Comparison of an in-house PCR assay, direct fluorescence assay and the Roche AMPLICOR *Chlamydia trachomatis* kit for detection of *C. trachomatis*

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To improve the control of *Chlamydia trachomatis* infection in India, a rapid, specific and cost-effective method is much needed. We developed an in-house PCR assay by targeting a unique genomic sequence encoding a protein from the *C. trachomatis* phospholipase D endonuclease superfamily that produces an amplified fragment of 368 bp. The specificity of the primers was confirmed using genomic DNA from other sexually transmitted disease-causing and related micro-organisms and from humans. The assay was highly sensitive and could detect as low as 10 fg *C. trachomatis* DNA. Clinical evaluation of the in-house-developed PCR was carried out using 450 endocervical specimens that were divided in two groups. In group I ($n=274$), in-house PCR was evaluated against the direct fluorescence assay. The resolved sensitivity of the in-house PCR method was 97.22 % compared with 88 % for the direct fluorescent antibody assay. In group II ($n=176$), the in-house PCR was compared with the commercial Roche AMPLICOR MWP CT detection kit. The resolved sensitivity of the in-house PCR assay reported here was 93.1 % and the specificity was 97.46 %, making it a cost-effective alternative for routine diagnosis of genital infection by *C. trachomatis*. The method should facilitate early detection leading to better prevention and treatment of genital infection in India.

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INTRODUCTION

Chlamydia trachomatis is one of the most common causative agents of sexually transmitted infections in developing countries including India. Infection with this agent is often asymptomatic (up to 80 % of women and 40 % of men) (Gaydos *et al.*, 2004), making diagnosis and treatment difficult. Undetected genital infections may evolve into complications such as ectopic pregnancy, pelvic inflammatory disease, salpingitis with tubal scarring and infertility in female patients (Black, 1997; Semeniuk *et al.*, 2002). In infected men, arthritis and epididymitis may result in urethral obstruction and decreased fertility. As asymptomatic and untreated patients can spread the disease to their partners, screening of all sexually active adolescents for *C. trachomatis* infection is recommended (CDC, 2002).

Detection methods for *C. trachomatis* infection include serology, culture method, ELISA, direct fluorescence assay (DFA) and nucleic acid amplification tests (NAATs). Although antigen-based diagnostic assays are as sensitive as the culture method, they show variability due to the methods of sample collection, transport and storage (Mårdh *et al.*, 1981; Mahony & Chernesky, 1985). PCR and ligase chain reaction are more sensitive and specific compared to other diagnostic methods (Black, 1998; Wylie *et al.*, 1998; Semeniuk *et al.*, 2002) and have facilitated the use of less-invasive procedures for detection of asymptomatic *C. trachomatis* infection in female patients. Currently, several commercial NAAT-based assays such as Gen-Probe APTIMA Combo 2 (AC2), BD Probe Tec ET and Roche AMPLICOR (COBAS and manual) PCRs are available but their high cost prevents their routine use in developing countries. There is an urgent need for the development of a rapid, highly sensitive and cost-effective detection method considering the high prevalence of *C. trachomatis* infection in India (Divekar *et al.*, 2000; Singh *et al.*, 2002, 2003; George *et al.*, 2003). In the present study, we have designed and established an in-house PCR assay for detection of *C. trachomatis* using primers against

Abbreviations: DFA, direct fluorescence assay; EBs, elementary bodies; FISH, fluorescence *in situ* hybridization; NAAT, nucleic acid amplification test; NPV, negative predictive value; PPV, positive predictive value; STD, sexually transmitted disease.

Supplementary figures are available with the online version of this paper.

genomic sequence. The performance of the in-house PCR method was evaluated against currently used diagnostic methods and was found to be cost-effective, highly specific and sensitive.

METHODS

Enrollment of patients. A total of 450 symptomatic female patients attending the Gynaecology Outpatient Clinic of Safdarjung Hospital and Hindu Rao Hospital, New Delhi, India, were enrolled in this study following institutional ethical committee clearance and informed oral consent of patients. Women with clinical features of chlamydial cervicitis (mucopurulent cervical discharge or cervical ectopy with inflammation), urethritis or infertility were also enrolled.

Specimen collection. The vulva was examined for lesions and vaginal/cervical discharge and the cervix was examined for ulcers, wart and ectopy. After cleaning the exocervix with a cotton swab (Hi Media), two endocervical swabs were taken from each patient in order to avoid the swab sample variation that can occur when multiple swabs are taken. The clinical samples were divided into two groups (I and II). In the first group of 274 samples, a sterile cotton swab was used to collect each specimen in 1 ml transport medium (130 mM NaCl, 2.6 mM KCl, 10 mM Na₂HPO₄, 1.7 mM KH₂PO₄, 10 mM EDTA, pH 7.4). The specimen from the second swab was smeared on a clean glass slide, and air-dried for the DFA and fluorescence *in situ* hybridization (FISH) assay.

For the second group of 176 samples, two endocervical swab specimens were obtained. The first swab was placed into a vial containing AMPLICOR Specimen Transport Medium and the second swab was placed in 1 ml transport medium described above. All specimens were transported to the microbiology laboratory on ice within 1 h of collection and tested within 24 h or stored at -80°C for subsequent use.

Primer design. More than 50 sequences of various genes of the *C. trachomatis* genome were obtained from GenBank. Short stretches of about 25–30 nucleotides were aligned using the BLAST program from NCBI to find matching sequence with other organisms if any. The sequence that was highly unique to *C. trachomatis* was selected for gene-specific primers. The selected sequence encoded a protein from the phospholipase D endonuclease superfamily (CT157; Entrez GeneID: 884104) producing an amplicon of size 368 bp. Primers were designed using Gene Runner 3.05 software. Their sequences are as follows: forward primer P1, TCTTTTAAACCTCCGGAACCCA-CTT, T_m 74.9; reverse primer P2, GGATGGCATCGCATAGCA-TTCTTTG, T_m 76.3 (US, UK, EU patent pending).

PCR amplification. For PCR assay, specimens were processed by a lysis method as described previously (Chaudhry & Saluja, 2002) and by an alternative method wherein 400 μl clinical specimen was centrifuged at 15 000 g at 4°C for 30 min, and the pellet was resuspended in 40 μl $1 \times$ Tris-EDTA (pH 8.0) and boiled for 10 min at 100°C (referred as crude lysate). Five microlitres of supernatant of processed sample or crude lysate was used for PCR in a reaction volume of 50 μl containing $1 \times$ *Taq* DNA polymerase buffer (50 mM KCl, 10 mM Tris/HCl pH 8.3, 1.5 mM MgCl₂), 200 μM each of the four dNTPs (New England Biolabs), 25 pmol each forward and reverse primer and 1.0 U *Taq* DNA polymerase (Bangalore Genei India). Purified genomic DNA of *C. trachomatis* (kindly provided by Lynn Olinger, Francis I. Proctor Foundation, University of California, San Francisco, USA) was used as a positive control for each set of assays. Amplification was performed in a thermal cycler (I Cycler; Bio-Rad) for 35 cycles: 95°C for 5 min for initial denaturation,

cycling of 95°C for 30 s, 65°C for 30 s, 72°C for 1 min, and final extension at 72°C for 10 min. The amplicons were analysed on a 1.5% agarose gel by electrophoresis. The amplicons from positive samples (10%) were eluted using a DNA isolation kit (Biological Industries) according to the manufacturer's instructions and sequenced using PCR primers (forward primer) with a *Taq* Dye Terminator Cycle Sequencing kit on a 377A autosequencer (Applied Biosystems) according to the manufacturer's instructions. The DNA sequence of the amplified product was compared to known phospholipase D endonuclease superfamily nucleotide sequences (January, 2009) in the GenBank databases using the BLAST program to determine the percentage identity.

Roche AMPLICOR MWP CT detection assay. One hundred and seventy-six endocervical specimens of group II were tested by the Roche AMPLICOR CT detection kit (Roche Diagnostic Systems) according to the manufacturer's instructions. For endocervical specimens, 1 ml specimen diluent was added to endocervical samples, mixed thoroughly by vortexing, and incubated for 10 min at room temperature. After overnight storage at 4°C , 50 μl of the clinical sample was added to each PCR tube containing 50 μl of the *Chlamydia* PCR master mix. The PCR master mix contains primers for internal control as well. The assay was developed as per the instructions given by the manufacturer. To resolve the discrepant samples, genomic DNA was isolated from the aliquots of frozen specimens. Samples were centrifuged at 15 000 g for 30 min. Pellet was resuspended in 500 μl lysis buffer (50 mM Tris-EDTA pH 8.0, 400 μg proteinase K ml^{-1}), incubated at 55°C for 2 h and then boiled at 100°C for 10 min with 1 mM DTT. Thereafter, it was extracted with phenol/chloroform and centrifuged at 12 000 g for 10 min. 2-Propanol with 1/10 volume of 3 M sodium acetate was added to the supernatant and incubated overnight at -20°C . The pellet was collected by centrifugation at 12 000 g for 10 min, washed with 70% ethanol, air-dried and dissolved in water.

Evaluation of specificity and sensitivity. To evaluate the specificity of the primer pair, DNA extracted from *C. trachomatis* serovar L2 (kind gift from Dr Peter Braun, Department of Molecular Biology, Max Plank Institute for Infection Biology, Berlin, Germany), known positive clinical isolates of *C. trachomatis* (20 clinical isolates of serovar A and D obtained from Dr Sudha Salhan, Department of Obstetrics and Gynaecology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi) and other sexually transmitted disease (STD)-causing and related micro-organisms, such as *Mycoplasma* sp. (6) (also a kind gift from Dr Sudha Salhan), *Chlamydia pneumoniae* (2), herpes simplex (1), *Candida* species (7), *Ureaplasma* (10), *Trichomonas* (6) (kind gift from the Department of Microbiology AIIMS, New Delhi, India), *Neisseria gonorrhoeae* (10), *Neisseria meningitidis* (genital isolates) (3), *Neisseria lactamica* 94D4 (1), *Neisseria sicca* 94C1 (1) and *Neisseria subflava* 86G7 (1) (kind gift from Professor J. W. Tapsall, WHO Collaborating Centre for STD and HIV, Department of Microbiology, The Prince of Wales Hospital, Randwick, New South Wales, Australia), was used as template for PCR. DNA was extracted from isolates of STD-causing and related micro-organisms as described above. Ten human genomic DNA samples were also used to evaluate the specificity of the primer pair.

To determine the sensitivity of the primer pair, serial dilutions from 100 pg to 1 fg of purified chlamydial genomic DNA and various dilutions of *C. trachomatis* positive clinical samples were used as template for PCR amplification. All assays were repeated at least five times.

DFA. For DFA (MicroTrak), specimens were centrifuged at 3000 g for 10 min and sediments were air-dried, fixed by incubation in methanol and stained with a FITC-conjugated anti-major outer-membrane protein monoclonal antibody. The slides were examined

for typical apple-green fluorescent elementary bodies (EBs) at $\times 1000$ magnification. The presence of more than 10 fluorescent EBs was considered to be a positive result.

FISH assay. A FISH assay was performed following the method described by Kapur *et al.* (2006). Briefly, smears from endocervical swab specimens were prepared on clean glass slides treated with diethyl pyrocarbonate (DEPC) water. Smears were fixed in 4% paraformaldehyde in PBS for 3 h, immersed in 70% (v/v) ethanol for 15 min and air-dried. The slides were rinsed briefly in DEPC water, air-dried and hybridized with 5-carboxyfluorescein (FAM) fluorochrome labelled oligonucleotide probes complementary to the 16S rRNA gene sequence of *C. trachomatis*.

Evaluation of assays. All assays on clinical specimens were performed blinded to the results of one another. The original samples were taken for resolution of discrepancy. In group I, all 274 samples were tested by in-house PCR and DFA. In order to resolve 24 discrepant results in group I where samples were PCR-positive and DFA-negative, a FISH assay was performed. A specimen was considered true positive for *C. trachomatis* if it was positive by any two methods (DFA/FISH/in-house PCR). For group II of 176 samples, discrepant results ($n=19$) were resolved by PCR-based detection of two housekeeping genes, *ompA* (CT681; Entrez GeneID: 884473) and *gyrA* (CT189; Entrez GeneID: 884941). The treatment response of patients with discrepant results was also obtained. The treatment response was measured based on the resolution of clinical symptoms and doctors' examination obtained from hospital records. A specimen was considered true positive when it was (i) in-house PCR and Roche PCR positive, (ii) Roche PCR positive and *ompA/gyrA* PCR positive or (iii) in-house PCR positive and *ompA/gyrA* PCR positive.

Data analysis. All statistical analysis was performed using SPSS v. 10.0 software. Sensitivities, specificities, positive predictive values (PPVs) and negative predictive values (NPVs) were calculated with 95% confidence intervals to test the significance of the estimates.

RESULTS AND DISCUSSION

Specificity and sensitivity of primers

Using the BLAST program from NCBI, sequences highly unique to *C. trachomatis* were selected for designing gene-specific primers. One of the sequences that was unique to *C. trachomatis* encoded a protein from the phospholipase D endonuclease superfamily (CT157, Entrez Gene ID: 884104) and was selected for primer designing. Amplicons of the desired size (~ 368 bp) were obtained when purified genomic DNA of different serovars of *C. trachomatis* (A, D and L2) was used as template for the in-house PCR (Supplementary Fig. S1 in JMM Online), while no amplicon was detected with the rest of the STD-causing

and related micro-organisms as well as with human genomic DNA. The amplicons obtained from clinical samples were sequenced for at least 10% of positive samples, which further established the specificity of the in-house PCR. The DNA sequences of the amplicons were aligned with the known sequences of serovar A, D and L2 in the GenBank databases (Supplementary Fig. S2). The sensitivity of the primer pair was determined by testing serial dilutions of purified chlamydial genomic DNA for PCR. Using P1/P2 primers, amplified product could be detected with as low as 10 fg chlamydial DNA (equivalent to 9 inclusion-forming units) (Fig. 1). The sensitivity of the primer pair was also checked with *C. trachomatis* positive clinical samples. P1/P2 primers could amplify the specific product when crude lysate of clinical samples was used as template. PCR has been observed to be significantly more sensitive than conventional methods such as culture and antigen-based methods for diagnosis of *C. trachomatis* in clinical specimens (Wu *et al.*, 1992; Mahony *et al.*, 1993; Smith *et al.*, 1993; Shattock *et al.*, 1998; George *et al.*, 2003). Many existing PCR assays, including the Roche MWP test, target the cryptic plasmid (Mahony *et al.*, 1993; Smith *et al.*, 1993; Shattock *et al.*, 1998; George *et al.*, 2003). The chromosomal targets most widely used are *ompA* (Mahony *et al.*, 1993; Smith *et al.*, 1993; Shattock *et al.*, 1998; Wu *et al.*, 1992) and rRNA encoding genes (Mahony *et al.*, 1993). Plasmid-based PCR detection assays are considered to be more sensitive than chromosomal gene-based assays, as plasmid copy number is generally 7–10 per cell (Palmer & Falkow, 1986; Sriprakash & Macavoy, 1987; Ostergaard, 2002). NAAT-based diagnostic kits such as the Roche COBAS AMPLICOR CT assay and the Ligase Chain Reaction kit from Abbott (no longer available) have proven to be highly sensitive (92.9–100%) and specific (99–100%) for *C. trachomatis* detection (Loeffelholz *et al.*, 1992; Bass *et al.*, 1993; Jaschek *et al.*, 1993; Smith *et al.*, 1993; Kessler *et al.*, 1994; Mahony *et al.*, 1994; Catry *et al.*, 1995; Chout *et al.*, 1995; Semeniuk *et al.*, 2002). However, a major obstacle in adopting these kits for routine diagnosis in clinical laboratories in India is the high cost (mean cost of a single assay is \$50). The available diagnostic kits use plasmid-based amplification (Singh *et al.*, 2002; George *et al.*, 2003). Along with others (An *et al.*, 1992; Schachter *et al.*, 1996; Farencena *et al.*, 1997), we have also observed that infection due to plasmid-free variants remains undetected (unpublished results) though it is still not a major clinical problem.

Comparison of the performance of different PCR assays including plasmid, *ompA* and rRNA gene targets has

Fig. 1. Sensitivity of in-house primers using purified chlamydial genomic DNA. PCR amplification of purified genomic DNA (100 pg–1 fg) of *C. trachomatis* serovar D was carried out by using P1/P2 primers.

suggested that in general plasmid primers are 10–1000 times more sensitive (0.1 fg for plasmid DNA) than the genomic DNA primers for *ompA* (0.1–10 pg genomic DNA) and the rRNA gene (1 pg genomic DNA) (An *et al.*, 1992; Joshi *et al.*, 1994; Mahony *et al.*, 1994; Crotchfelt *et al.*, 1998; Niederhauser & Kaempf, 2003; Kapur *et al.*, 2006). In contrast, our assay targeting the phospholipase D endonuclease superfamily gene showed higher sensitivity (10 fg–0.1 pg genomic DNA) than assays with other genomic targets.

Clinical performance of the in-house PCR and comparison with DFA and the Roche AMPLICOR MWP kit

Among 274 cases enrolled in group I, the median age of the patient population was 29 (range 18–57) years. Of 274 samples, 61 (22%) were positive and 189 (69%) were negative by PCR assay using the P1/P2 primer set and by DFA (Table 1). Of 24 discrepant samples, 11 samples were considered true positive, as 9 of these were PCR- and FISH-positive but DFA-negative while 2 were DFA- and FISH-positive but PCR-negative (Table 2). Thirteen samples were PCR-positive with the P1/P2 primer set but tested negative by DFA and FISH assay and were therefore considered true negatives. These 13 cases could not be reconfirmed because samples were insufficient. Follow-up specimens could not be collected because the patients had received antibiotic treatment by that time. Thus, in all, the P1/P2 primer pair detected a significant number of true-positive samples (70 out of 72) ($P < 0.0001$). In contrast, DFA detected 63 out of 72 true-positive samples. Based on our results, the prevalence of *C. trachomatis* infection was 27% among women visiting the gynaecology outpatient clinic when DFA was selected as the gold standard method.

Of 176 samples of group II, 42 were positive and 115 were negative by in-house PCR and commercial PCR assay (Table 3). Of 19 discrepant samples, 11 were positive by in-house PCR but negative by commercial PCR when processed as per the manufacturer's instructions. However, all of them scored positive when purified DNA was used as a template and were considered true positives. Three samples were positive by in-house PCR but negative by Roche PCR. To resolve the discrepancy, purified genomic DNA of the samples was tested for two housekeeping genes: *ompA* and *gyrA*. The three samples

tested negative for both *ompA* and *gyrA* and were treated as true negatives. One sample was positive by in-house PCR and negative by the Roche kit but found to be positive for *ompA* and *gyrA*, so it was taken as a true positive. Four samples were positive by the Roche AMPLICOR MWP kit and negative by in-house PCR but all of these tested positive for *ompA/gyrA* (Table 4). The response to therapy administered to the patients with discrepant results obtained from hospital records also supported the results obtained after resolving the discrepant samples. Thus, in all, the P1/P2 primer pair detected a significant number of samples (54 out of 58, 93%). The commercial PCR assay detected 98% of positive samples only when DNA was purified from 11 samples out of 58. The prevalence of *C. trachomatis* infection was 33% among women patients visiting the gynaecology clinic taking the Roche AMPLICOR MWP kit as the gold standard method.

Sensitivity, specificity, PPV and NPV

Compared with DFA, the in-house PCR with the P1/P2 primers demonstrated a sensitivity of 96.83%. After discrepant analysis by FISH, the sensitivity of the P1/P2 primers increased to 97.22% (Table 5). The specificity of PCR with the P1/P2 primers increased from 89.57% to 93.56%. The PPV of PCR with the P1/P2 primers increased from 73.5% to 84.33%. The NPV of PCR with the P1/P2 primers remained unchanged at 99%.

In the second part of the study, compared with the Roche MWP PCR assay, the sensitivity and specificity of the in-house PCR with P1/P2 was 91.3% and 88.46%. After discrepant analysis, the sensitivity of the PCR method increased to 93.1% and the specificity increased to 97.46%. The PPV of the in-house PCR increased from 73.7% to 94.73% while the NPV remained unchanged at 96.6% (Table 5).

The specificity of DFA is normally less than 90% in actual practice, thus to avoid the tendency to underestimate the specificity of the NAAT-based assay developed by us, the performance of discrepant samples was analysed with alternative methods of detection in order to determine whether they were true positives. Following discrepant analysis, the sensitivity and specificity of the in-house PCR improved considerably. All the discrepant samples (see Table 2) when retested by the in-house PCR method gave 100% reproducible results. The specificity of the in-house

Table 1. Comparison of in-house PCR with DFA: results for *C. trachomatis* before and after discrepant analysis

PCR result (P1/P2)	DFA			After resolution of discordant results	
	Positive	Negative	Total	Positive	Negative
Positive	61	22	83	70 (61+9)	13 (22–9)
Negative	2	189	191	2	189
Total	63	211	274	72	202

Table 2. Resolution of discrepant results by FISH assay for *C. trachomatis* (n=24)

No. of samples	Test results			Conclusion
	P1/P2 PCR	DFA	FISH	
9	+	–	+	True positive
2	–	+	+	True positive
13	+	–	–	True negative

PCR might have improved further if the follow-up samples from patients could be tested.

The estimated sensitivity and specificity range of DFA is 61–92 % and 99–100 %, respectively, in different labor-

atory settings when compared to those of the culture or non-culture method (Thejls *et al.*, 1994; Schachter *et al.*, 1996). The overall sensitivity of DFA in the present study was 88 %. Since the cut-off for DFA is established to get the best combination of sensitivity and specificity, most assays will miss some positive samples by compromising sensitivity to achieve specificity. Although DFA is widely used as a reference method for evaluating diagnostic kits and for checking inter-laboratory variation, Thomas *et al.* (1993) reported that the DFA kit has its limitations. According to the report, about 30 % of the clinical samples contain ≤ 10 EBs as judged by examining the smears stained in the assay. Therefore, the diagnostic performance of the DFA test is highly dependent on the number of chlamydial EBs that should be seen in order to obtain a positive sample. Similar

Table 3. Comparison of in-house PCR with the Roche AMPLICOR MWP kit: results for *C. trachomatis* before and after discrepant analysis

PCR result (P1/P2)	Roche			After resolution of discordant results	
	Positive	Negative	Total	Positive	Negative
Positive	42	15	57	54 (42 + 12)	3 (15 – 12)
Negative	4	115	119	4	115
Total	46	130	176	58	118

Table 4. Resolution of group II discrepant results by PCR-based detection of *ompA* and *gyrA* of *C. trachomatis* (n=19)

No. of samples	P1/P2	Processed and developed with Roche	Purified DNA, developed with Roche*	<i>ompA</i>	<i>gyrA</i>	Symptoms	Treatment response	Conclusion
11†	+	–	+	NR	NR	+	+	True positive
3‡	+	–	–	–	–	–	–‡	True negative
1	+	–	–	+	+	+	+	True positive
3	–	+	NR	+	+	+	+	True positive
1	–	+	NR	+	–	+	+	True positive

NR, Not required.

*Total DNA from the patient sample was purified, followed by NAAT-based detection of *C. trachomatis* using the COBAS AMPLICOR MWP kit.

†The patient samples when processed and tested with the COBAS AMPLICOR MWP did not give positive results, even for the internal control, suggesting that the PCR was inhibited due to the presence of inhibitors in the processed patient samples.

‡Patients were asymptomatic, hence they were not given treatment for *C. trachomatis* infection.

Table 5. Performance of in-house PCR assays based on expanded spectrum of positivity after confirmatory FISH assay and treatment response

CI, Confidence interval.

In-house PCR assay	Sensitivity		Specificity		PPV		NPV	
	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI
Group I	97.22	93.4–100	93.56	90.2–96.9	84.3	76.5–92.2	99	96.8–100
Group II	93.1	86.6–96.6	97.46	94.6–100	94.7	88.9–100	96.6	92–100

results were also observed by Shattock *et al.* (1998), wherein they compared various detection methods for *C. trachomatis*. This can present a problem in a sample that is scored DFA-negative but gives an amplicon by PCR method. In our study, we observed that 13 samples out of 274 were positive with the P1/P2 primers but negative by DFA and FISH assay. These samples, which repeatedly were positive by our PCR, may have contained chlamydial genomic DNA, but unfortunately no follow-up samples were available to confirm this as patients either did not return or had taken the therapy.

We also evaluated our in-house PCR method against the commercially available and widely used Roche MWP kit. It is pertinent to mention that to avoid the swab sample variation that may occur when multiple swabs are taken (especially when infection load is low), only two swabs were taken for each patient. After discrepant analysis, the number of true-positive samples increased from 42 to 58, resulting in increased sensitivity and specificity of the in-house PCR. More importantly, the PPV improved significantly. The Roche AMPLICOR MWP kit gave positive results for four samples that tested negative by the in-house PCR. Twelve samples were positive by in-house PCR but negative by the Roche AMPLICOR MWP kit.

One major problem that we encountered during this study was occurrence of inhibition of the amplification reaction when the Roche MWP kit was used, as has also been observed by others (Shattock *et al.*, 1998; Niederhauser & Kaempf, 2003). When DNA was purified for all discrepant samples (19 samples) and assayed again by the Roche MWP kit, the 11 samples which initially tested negative turned out to be positive (Table 4). These practical limitations prevented us from confirming the four samples that tested positive by in-house PCR but were negative by the Roche MWP kit. One of these patients was symptomatic and also gave an amplicon when PCR was performed for *ompA* and *gyrA*, while the other three were asymptomatic and were negative for *ompA* and *gyrA*. It is also possible that a plasmid-free variant of *C. trachomatis* may be present in the population.

The major objective of this study was to develop an in-house PCR method that is at least as sensitive and as specific as the commercial method. An additional advantage of the in-house PCR method would be its low cost. Since our studies also suggest a high prevalence of *C. trachomatis* (25–30% in females visiting the gynaecology department), there is a definite need to have a cost-effective method for routine diagnosis in India. We consider that implementation of the cost-effective and sensitive PCR assay developed in the present study may allow clinical microbiology laboratories in developing countries to detect *C. trachomatis* rapidly, which would be of great consequence in disease management.

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