A novel real-time PCR to detect *Chlamydia trachomatis* in first-void urine or genital swabs

Katia Jaton, Jacques Bille and Gilbert Greub

Institute of Microbiology, University of Lausanne, Lausanne, Switzerland

Correspondence

Gilbert Greub

gilbert.greub@chuv.ch

Screening for *Chlamydia trachomatis* infections can be performed on urine samples and genital swabs using molecular techniques. A novel approach was developed that combined an automated extraction procedure, an automated liquid-handling system and real-time PCR to detect *C. trachomatis* from urine or swabs. This novel real-time PCR approach was compared to the commercial Cobas Amplicor system on 628 specimens. In a retrospective analysis, 51 samples that tested positive using the Cobas assay were also positive with the real-time PCR, whereas the 49 samples negative with Cobas were also negative with the real-time PCR, for an overall agreement of 100 %. Among 528 prospective samples consecutively received at the authors' laboratory with a request for *C. trachomatis* PCR, five PCR reactions were inhibited when tested with Cobas. These five inhibited samples were found negative with the real-time PCR. Among the remaining 523 samples, 45 (8.6 %) were positive with both methods, 476 (91 %) were negative with both methods, and 2 (0.4 %) were positive with Cobas but negative with the real-time PCR. Thus, when considering Cobas as the gold standard, the overall agreement was 99.6 %, the sensitivity of the real-time PCR was 95.7 % and the specificity was 100 %. The two discrepant samples were retested in parallel and were found negative with both methods. When testing a batch of 25 samples, both reagent costs and laboratory technician time were reduced with the new technique (7.30 euros per sample and 134 min) compared to Cobas (11.20 euros per sample and 232 min). Moreover, due to reduced organizational constraints, the median time from sample reception to result was only 24 h using the automated platform. Overall, this novel real-time PCR approach exhibited an excellent specificity and a sensitivity similar to that of Cobas Amplicor PCR for the detection of *C. trachomatis*. Given its high throughput potential and low costs/laboratory technician time requirement, it may be useful for future use in large *C. trachomatis* screening programs.

Received 12 April 2006

Accepted 23 August 2006

INTRODUCTION

Infections due to *Chlamydia trachomatis* are the most common bacterial sexually transmitted disease in Europe, where a high prevalence of infection has been documented among otherwise healthy women (Wilson *et al.*, 2002). *C. trachomatis* infection may cause urethritis, cervicitis and pelvic inflammatory disease in women. Since more than 50 % of *C. trachomatis* infections are asymptomatic, they may remain undetected, and thus untreated, for extended periods of time. Untreated *C. trachomatis* infection has been linked to serious long-term sequelae, such as ectopic pregnancy and tubal infertility (Gonzales *et al.*, 2004; Hu *et al.*, 2004; Paavonen *et al.*, 1998; Paavonen & Eggert-Kruse, 1999; Schneede *et al.*, 2003; Scholes *et al.*, 1996). This infection can be reliably diagnosed and, in uncomplicated cases, may be treated with a single dose of azithromycin (Egger *et al.*, 1998; Welte *et al.*, 2003; Senn *et al.*, 2005). It is

thus important to identify individuals with *C. trachomatis* infections. However, for many years, the significant cost and laboratory workload associated with conventional diagnostic approaches has prevented their application in many microbiology laboratories.

Nucleic acid amplification techniques are more and more used to diagnose chlamydial infections. In addition to cervicovaginal and urethral swabs, DNA amplification methods may be applied to urine samples, and this is associated with increased acceptance of *C. trachomatis* screening programmes among asymptomatic persons (Black, 1997; Jalal *et al.*, 2006a; Morre *et al.*, 1999; Quinn *et al.*, 1996; Van Der Pol *et al.*, 2000; van Doornum *et al.*, 2001).

The Amplicor PCR test, developed by Roche Diagnostics to detect *C. trachomatis* DNA, was the first US Food and Drug Administration (FDA)-approved *C. trachomatis* molecular test, and many studies have attested its reliability in comparison with culture (Morre *et al.*, 1999). The Cobas

Abbreviation: Ct, threshold cycle.

Amplicor (Roche) is currently the most widely used system in Switzerland. Other systems, such as APTIMA Combo 2 (Gen-Probe) using transcription-mediated amplification (Chernesky *et al.*, 2005), BDProbeTec (Becton Dickinson Diagnostics) using strand-displacement amplification (Van Der Pol *et al.*, 2001), and Abbott real-time PCR, are also currently supplied in Europe to detect *C. trachomatis* DNA. However, these systems remain expensive and are not fully automated. On the other hand, a large number of in-house molecular tests for the detection of *C. trachomatis* have been described (Ostergaard, 1999; Roymans *et al.*, 1996). All these in-house tests are manual and gel based, and use open systems with separate steps for amplification and amplicon analysis. Due to the risk of contamination and a lack of automation, these assays are inappropriate for diagnostic laboratories with high workload.

In recent years, real-time PCR has increasingly been used, since it is easier to perform and faster, and since it is performed in a closed system, less prone to contamination than conventional PCR. A platform concept was set up in our molecular diagnostic laboratory to detect nucleic acid of many different pathogens. Real-time PCR (TaqMan technology, ABI 7900, Applied Biosystems) was coupled with an automated DNA extraction method (MagNA Pure, Roche) and an automated pipetting system (Tecan EVO 150, Tecan Trading). This made it possible to process a large number of specimens in 384-well plates, in a nearly fully automated procedure, with a low requirement for laboratory technician time.

The aims of the study were as follows. (i) To develop a *C. trachomatis* PCR approach that could be run on our automated platform; such a platform concept being associated with a rapid turn-around time, low cost (as a small volume per reaction is possible), low hands-on time and flexibility (since it is possible to target other bacteria and viruses in the same run). (ii) To compare this modern approach with a conventional commercial PCR detection system, the Cobas Amplicor (Roche).

METHODS

Samples. A total of 628 specimens were submitted to our diagnostic laboratory for *C. trachomatis* DNA detection. The samples included 281 cervical or vaginal swabs and 347 urine specimens taken from 562 patients (389 women and 173 men). Among the 628 specimens, 100 specimens, enriched in positive samples to be about 50% positive, were tested retrospectively, and 528 samples were collected prospectively (from March to August 2005), and all were submitted for both Cobas Amplicor and real-time *C. trachomatis* PCR.

To compare the time from reception of a sample at our molecular diagnostic laboratory to the availability of a result, we extracted from our informatics system these two time points for two sets of 100 consecutive samples routinely analysed during two comparable periods (spring 2005 for Cobas Amplicor and spring 2006 for real-time PCR).

***C. trachomatis* Cobas Amplicor test.** Swab samples were collected, transported to the laboratory at room temperature in the transport medium of the Amplicor Transport kit (Roche), and

processed according to the manufacturer's instructions for Cobas Amplicor. They were then frozen at -20°C until further use, or DNA was directly extracted with MagNA Pure (Roche). Urine samples were collected in sterile containers, transported at room temperature, and 10 ml was centrifuged upon receipt for 20 min at 1680 g. The supernatant was discarded, and the pellet processed according to the manufacturer's instructions (Amplicor CT/NG specimen preparation kit). The pellet was frozen at -20°C until further use, or DNA was directly extracted with MagNA Pure.

PCR amplification and detection of positive samples were performed using the automated Cobas Amplicor system as recommended by the manufacturer. The inhibition control (a plasmid containing the primer sequences used for the amplification of *C. trachomatis*) was always run in parallel for each sample to ensure the validity of each negative result.

DNA extraction for the real-time PCR test. Using the MagNA Pure LC automated system (Roche) and the MagNA Pure LC DNA isolation kit I (Roche), DNA was extracted from 200 μl thawed or fresh swab sample suspended in the transport medium or from a 200 μl aliquot of the pellet from 10 ml of centrifuged urine (1680 g for 20 min). The DNA was then eluted in a final volume of 100 μl of the elution buffer provided in the kit. One negative extraction control was tested in each extraction run (32-well plate).

Real-time PCR assay. Primers and probe were selected from sequences of the cryptic plasmid (GenBank accession nos M19487, Y00505, J03321, X06707 and X07547) of five different *C. trachomatis* strains (serotypes A, B, D, L1 and L2, respectively), and designed using Primer Express software (Applied Biosystems). A forward primer Ctr_F (5'-CATGAAAACCTCGTTCCGAAATAGAA-3'), a reverse primer Ctr_R (5'-TCAGAGCTTTACCTAACAACGCATA-3') (which amplify a 71 bp DNA segment of *C. trachomatis*) and a minor-groove binder probe labelled with 5' FAM (6-carboxyfluorescein) Ctr_P (5'-TCGCATGCAAGATATCGA-3') were selected. The melting temperature (T_m) of the probe was chosen to be $10-11^{\circ}\text{C}$ higher than that of the corresponding primers, in order to ensure probe hybridization during primer extension. The primers were prepared by Eurogentec and the probe by Applied Biosystems.

The reactions were performed in a final volume of 20 μl , including 0.2 μM each primer, 0.1 μM Ctr_P probe, 10 μl 2 $\times$ TaqMan Universal Master Mix (Applied Biosystems) and 5 μl DNA sample. Cycling conditions were 2 min at 50°C , 10 min at 95°C , followed by 45 cycles of 15 s at 95°C and 1 min at 60°C . Amplification and PCR product detection were performed with the ABI Prism 7900 Sequence Detection system (Applied Biosystems).

An automated liquid-handling system (Tecan Freedom EVO 150, 8 tips, Tecan Trading), which enables the preparation of 384-well plates with high precision and accuracy, was coupled with the ABI 7900 automated sequence-detection system. Each sample was amplified in duplicate. An inhibition control was tested for each sample. This control was obtained by adding to the 15 μl PCR master mixture, 4 μl DNA sample and 1 μl containing 20 copies of the positive control plasmid. A negative control, starting from the extraction step, was included for each run of extraction, and three additional no-template controls were added for each run of amplification.

Positive control. Initially, we used a commercial positive control (Bio-Rad Amplichek CT/GC Controls). To facilitate bacterial quantification, a plasmid containing the target gene was constructed. DNA was extracted from this *C. trachomatis*-positive control and the target sequence was amplified with CTr_F and CTr_R primers. The final 50 μl reaction mixture contained 0.4 μM each primer, $1.25\text{ U }\mu\text{l}^{-1}$ AmpliTaq Gold DNA polymerase (Taq) (Applied Biosystems), 5 μl 10 $\times$ PCR Buffer containing 20 mM Tris/HCl, 100 mM KCl, 3 mM MgCl_2 , 0.02% gelatin, 400 μM dNTPs

(Microsynth) and 5 µl *C. trachomatis* DNA. PCR was performed in a GeneAmp PCR system 9700 (Applied Biosystems) according to the following procedure: 2 min at 50 °C, 10 min at 95 °C, 50 cycles at 95 °C for 15 s, 60 °C for 1 min, 72 °C for 30 s. PCR products were then purified with the QIAquick PCR purification kit (Qiagen) and cloned into pCR^R 2.1-TOPO vector (Invitrogen) according to the manufacturer's protocol. Isolation of recombinant plasmid DNA was performed with QIAprep Miniprep (Qiagen), and the presence of the correct insert was confirmed by sequencing. The plasmid was then quantified spectrophotometrically (GeneQuant *pro* RNA/DNA calculator, Amersham Pharmacia Biotech). These positive controls were 10-fold diluted, and stabilized by adding human genomic DNA (10 ng µl⁻¹; Roche) and stored at -20 °C. At least three positive controls, corresponding to 100, 10 and one DNA copies, were included in each run. Inter-run variability was assessed based on the results obtained from 60 successive runs.

Interpretation of the real-time TaqMan PCR. During amplification, the reporter dye (FAM) was measured against the passive reference dye (ROX) signal to normalize fluorescence fluctuations not related to PCR amplification and that may occur with increasing cycle numbers. A positive result was determined by identifying the threshold cycle (Ct), i.e. the cycle number at which normalized reporter dye emission was above the background noise (corresponding to ten times the standard deviation of the mean baseline emission calculated for PCR cycles 3–15). If the fluorescent signal did not increase within 45 cycles, the sample was considered negative.

In order to perform quantification, a standard curve was generated by Taqman software from the Ct of serial dilutions of known quantities of cloned plasmids. These standard curves were generated using serial 10-fold dilutions of the plasmid solutions (ranging from 100 to 1 plasmids) and allowed quantification of positive samples in copies per five microlitres of DNA. The standard curve also provided information on the analytical sensitivity and reproducibility of PCR.

An amplification was considered efficient for a specimen if the corresponding inhibition control exhibited about the same Ct (± 1) as the Ct expected for 20 copies, as deduced from the Ct obtained for the 10- and 100-copy positive controls.

Organisms used to assess the specificity of the assay are listed in Table 1. For each pathogen, PCR assays were performed in duplicate with 10 ng of DNA extracted as described above and quantified spectrophotometrically. An inhibition control (DNA of each pathogen spiked with positive control) was simultaneously tested for each organism. Human DNA was also tested.

Economic analyses. To compare the cost of our novel real-time PCR with that of Cobas Amplicor, only direct costs were taken into account, as extracted from our records for 2005. The costs in Swiss francs were converted to euros according to the current exchange rate of 1 Swiss franc = 0.636715 euros on 20 July 2006 (<http://www.xe.com/ucc/convert.cgi>). The model tested a situation in which one to 50 samples were tested in duplicate with three positive and three negative controls per run, one inhibition control per sample, and one negative extraction control per extraction run. The cost of extraction, liquid distribution of reagents and PCR amplification comprised reagents, tubes, tips and other plastic disposables.

In a second model, the cost of laboratory technician time was also taken into account, assuming that 1 h corresponds to about 38 euros (this cost includes holidays and social charges supported by the employer). Finally, since several pathogens may be extracted in the same MagNA Pure run and detected in the same TaqMan run, another economic model was also conducted. In this so-called 'corrected platform approach', we considered that even if only one sample is submitted for detection of *C. trachomatis* DNA, the extraction run and TaqMan run

Table 1. Organisms used for testing the specificity of the real-time PCR

Species	Source
<i>Candida albicans</i>	ATCC 10231
<i>Chlamydomydia pneumoniae</i>	ATCC VR-1310
<i>Enterococcus faecalis</i>	ATCC 29212
<i>Escherichia coli</i>	ATCC 35218
<i>Gardnerella vaginalis</i>	Clinical specimen
<i>Haemophilus influenzae</i>	ATCC 49247
<i>Klebsiella pneumoniae</i>	ATCC 27736
<i>Lactobacillus</i> sp.	Clinical specimen
<i>Neisseria gonorrhoeae</i>	ATCC 49226
<i>Neisseria weaveri</i>	Clinical specimen
<i>Neochlamydia hartmanellae</i>	ATCC 50802
<i>Parachlamydia acanthamoebae</i>	ATCC VR-1476
<i>Protochlamydia amoebophila</i>	ATCC PRA-7
<i>Pseudomonas aeruginosa</i>	ATCC 27853
<i>Simkania negevensis</i>	ATCC VR-1471
<i>Staphylococcus saprophyticus</i>	ATCC 15305
<i>Streptococcus agalactiae</i>	ATCC 13813
<i>Streptococcus intermedius</i>	Clinical specimen
<i>Streptococcus pyogenes</i>	ATCC 19615
<i>Waddlia chondrophila</i>	ATCC VR-1470

will be complete due to the extraction and/or amplification of additional samples submitted for other diagnostic purposes. The cost of the ABI 7900, Tecan EVO 150, MagNA Pure and Cobas Amplicor were not taken into account in the analysis.

RESULTS AND DISCUSSION

Analytical sensitivity and specificity of the real-time PCR

A plasmid containing the target sequence was constructed and used as a positive control. To determine the analytical sensitivity of the PCR, 10-fold serial dilutions (1000 to 1 copies) of the positive plasmid control were run with the Cobas Amplicor system and the novel real-time PCR in parallel. When the Cobas Amplicor was found positive, the real-time PCR was also found positive. Thus, the level of sensitivity of the two methods was considered equivalent. Multiple investigators have used the Cobas Amplicor system, which targets the cryptic plasmid (Morre *et al.*, 1999; van Doornum *et al.*, 2001). In other reports, conventional PCR methods that target other genes have been used (Jalal *et al.*, 2006b; Whitley & Sloots, 2005). Since seven to 10 copies of the cryptic plasmid are present in *C. trachomatis*, a PCR targeting the cryptic plasmid should be more sensitive than a PCR targeting the MOMP gene or the gene for the cysteine-rich outer-membrane protein, both of which are present at only one copy per genome (Jalal *et al.*, 2006b; Mahony *et al.*, 1993; Ossewaarde *et al.*, 1992; Ostergaard, 1999; Roosendaal *et al.*, 1993). Although some isolates have been described that lack the cryptic plasmid, with a risk of producing false-negative results (Farencena

et al., 1997; Peterson *et al.*, 1990; Stothard *et al.*, 1998), the plasmidless strains are relatively rare. We thus decided to target the cryptic plasmid. Primers and probes were designated to detect all serotypes and to be used at 60 °C. This annealing temperature was chosen to adapt the test to our platform that can detect other micro-organisms in the same run. The level of sensitivity of the real-time PCR was one copy per reaction or 100 copies ml⁻¹. When testing the reproducibility of these results by testing triplicates of the 10-fold dilutions in five different experiments, 100 and 80 % of replicates were positive for *C. trachomatis* at concentrations of 10 and one copy per reaction, respectively, showing an excellent reproducibility with 10 copies and consistent with a stochastic distribution of DNA with one copy per reaction.

No amplification was observed when testing the real-time PCR with human genomic DNA or with DNA of 20 different micro-organisms related to *C. trachomatis* or that may be present in urogenital samples (Table 1). This indicates a high specificity of the novel real-time PCR.

Retrospective analysis

To test the reliability of the new real-time PCR on clinical specimens, a total of 100 samples previously tested by Cobas Amplicor were analysed blind by real-time PCR. These samples, collected between August 2003 and August 2005 and taken from 67 women and 32 men, were enriched in positive samples (51 positive samples by Cobas Amplicor).

These 51 positive samples (22 swabs and 29 urines) were also found positive with the real-time PCR (Table 2). Conversely, all 49 samples that were negative by Cobas Amplicor (29 swabs and 20 urines) were also found negative with the real-time PCR. Inhibition of the real-time PCR was not observed, as shown by the efficient amplification of all spiked specimens. Thus, the real-time PCR exhibited a high sensitivity and an excellent specificity when considering the Amplicor test as the gold standard, with 100 % agreement on the 100 samples retrospectively studied. Moreover, the

Table 2. Comparison of real-time PCR and Cobas Amplicor for 100 specimens tested retrospectively and 523 specimens tested prospectively

Tested retrospectively	Real-time PCR	Cobas Amplicor	
		Positive	Negative
	Positive	51	0
	Negative	0	49
Tested prospectively	Real-time PCR	Cobas Amplicor	
		Positive	Negative
	Positive	45	0
	Negative	2	476

intra-run variability of the real-time PCR was excellent, with an R^2 of 0.969 when plotting the Ct of both duplicates on the x and y axes (Fig. 1a). For a single sample, only one duplicate was positive (not plotted on the graph).

Prospective analysis

Of the 528 samples received at the laboratory during the 6-month prospective study period, 298 (56 %) were urine samples and 230 (44 %) were swab specimens. These were collected from 323 women and 140 men. The samples were tested in parallel and blind with Cobas Amplicor and the real-time PCR. With Cobas Amplicor, 476 specimens were found negative and 47 positive for *C. trachomatis*. Fifteen specimens were found to be inhibited in a first run. When retested after 24 h at -20 °C, one specimen was found positive, nine were found negative and five (0.9 %) remained inhibited. These five samples were negative when tested with the real-time PCR. The results of both procedures were then compared for the remaining 523 specimens (Table 2). The prevalence of positivity was 8.9 % (47/523) and 8.5 % (45/528) with Cobas Amplicor and real-time PCR, respectively. Overall, 45 specimens (35 urine and 10 swabs) were positive and 476 specimens negative with

Fig. 1. Intra-run variability of the real-time PCR between duplicates of positive samples tested in the retrospective (a) and prospective (b) parts of the study.

both methods. Thus, overall agreement was 99.6 %, and the sensitivity and the specificity of the real-time PCR were 95.7 and 100 %, respectively, when considering Cobas Amplicor as the gold standard. Two specimens (0.4 %), one urine and one swab, were positive with Cobas Amplicor but negative with the real-time PCR. These two samples with discrepant results were retested in parallel and were found negative by both methods, suggesting that the discrepant results corresponded to false-positive results of the Cobas Amplicor or to a stochastic distribution of DNA in specimens with a very low amount of *C. trachomatis* DNA. Carry-over contamination during extraction is unlikely to explain these discrepancies, since all extraction-negative controls were negative. Intra-run variability was also excellent, with an R^2 of 0.956 when plotting the Ct of both duplicates of positive samples prospectively tested on the x and y axes (Fig. 1b). Inter-run variability was also good. Indeed, if we except two positive controls that were negative, all 100 copies of the positive controls gave values that were within 2SD of the mean (Fig. 2). Three 10-copy positive controls exhibited a variation that was above 2SD from the mean, and two additional 10-copy positive controls were found negative (Fig. 2).

Economic analysis

According to Fig. 3(a), the costs of reagents and plastic disposables used to analyse 25 urogenital swabs or urines in a single run for *C. trachomatis* were reduced by about 35 % when using the real-time system instead of Cobas Amplicor (7.30 versus 11.20 euros per sample). Moreover, it took 134 min (mean 5.4 minutes per sample, i.e. 3.45 euros per sample) for the 25 urogenital swab analyses to be completed with the real-time PCR system (from the extraction of DNA to the results), compared with 232 min (mean 9.27 minutes per sample, i.e. 5.90 euros per sample) for the Cobas Amplicor system. When taking into account reagents, plastic disposables, and laboratory technician time, the cost per sample for a batch of 25 samples was 10.75 euros for the real-time PCR system compared with 17.10 euros for the Cobas Amplicor system (see Fig. 3b). Thus, when 25 samples were tested in the same batch, molecular diagnosis of *C. trachomatis* using the automated extraction procedure

associated with the liquid-handling system and real-time PCR allowed a 35 % reduction of reagent costs and a 42 % reduction of laboratory technician time compared with Cobas Amplicor, corresponding to an overall 37 % reduction in costs. Such a reduction in costs was already present when 15 samples were tested in the same run.

Moreover, due to the versatility of our molecular diagnostic platform and the fact that DNA of other pathogens may be extracted and/or amplified in the same run, the true cost of the real-time PCR system has to be corrected for the price of plastic disposables and technician time shared with analyses submitted for other pathogens and tested in the same batch (see corrected platform approach data in Fig. 3a, b). Thus, as shown by an arrowhead in Fig. 3(a, b), the cost to test a single sample for the presence of *C. trachomatis* DNA is 60 % lower with the platform approach than with Cobas Amplicor (12.70 versus 32.15 euros for reagents and plastic disposables; 22.40 versus 56.50 euros taking into account also the costs associated with laboratory technician time).

Advantages of the novel real-time PCR

Horizontal and vertical PCR contamination with amplicons is an important drawback of PCR (Greub *et al.*, 2005), especially when processing large numbers of samples. This contamination may be prevented by using closed systems, such as the LightCycler (Roche) and the MyiQ (Bio-Rad). However, the ABI 7900 has the additional advantages of a 384-well format and compatibility with an automated liquid-handling system (Tecan). This fully automated system ensures secure sample handling and efficient PCR plate preparation, and may be an ideal tool for large screening programmes, given its high-throughput potential. Moreover, this integrated system decreases the time to obtain a result, due to a lower hands-on time per run and the fact that it allows the simultaneous analysis of different targets (e.g. other bacteria, virus, fungi and parasites) (Rougemont *et al.*, 2004; Welti *et al.*, 2003). Indeed, since the cost to analyse a single sample using the platform approach is 60 % lower than that using Cobas Amplicor (see above), and approximately the same as the cost per sample to process five to six samples simultaneously, some small laboratories might prefer to test *C. trachomatis* only in

Fig. 2. Inter-run variability using 10 and 100 plasmid copies, as obtained during 60 successive runs. The solid black lines show the mean Ct obtained with the 10- (upper line) and 100-copy (lower line) positive controls. The dashed lines each side of the means represent 2SD.

Fig. 3. Economic analysis for one to 50 samples analysed as a batch, showing the lower cost of the real-time PCR system, especially when corrected for batch analyses (corrected platform approach) with samples submitted for other pathogens. (a) Costs of reagents and plastic disposables; (b) total costs, including laboratory technician time. Grey arrows show increased costs every 10 samples for Cobas Amplicor (12 places available on each ring, with a need for positive and negative controls). The black arrow shows the increased costs every 31 samples when using the real-time PCR system (32 places per extraction batch available, with a need for a negative extraction control). The arrowhead shows the cost to analyse a single sample using the corrected platform approach.

batches to reduce costs further, although this may prolong the time taken to obtain results. In our molecular diagnostic laboratory, results were available within a median of 24 and

30 h by the automated molecular platform and Cobas, respectively. A lower time to results is thus an additional marginal advantage of our system.

Real-time PCR also has the advantage of providing quantitative results. Fig. 4 shows the distribution of the number of *C. trachomatis* DNA copies per five microlitres of DNA sample in all 96 positive samples. As many as 16 % (15/96) of positive samples exhibited a very low copy number (<10 copies per five microlitres of DNA), demonstrating the need for a high sensitivity to reliably diagnose *C. trachomatis* infection. In the future, this quantitative information might be useful to determine whether chlamydial load correlates with disease severity. However, to date, published studies on the usefulness of quantitative results in clinical settings are not really convincing. Indeed, chlamydial loads do correlate with male urethral discharge, cervical ectopy (Hobson *et al.*, 1980; Arno *et al.*, 1994; Geisler *et al.*, 2003), mucopurulent cervicitis and pelvic inflammatory diseases in some studies (Geisler *et al.*, 2001), whereas in other more recent studies, the number of *C. trachomatis* organisms does not correlate with the patient's age and clinical symptoms (Gomes *et al.*, 2005; Jalal *et al.*, 2006b). One of the main limitations of our PCR is that, unlike some commercial molecular assays, it only detects the DNA of *C. trachomatis*. In the future, we will develop a *Neisseria gonorrhoeae* PCR that may detect both *C. trachomatis* and *N. gonorrhoeae* in a duplex assay.

Given the long-term complications associated with the frequently asymptomatic urogenital chlamydial infection, it is important to have a specific and sensitive diagnostic test. In previous studies, the *C. trachomatis* Cobas Amplicor test has shown a high sensitivity (90–100 %) and an excellent specificity (98–100 %) on urine and endocervical specimens (Black, 1997; Quinn *et al.*, 1996; Morre *et al.*, 1999; Van Der Pol *et al.*, 2000). Nevertheless, this commercial method is rather expensive, and is not compatible with an automated molecular platform. We have developed a high-throughput,

Fig. 4. Distribution of *C. trachomatis* DNA copy load in the 96 positive samples.

highly specific quantitative real-time PCR for the detection of *C. trachomatis*, the sensitivity of which was equivalent to that of Cobas Amplicor. Our system is more cost effective than other commercial nucleic-acid-based systems for the following reasons. (i) Thanks to the use of an automated liquid-handling system, it is possible to work with 20 µl volumes and in a 384-well format; this reduces costs associated with the master mix and plastic disposables. (ii) Automation (for extraction, pipetting and PCR amplification) strongly reduces laboratory technician time (i.e. salary costs). Commercial tests currently available are not amenable to such a level of automation.

In the absence of suitable vaccines, efforts towards the prevention of *C. trachomatis* infection and its associated long-term complications rely mainly on education, screening, and the treatment of cases. Based on its efficacy and reliability, the novel real-time PCR has the potential to contribute to future large-scale *C. trachomatis* screening programmes.

ACKNOWLEDGEMENTS

We thank René Brouillet, Lara Campa and Cyril André (Molecular Diagnostic Laboratory of the Institute of Microbiology, University of Lausanne) for technical help, and Philip Tarr (Infectious Diseases Service, Lausanne, Switzerland) for reviewing the manuscript.

REFERENCES

- Arno, J. N., Katz, B. P., McBride, R., Carty, G. A., Batteiger, B. E., Caine, V. A. & Jones, R. B. (1994). Age and clinical immunity to infections with *Chlamydia trachomatis*. *Sex Transm Dis* **21**, 47–52.
- Black, C. M. (1997). Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Microbiol Rev* **10**, 160–184.
- Chernesky, M. A., Martin, D. H., Hook, E. W., Willis, D., Jordan, J., Wang, S., Lane, J. R., Fuller, D. & Schachter, J. (2005). Ability of new APTIMA CT and APTIMA GC assays to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male urine and urethral swabs. *J Clin Microbiol* **43**, 127–131.
- Egger, M., Low, N., Smith, G. D., Lindblom, B. & Herrmann, B. (1998). Screening for chlamydial infections and the risk of ectopic pregnancy in a county in Sweden: ecological analysis. *BMJ* **316**, 1776–1780.
- Farencena, A., Comanducci, M., Donati, M., Ratti, G. & Cevenini, R. (1997). Characterization of a new isolate of *Chlamydia trachomatis* which lacks the common plasmid and has properties of biovar trachoma. *Infect Immun* **65**, 2965–2969.
- Geisler, W. M., Suchland, R. J., Whittington, W. L. & Stamm, W. E. (2001). Quantitative culture of *Chlamydia trachomatis*: relationship of inclusion-forming units produced in culture to clinical manifestations and acute inflammation in urogenital disease. *J Infect Dis* **184**, 1350–1354.
- Geisler, W. M., Suchland, R. J., Whittington, W. L. & Stamm, W. E. (2003). The relationship of serovar to clinical manifestations of urogenital *Chlamydia trachomatis* infection. *Sex Transm Dis* **30**, 160–165.
- Gomes, J. P., Borrego, M. J., Atik, B., Santo, I., Azevedo, J., Brito de, S. A., Nogueira, P. & Dean, D. (2005). Correlating *Chlamydia trachomatis* infectious load with urogenital ecological success and disease pathogenesis. *Microbes Infect* **8**, 16–26.
- Gonzales, G. F., Munoz, G., Sanchez, R. & 8 other authors (2004). Update on the impact of *Chlamydia trachomatis* infection on male fertility. *Andrologia* **36**, 1–23.
- Greub, G., Lepidi, H., Rovey, C., Casalta, J. P., Habib, G., Collard, F., Fournier, P. E. & Raoult, D. (2005). Diagnosis of infectious endocarditis in patients undergoing valve surgery. *Am J Med* **118**, 230–238.
- Hobson, D., Karayiannis, P., Byng, R. E., Rees, E., Tait, I. A. & Davies, J. A. (1980). Quantitative aspects of chlamydial infection of the cervix. *Br J Vener Dis* **56**, 156–162.
- Hu, D., Hook, E. W., III & Goldie, S. J. (2004). Screening for *Chlamydia trachomatis* in women 15 to 29 years of age: a cost-effectiveness analysis. *Ann Intern Med* **141**, 501–513.
- Jalal, H., Stephen, H., Al-Suwaine, A., Sonnex, C. & Carne, C. (2006a). The superiority of polymerase chain reaction over an amplified enzyme immunoassay for the detection of genital chlamydial infections. *Sex Transm Infect* **82**, 37–40.
- Jalal, H., Stephen, H., Curran, M. D., Burton, J., Bradley, M. & Carne, C. (2006b). Development and validation of a rotor-gene real-time PCR assay for detection, identification, and quantification of *Chlamydia trachomatis* in a single reaction. *J Clin Microbiol* **44**, 206–213.
- Mahony, J. B., Luinstra, K. E., Sellors, J. W. & Chernesky, M. A. (1993). Comparison of plasmid- and chromosome-based polymerase chain reaction assays for detecting *Chlamydia trachomatis* nucleic acids. *J Clin Microbiol* **31**, 1753–1758.
- Morre, S. A., van Valkengoed, I., Moes, R. M., Boeke, A. J., Meijer, C. J. & van den Brule, A. J. (1999). Determination of *Chlamydia trachomatis* prevalence in an asymptomatic screening population: performances of the LCx and COBAS Amplicor tests with urine specimens. *J Clin Microbiol* **37**, 3092–3096.
- Ossewaarde, J. M., Rieffe, M., Rozenberg-Arska, M., Ossenkoppele, P. M., Nawrocki, R. P. & van Loon, A. M. (1992). Development and clinical evaluation of a polymerase chain reaction test for detection of *Chlamydia trachomatis*. *J Clin Microbiol* **30**, 2122–2128.
- Ostergaard, L. (1999). Diagnosis of urogenital *Chlamydia trachomatis* infection by use of DNA amplification. *APMIS Suppl* **89**, 5–36.
- Paavonen, J. & Eggert-Kruse, W. (1999). *Chlamydia trachomatis*: impact on human reproduction. *Hum Reprod Update* **5**, 433–447.
- Paavonen, J., Puolakkainen, M., Paukku, M. & Sintonen, H. (1998). Cost-benefit analysis of first-void urine *Chlamydia trachomatis* screening program. *Obstet Gynecol* **92**, 292–298.
- Peterson, E. M., Markoff, B. A., Schachter, J. & de la Maza, L. M. (1990). The 7.5-kb plasmid present in *Chlamydia trachomatis* is not essential for the growth of this microorganism. *Plasmid* **23**, 144–148.
- Quinn, T. C., Welsh, L., Lentz, A., Crotchfelt, K., Zenilman, J., Newhall, J. & Gaydos, C. (1996). Diagnosis by AMPLICOR PCR of *Chlamydia trachomatis* infection in urine samples from women and men attending sexually transmitted disease clinics. *J Clin Microbiol* **34**, 1401–1406.
- Roosendaal, R., Walboomers, J. M., Veltman, O. R., Melgers, I., Burger, C., Bleker, O. P., MacClaren, D. M., Meijer, C. J. & van den Brule, A. J. (1993). Comparison of different primer sets for detection of *Chlamydia trachomatis* by the polymerase chain reaction. *J Med Microbiol* **38**, 426–433.
- Rougemont, M., Van, S. M., Sahli, R., Hinrikson, H. P., Bille, J. & Jaton, K. (2004). Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. *J Clin Microbiol* **42**, 5636–5643.

- Roymans, R. T., Onland, G. & Postma, B. H. (1996).** One-day detection of PCR amplified *Chlamydia trachomatis* DNA in clinical samples: ELISA versus Southern blot hybridisation. *J Clin Pathol* **49**, 581–583.
- Schneede, P., Tenke, P. & Hofstetter, A. G. (2003).** Sexually transmitted diseases (STDs) – a synoptic overview for urologists. *Eur Urol* **44**, 1–7.
- Scholes, D., Stergachis, A., Heidrich, F. E., Andrilla, H., Holmes, K. K. & Stamm, W. E. (1996).** Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N Engl J Med* **334**, 1362–1366.
- Senn, L., Hammerschlag, M. R. & Greub, G. (2005).** Therapeutic approaches to *Chlamydia* infections. *Expert Opin Pharmacother* **6**, 2281–2290.
- Stothard, D. R., Williams, J. A., Van Der Pol, B. & Jones, R. B. (1998).** Identification of a *Chlamydia trachomatis* serovar E urogenital isolate which lacks the cryptic plasmid. *Infect Immun* **66**, 6010–6013.
- Van Der Pol, B., Quinn, T. C., Gaydos, C. A. & 8 other authors (2000).** Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. *J Clin Microbiol* **38**, 1105–1112.
- Van Der Pol, B., Ferrero, D. V., Buck-Barrington, L. & 10 other authors (2001).** Multicenter evaluation of the BDProbeTec ET Syst for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. *J Clin Microbiol* **39**, 1008–1016.
- van Doornum, G. J., Schouls, L. M., Pijl, A., Cairo, I., Buimer, M. & Bruisten, S. (2001).** Comparison between the LCx Probe system and the COBAS AMPLICOR system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in patients attending a clinic for treatment of sexually transmitted diseases in Amsterdam, The Netherlands. *J Clin Microbiol* **39**, 829–835.
- Welte, R., Kretzschmar, M., van den Hoek, J. A. & Postma, M. J. (2003).** A population based dynamic approach for estimating the cost effectiveness of screening for *Chlamydia trachomatis*. *Sex Transm Infect* **79**, 426.
- Welti, M., Jatón, K., Altwegg, M., Sahli, R., Wenger, A. & Bille, J. (2003).** Development of a multiplex real-time quantitative PCR assay to detect *Chlamydia pneumoniae*, *Legionella pneumophila* and *Mycoplasma pneumoniae* in respiratory tract secretions. *Diagn Microbiol Infect Dis* **45**, 85–95.
- Whiley, D. M. & Sloots, T. P. (2005).** Comparison of three in-house multiplex PCR assays for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using real-time and conventional detection methodologies. *Pathology* **37**, 364–370.
- Wilson, J. S., Honey, E., Templeton, A., Paavonen, J., Mardh, P. A. & Stray-Pedersen, B. (2002).** A systematic review of the prevalence of *Chlamydia trachomatis* among European women. *Hum Reprod Update* **8**, 385–394.