and storage problems play a major role. These can be the cause of a decrease in the viability of the microorganism, thereby lowering the sensitivity of cell culture. For this study, separate endocervical and urethral specimens were collected and cultured instead of having the two samples in one transport medium (8).

The PCR test was performed directly on clinical samples without performing phenol-chloroform extraction and ethanol precipitation of DNA. It has been suggested by others that the use of this form of DNA extraction might decrease sensitivity because of a loss of DNA (5). A further advantage of our direct technique is that it is much easier to handle, saves valuable time (which might be an important factor in cost-effectiveness analysis), and decreases the risk of contamination.

The sensitivity of cell culture for all samples from all patients in this study compared with true-positive samples was 77.5 to 78.4%. Sensitivity was lowest when only the samples from the female urethra (57.7%) were used and higher when samples from the female cervix (84.4 to 87.5%) and the male urethra (89.2%) were used. For the PCR test, these numbers were 99.0 to 100.0, 100.0, 96.9 to 100.0, and

100.0%, respectively. In other recent studies (5, 13, 14, 22), the authors also found a lower sensitivity for cell culture than for the PCR test.

In 23 of 814 samples from 23 patients, a discrepancy between cell culture and the PCR test was found. Of these samples, 20 of 23 were pretreatment samples. More discrepancies occurred in urethral samples (17 samples) than in cervical samples (6 samples). Regarding the urethral samples, more discrepancies occurred in female urethral samples (13 samples) than in male urethral samples (4 samples). This could be explained by looking at the results of a study performed by Jones et al. (8). They found that the geometric mean of the number of inclusions from the male urethra was higher than that for female urethral specimens. Both means were lower than the geometric mean found in endocervical specimens. These data indicate that the probability of detecting *C. trachomatis* by cell culture is highest in cervical samples and lowest in female urethral samples. With a more sensitive test like the PCR, the difference in detection rate between the PCR test and cell culture would thus be much higher for the urethra than for the cervix of female patients, as found in our study. In a cost-effectiveness analysis, the rise in prevalence of *C. trachomatis*-positive female patients by performing the PCR test on samples from the urethra as well as from the cervix might be relevant in a high-risk population. To lower the cost, the two samples can be put together in one transport medium for one PCR test.

Our hypothesis that the positive PCR tests were clinically relevant is supported by the fact that 10 female patients (Table 4, patients 6 through 15) were positive for PCR test and cell culture in one of the samples and so had a proven chlamydial infection but had a discrepancy (positive PCR test but negative cell culture) in the other sample. Furthermore, the clinical relevance of the positive PCR test results for the patients mentioned above and for the remainder of the cell culture-negative but PCR test-positive patients (Table 4, patients 1, 2, 5, and 16 through 21) is supported by the medical histories (except for patient 17) and the negative PCR tests after treatment.

Patient 23 (Table 4) was the only patient with a specimen (endocervical) which was cell culture positive and PCR test negative. The explanation for this result could be either a false-positive cell culture, because of contamination, or a false-negative PCR test. As suggested by Ossewaarde et al. (13), a disadvantage of the use of plasmid primers might be the lack of the detectable presence of the plasmid, as described by Peterson et al. for one clinical isolate (15). This could have been the cause of the negative PCR test in this particular specimen.

The results from our follow-up study show a discrepancy for 3 of 70 treated patients. The samples from these patients remained PCR test positive while cell culture samples became negative. This is in contrast to the results of the follow-up study done by Claas et al. (6). All of their samples were PCR test and cell culture negative 1 week after treatment. *C. trachomatis* is highly susceptible to tetracyclines, as shown by in vitro assays. Resistance of chlamydiae to these antibiotics has never been described. The positive PCR test results in our follow-up study might be due to detection of nonviable *C. trachomatis*. Indeed, after three more weeks without further treatment, the PCR test of one of these patients was negative, suggesting that, at that time, chlamydial DNA was no longer present. This is consistent with the results of Lefebvre et al. (11), who found a 100% correlation between culture, enzyme immunoassay, and direct immunofluorescence when used for "test of cure" 4 weeks or longer after treatment. However, they found false-positive results from the enzyme immunoassay and direct immunofluorescence (capable of demonstrating nonviable *C. trachomatis*) in the first week after treatment. A test of cure is not normally necessary after treatment of a chlamydial infection. If it is nevertheless performed, our data suggest that an interval of 2 weeks after the treatment course may sometimes be too short when using the PCR test.

In conclusion, our results show that the PCR test is more sensitive than the cell culture method in detecting chlamydial infection in a high-risk population, which is in agreement with other investigators, and that positive PCR tests are generally clinically relevant in such a population. Therefore, we think that the PCR test can replace cell culture.

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