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COMPARISON OF PCR BASED ASSAY AND CELL CULTURE IN DETECTION OF UROGENITAL *CHLAMYDIA TRACHOMATIS* INFECTION AND DETERMINATION OF THE INHIBITORY RATE OF SPECIMENS UNDER STUDY

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ABSTRACT

An amplification polymerase chain reaction (PCR) test for the direct detection of *Chlamydia trachomatis* in urethral and endocervical swab specimens from symptomatic and asymptomatic women and men were compared to standard culture technique.

During 6 months, 300 endocervical swab specimens from 205 asymptomatic women (64.4%) and 95 symptomatic women (31.6%), and 187 urethral swab specimens from 79 asymptomatic men (42.3%) and 108 symptomatic men (57.7%) attending the Gynecology Dept. and Genitourinary Clinic of Royal Victoria Hospital, Montreal, were collected. Processed specimens were cultured in McCoy cells and PCR was performed in a tube containing primer for *C. trachomatis* and internal control (IC). PCR products were detected by colorimetric and hybridization assay. Discrepant analysis for any specimens without unanimous results were performed by direct fluorescent antibody (DFA) or major outer membrane gene test (MOMP) with the 2SP medium sediment. In this study culture detected 13.1% of asymptomatic and 33.6% of symptomatic infected women. By PCR, 16% of asymptomatic and 45.2% of symptomatic infected women exhibited positive results. By culture, 36.6% of asymptomatic and 45.3% of symptomatic men were positive, whereas 50.6% of asymptomatic and 51.8% of symptomatic men were positive by PCR. Sensitivity and specificity of PCR for asymptomatic and symptomatic women were 82.5% and 99.3%, and 89.5% and 97.8% respectively. Sensitivity and specificity of PCR for asymptomatic and symptomatic men were 93% and 100%, and 93.3% and 97.9% respectively. Sensitivity and specificity of culture for asymptomatic and symptomatic women and men were 67.5% and 100%, 66.6% and 100%, 67.4% and 81.6%, and 100% and 100% respectively. The overall sensitivity and specificity of PCR and culture were 90% and 98%, and 75.6% and 100%. The internal control revealed that 3.9% of specimens were inhibitory, but when an aliquot of 10 fold dilution of these specimens was retested, 73.6% of them were non-inhibitory. In this study PCR exhibited higher sensitivity than culture for detection of *C. trachomatis* in both endocervical and urethral swab specimens and can be recommended for use in the clinical laboratory.

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INTRODUCTION

Chlamydia trachomatis is the most common bacterial sexually transmitted pathogen in developed countries. Infection with the genital serovars of *C. trachomatis* is sexually acquired through unprotected vaginal or anal sex or vertically at birth.[1] According to the World Health Organization approximately 89 million new *Chlamydia trachomatis* infections occur annually worldwide.[2,3] Nonspecific clinical signs and symptoms of *C. trachomatis* infection and the frequent occurrence of asymptomatic infection render the clinical diagnosis of *C. trachomatis* infections difficult.[4]

Since asymptomatic individuals serve as a reservoir of infection and since complications also occur after silent infection, detection of infected individuals is a major challenge to the preventive health care system.[5] Culture of swab specimens collected from symptomatic and asymptomatic male and females has been the approach to diagnosis for the past 20 years. With advances in DNA technology, laboratory methods for the amplification and detection of plasmid DNA present in all *C. trachomatis* serovars have been introduced for the diagnosis of *C. trachomatis* infection.[6] Because of its ability to specifically amplify minute quantities of nucleic acid, polymerase chain reaction (PCR) has been applied with great success in clinical diagnostics.[7,8,9,10] Relatively simple procedures for extracting nucleic acids from clinical specimens provide samples of reasonable purity without requiring hazardous chemicals and extensive manipulation.[11] Nevertheless extracted clinical specimens may contain impurities that inhibit enzyme based nucleic acid amplification processes.[12,13,5] Several investigators have detected false negative PCR results and suggested that certain inhibitory factors present in specimens may compromise the sensitivity of the assay.[12] Because of the presence of inhibitory factors in the specimens, negative amplification test results do not indicate absence of infection. Inhibitory specimens can be identified by monitoring amplification of a second target nucleic acid which serves as an internal control (IC).[14,15,16] When introduced into the amplification reaction mixture, IC coamplifies with target nucleic acid, thus a positive IC signal assures amplification sufficient to generate a positive signal from very small quantities of target, and can therefore monitor amplification and detection.[17,9,18] Our study was to compare the ability of automated polymerase chain reaction test (Cobas Amplicor) with routine culturing method using endocervical and urethral swab specimens of symptomatic and asymptomatic women and men and to assess the rate of inhibition of the amplification reaction in our materials using PCR test which includes an internal control.

MATERIAL AND METHODS

Between July 1999 and February 2000, 300 women and 187 men attending the Gynecology Dept. and Genitourinary Clinic of Royal Victoria Hospital of Montreal, Canada,were included in the study. During clinical examination,specimens were collected for *C. trachomatis* detection and participants were questioned about the reason for their visit.Both symptomatic and asymptomatic patients were included. Symptomatically infected patients were defined as those presenting with one or more genitourinary clinical symptoms (men with urethral discharge and dysuria, women with abnormal vaginal discharge, spotting, postcoital bleeding, dysuria, lower abdominal pain, dysmenorrhea and dyspareunia). Asymptomatic infected patients were defined as persons who did not contact the physician for urogenital complaints, but contacted the physician for a variety of other reasons, including a

Table I: Results of *C. trachomatis* testing in patients under study.

Category	PCR results	Asym.F.	Sym.F.	Asym.M.	Sym.M.	Total
Culture positive	+	25	30	28	47	130
	*-	2	2	1	2	7
Culture negative DFA or MOMP positive	+	8	13	12	9	42
	-	5	3	2	2	12
Culture negative DFA and MOMP Negative	+	1	1	0	1	3
Culture negative	-	164	46	36	47	293
Total		205	95	79	108	487

* : Inhibitory

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Table II: Frequency of inhibitory specimens in PCR in symptomatic and asymptomatic attendances.

Sex	Symptom category	Swab specimens	% of inhibitory specimens
Women	Asymptomatic	Endocervical	3.4% (7/205)
	Symptomatic		5.2% (5/95)
Men	Asymptomatic	Urethral	3.8% (3/70)
	Symptomatic		3.7% (4/108)
		Total	3.9% (19/487)

Table III: Number of positive results after retesting of inhibitory specimens in culture positive and culture negative symptomatic and asymptomatic women and men.

Results	Asym.F.	Sym.F.	Asym.M.	Sym.M.	Total
PCR Positive Culture Positive	2	2	1	2	7
PCR Positive Culture Negative	5	3	2	2	12
Total	7	5	3	4	19

Asym.: Asymptomatic, Sym.: Symptomatic, F.: Female, M.: Male

Table IV: Rate of culture and PCR positive specimens in symptomatic and asymptomatic women and men.

Results	Asym.F. (%)	Sym.F. (%)	Asym.M. (%)	Sym.M. (%)	Total (%)
Culture	27/205 (13.1)	32/95 (33.6)	29/79 36.7)	49/108 (45.3)	137/487 (28.1)
PCR	38/205 (18.5)	48/75 (50.5)	42/79 53.1)	58/108 (53.7)	186/487 (38.1)

Chlamydia trachomatis positive partner or pregnancy control and *C. trachomatis* testing before intrauterine device insertion. None of the participants had received antibiotics one week prior to the visit.

Specimen collection

One endocervical swab specimen obtained from each woman and one urethral swab specimen obtained from each man was placed into *C. trachomatis* transport medium (2SP containing minimal essential medium with 5% sorbitol , 3% fetal bovine serum and L-glutamine and sent to the microbiology lab during 24 hours. The specimens in 2SP medium were aliquoted for culture, PCR, direct fluorescent antibody (DFA) and /or MOMP gene tests.

Culture for *C. trachomatis*

Specimens in 2SP medium were vortexed vigorously and transferred for Chlamydia culture into cycloheximide treated Mc-Coy cell monolayers growing on glass coverslips in a 24-well plate, and centrifuged at 1000×g (at 35•C) and it's medium was replaced before incubation at 37°C in a 5% CO_2 atmosphere. After 48 hours the inoculated cell monolayer was washed with phosphate -

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Table V: Sensitivity and specificity of PCR before retesting inhibitory specimens.

Sex and Symptoms	Specimen (Swab)	n	Prevalence (%)	Sensitivity PCR/Culture	Specificity PCR/Culture
Female					
Asymptomatic	Endocervical	205	18.5%	82.5/67.5	99.3/100
Symptomatic		95	50.5%	89.5/66.6	97.8/100
Male					
Asymptomatic	Urethral	79	53.1	93/67.4	100/100
Symptomatic		108	53.7	93.3/81.6	97.9/100
Overall		487	38.1	90/75.6	98.9/100

buffer saline and fixed with methanol and stained with fluorescein isothiocynate *C. trachomatis* antibody (Kallestad) for 30 min. The unbound antibodies were removed by washing with phosphate- buffered saline for 10 min., and 25 µL of 90% glycerol in Tris buffer (pH=8.6) was then added to each well. The microwell cell cultures were examined microscopically for the presence of *C. trachomatis* inclusions. The presence of one or more infected cells showing intensely fluorescent cytoplasmic inclusion bodies per well was considered *C. trachomatis* culture positivity.

PCR (Cobas Amplicor *C. trachomatis*) test

PCR test was performed according to the manufacturer's instructions (Cobas Amplicor, Roche Diagnostic System Inc., Branchburg, N.J.).[8] The endocervical and urethral swab specimens collected in 2SP medium were processed as follows:

100µL of CT lysis buffer was mixed with 100µL of the 2SP medium and the mixture was incubated at room temperature for 10 minutes. A total of 200µL of CT specimen diluent was added to each tube, and the tubes were incubated at room temperature for 10 minutes. Of the processed specimens, an aliquot of 50µL was transferred to tubes containing the working Master Mix. The PCR Master Mix contained primers for the 207-bp sequence of the *C. trachomatis* cryptic plasmid. The primers are labelled with Biotin, Taq polymerase and deoxynucleoside triphosphate in a buffered solution.Uracil-N-glycosylate was added to the PCR Master Mix prior to amplification to prevent carry over contamination.[4] The resulting amplification products were captured and detected colorimetrically by hybridization to magnetic microparticles coated with *C. trachomatis* and IC specific oligonucleotid probe. Amplification, hybridization and detection of *C. trachomatis* and IC were automatically performed by the Cobas Amplicor system.[8] Results were expressed as A660 (OD in 660 nm) and were regarded as positive for *C. trachomatis* with OD>0.8 regardless of IC results, and as negative with OD<0.2 and IC>0.2. Specimens yielding OD below the cut-off values for both *C. trachomatis* and IC (OD<0.2) were interpreted as inhibitory. Inhibitory specimens were retested by processing of another aliquot of a 10-fold dilution of 2SP medium, which could remove inhibitors before processing for PCR. Test results were classified using the above mentioned criteria. Specimens yielding *C. trachomatis* results between the negative and positive cutoffs (0.2<OD<0.8) were considered equivocal regardless of IC and OD values and were resolved by retesting of the processed specimen.

Resolution of discrepant results

Specimens that were positive by PCR but negative by culture were considered discrepant. To check for the presence of nonviable *C. trachomatis* EBs in culture-negative samples with discrepant results, direct fluorescent antibody testing (Micro-Trak) was performed with the transport medium sediment obtained following centrifugation. If the DFA test was negative, the specimen was tested with PCR for an alternative target DNA sequence, a portion of the major outer membrane protein (MOMP) gene (Roche).

A patient was considered infected if the culture was positive or the PCR test and either of DFA or MOMP tests were positive.

RESULTS

Table I shows the results of culture, PCR, DFA and /

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or MOMP tests in asymptomatic and symptomatic patients. 38.1% of swab specimens (186 out of 487) were PCR positive and 28.1 % (137 out of 487) were culture positive. Culture detected 13.1 % of asymptomatic and 33.6% of symptomatic infected women. By PCR, 18.5% of asymptomatic and 50.5% of symptomatic infected women exhibited positive results. By culture 36.7% of asymptomatic and 45.5% of symptomatic men were positive whereas 53.1% of asymptomatic and 53.7% of symptomatic men were positive by PCR (Table IV). Sensitivity and specificity of PCR for asymptomatic and symptomatic women were 82.5% and 99.3%, and 89.5% and 97.8% respectively. Sensitivity and specificity of PCR for asymptomatic and symptomatic men were 93% and 100%, and 93.3% and 97.9% respectively. Sensitivity and specificity of culture for asymptomatic and symptomatic women and men were 67.5% and 100% , 66.6% and 100%, 67.4% and 81%, and 100% and 100% respectively. The overal sensitivity and specificity of PCR and culture were 90% and 98.9%, and 75.6% and 100% respectively (Table V). In this study discrepant results were observed in 42 specimens, which after retesting by DFA or MOMP PCR testing, presence of *C. trachomatis* EBs or DNA was confirmed in all of these specimens. The internal control revealed that 3.9 % of specimens was inhibitory of amplification when initially tested, but when an aliquot of 10 fold dilution of these specimens were retested, 73.6 % of them were non-inhibitory therefore taking these numbers to account increased test sensitivity of PCR, but specificity of the test was not affected. Tables II and III show the frequency of inhibitory specimens in PCR and the number of positive results after retesting the inhibitory specimens of culture positive and culture negative symptomatic and asymptomatic patients.

DISCUSSION

The results of this study demonstrated that PCR exhibited excellent sensitivity and specificity for detection of *C. trachomatis* (90% and 98.9%) with urogenital specimens of symptomatic and asymptomatic men and women which is similar to other investigator's reports.[19] Culture was earlier considered the gold standard but PCR studies suggest that the sensitivity of culture even in expert laboratories is as low as 75% to 85%.[12,16] Some investigators reported even lower sensitivity of culture.[9,19] In this study culture missed 24.4% of infected specimens and it's sensitivity was 75.6% which is similar to other reports.[17] So, it is universally accepted that culture can no longer serve as a reference method in the diagnosis of *C. trachomatis*.[20,9]

Use of discrepant analysis is one attempt to improve the performance characteristics of PCR test.[21] Discrepant analysis aims to identify, by an alternative method, true positive specimens originally missed by culture or PCR.[21] Our retesting showed that 8.5% of the swab specimens that were obviously culture negative but PCR positive, did contain *C. trachomatis* EBs or *C. trachomatis* DNA when the specimens were analysed by DFA staining or MOMP PCR test. In one study the rate of discrepancy was 60%,[22] which shows low sensitivity of culture. The specimens are shown to contain several factors that inhibit DNA polymerase reaction.[22] Recently a system has been developed in PCR systems to detect such polymerase reaction inhibitors.[17] Detection of amplification of an IC in the PCR test, ensures that clinical specimens are successfully amplified and detected, hence maximizing test sensitivity by monitoring amplification in specimens yielding negative PCR test results for *C. trachomatis*. In this study the IC enabled us to definitively determine that the frequency of inhibition in our specimens was 3.9% (ranging from 3.4% in endocervical swab specimens of asymptomatic to 5.2% in swab specimens of symptomatic women) , which is similar to Bass et al's report.[12] Some investigators have noted higher inhibition rates detected by the IC system with clinical specimens,[15,23] which may be due to differences in specimen purity. Some specimens contain more inhibitory substances like invisible blood, hormone or enzyme which inhibit amplification.[13] Some of these inhibitors can be removed by dilution or heat treatment of the specimens prior to processing for PCR.[24] Weak inhibition may go undetected in positive specimens that contain relatively high target concentration.[15] In this study taking 14 PCR positive specimens which were initially inhibitory into account increased PCR test sensitivity to 100%. In this survey we have found 3 false positive results. Since this assay is highly sensitive, the potential for false positive due to inadvertent contamination exists. In the Roche PCR assay, this is minimized by the use of Uracil-N-glycosylase which degrades previously amplified DNA that may have contaminated the new samples as a result of carryover. However , separation of preamplification and postamplification areas with unidirectional work flow procedures needs to be done to minimize contamination.

In summary, PCR exhibited high sensitivity and specificity for detection of *C. trachomatis* with urogenital swab specimens and is thus well suited for detection of symptomatic and asymptomatic infected men and women and can be recommended for use in clinical laboratories.

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