Comparative performance of the Roche COBAS Amplicor assay and an in-house real-time PCR assay for diagnosis of *Chlamydia trachomatis* infection

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This study investigated the comparative performance of the Amplicor assay and an in-house semi-automated, multiplex real-time PCR for the diagnosis of genital chlamydial infection. Four different assays, the COBAS Amplicor CT test (Amplicor PCR), in-house real-time PCR (IHRT-PCR), in-house nested cryptic plasmid PCR and in-house nested major outer membrane protein PCR, were performed on genital swabs from 1000 consecutive patients attending a genitourinary medicine clinic. The samples were designated true positive if *Chlamydia trachomatis* DNA was detected by at least two of the four above-mentioned assays while a sample was defined as true negative if *C. trachomatis* DNA was detected in only one or none of the assays. By this criterion, there were 129 true positive and 871 true negative samples for *C. trachomatis* DNA in this cohort. Amplicor PCR designated 144 samples positive: 128 (89 %) of 144 samples were true positive and 16 (11 %) were false positive. IHRT-PCR detected 126 of 129 true positive samples and did not generate any false positive results. The sensitivity of IHRT-PCR was comparable with, and specificity was higher than, Amplicor PCR for the diagnosis of genital chlamydial infection.

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INTRODUCTION

Chlamydia trachomatis is the most common sexually transmitted bacterial infection in the UK (Health Protection Agency, 2005a). *C. trachomatis* infection is asymptomatic in at least 70 % of women and 50 % of men (Cates & Wasserheit, 1991). Asymptomatic individuals do not seek medical care and untreated infection can cause serious sequelae, i.e. pelvic inflammatory disease, ectopic pregnancy, infertility and reactive arthritis (Centers for Disease Control and Prevention, 2002). Accurate and timely diagnosis of infection is essential not only for the treatment of infected individuals but also to reduce the transmission of infection among the sexually active population. Nucleic acid amplification tests (NAATs) have demonstrated their superiority over antigen detection and cell culture for

screening as well as for the diagnosis of *C. trachomatis* infection (Østergaard, 1999; Jalal *et al.*, 2006a).

According to the National Strategy for Sexual Health and HIV for England, sexually active individuals aged under 25 years should be screened and asymptomatic infections should be treated (Department of Health, 2002). The phased implementation of this policy began in 2002. Apart from screening, in 2005, the Department of Health provided funding to at least one central laboratory in each Strategic Health Authority to convert to nucleic acid amplification testing technology for the diagnosis of *C. trachomatis* infection. A number of highly sensitive commercial NAATs for the diagnosis of genital chlamydial infection are available. However, up to 15 % of positive results generated by some of these assays were reported to be false (Culler *et al.*, 2003; Castriciano *et al.*, 2002; Vincelette *et al.*, 1999; Van Dyck *et al.*, 2001). We have developed a highly sensitive and specific in-house real-time PCR (IHRT-PCR) for the diagnosis of *C. trachomatis* infection. This study investigated the comparative performance of IHRT-PCR and Amplicor PCR for the diagnosis of genital chlamydial infection.

Abbreviations: IHNCP-PCR, in-house nested cryptic plasmid PCR; IHNMOMP-PCR, in-house nested major outer membrane protein PCR; IHRT-PCR, in-house real-time PCR; NAATs, nucleic acid amplification tests.

METHODS

IHRT-PCR was performed on 1000 coded DNA samples as described previously (Jalal *et al.*, 2006b) with one change. Due to the limited quantity of stored DNA, 5 µl DNA instead of 10 µl from each sample was used per reaction. These samples were obtained from a cohort of patients described previously (Jalal *et al.*, 2006a). Briefly, the cohort included 1000 consecutive patients attending the genitourinary medicine clinic, in late 2003, at Addenbrooke's Hospital, Cambridge. There were 437 males, median age 26 years (range 15–77), and 563 females, median age 23 years (range 15–58), in this cohort. Genital swabs were collected with the IDEIA chlamydia collection kit, and DNA was extracted using the MagNA Pure LC total nucleic acid isolation kit and MagNA Pure LC Robot. Three different PCR assays were performed on each sample in the previous study: Amplicor PCR, in-house nested cryptic plasmid PCR (IHNC-PCR) and in-house nested major outer membrane protein PCR (IHNMOMP-PCR) (Jalal *et al.*, 2006a). The data from the previous study and the results generated by this study were used to investigate the comparative performance of IHRT-PCR and Amplicor PCR for the diagnosis of *C. trachomatis* infection.

RESULTS AND DISCUSSION

The samples were designated true positive if *C. trachomatis* DNA was detected by at least two of the four above-mentioned assays while a sample was defined as true negative if *C. trachomatis* DNA was detected in only one or none of the assays. By this criterion, there were 129 true positive and 871 true negative samples for *C. trachomatis* DNA in this cohort. IHNMOMP-PCR identified 117 (91 %) of 129 true positive results. IHNC-PCR identified all of the 129 true positive results. Eleven (8 %) of 140 positive results generated by IHNC-PCR were designated false positive. Amplicor PCR identified 128 of 129 true positive results, but 16 (11 %) of 144 positive results generated by Amplicor PCR were designated false positive. IHRT-PCR detected *C. trachomatis* DNA in 126 of 129 true positive samples, but not in any of 871 true negative samples, thus achieving a sensitivity of 98 % and a specificity of 100 %. IHRT-PCR detected DNA from both cryptic plasmid and major outer membrane protein gene in 108 samples and only cryptic plasmid DNA in 18 samples. Repeat testing in duplicate on these 18 samples confirmed this finding. Overall, only 18

(2 %) of 1000 samples required repeat testing by IHRT-PCR to generate a confirmed positive or negative result. The positive results generated by the four assays are summarized in Table 1.

Cell culture is considered the 'gold standard' in assessing the performance of newly developed NAATs for the diagnosis of *C. trachomatis* infection. However, a recent publication has cast doubt about the validity of this approach due to the poor sensitivity of culture compared with NAATs (Jespersen *et al.*, 2005). The use of multiple molecular assays provides an appropriate reference for investigating the performance of new NAATs (Martin *et al.*, 2004). This principle was used to define the new gold standard used for the present study. Different primer binding sites within the same gene, different targets of amplification and different formats of the assays contributed to the accuracy of this gold standard.

There are certain shortcomings in the study design. The study compared the performance of IHRT-PCR and Amplicor PCR for the detection of *C. trachomatis* DNA in genital swabs only. The analysis was not done on urine, an important type of sample especially for *C. trachomatis* screening. At the time of this study, the in-house assay was in an early developmental phase and was not optimized for processing urine. Investigations are under way to optimize the assay for urine testing. Amplicor PCR was performed on fresh samples as part of a routine diagnostic service while IHRT-PCR was performed on stored DNA. The justification for this shortcoming was to save cost and labour in a financially overstretched diagnostic laboratory. The availability of DNA and data about the accurate diagnosis of *C. trachomatis* infection among 1000 consecutive patients saved the cost and labour of sample collection, DNA extraction, IHNC-PCR, IHNMOMP-PCR and Amplicor PCR. As all samples were coded before IHRT-PCR, the use of data and samples which were collected for a previous study was justified (Jalal *et al.*, 2006a). IHRT-PCR missed three positive samples while Amplicor PCR missed one. The possible explanations for these three false negative results by IHRT-PCR are: a very small quantity of *C. trachomatis* DNA in these samples, some degree of degradation of DNA in

Table 1. Performance of molecular assays for diagnosis of *C. trachomatis* infection

Abbreviations: F, female; M, male; IHNC-PCR, in-house nested cryptic plasmid PCR; IHNMOMP-PCR, in-house nested major outer membrane protein PCR; IHRT-PCR, in-house real-time PCR.

Assay	No. of true positives		No. of false positives		No. of true negatives		No. of false negatives		Sensitivity (%)		Specificity (%)		Positive predictive value (%)		Negative predictive value (%)		Efficiency (%)	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
IHNC-PCR	79	50	5	6	479	381	0	0	100	100	99	98	94	89	100	100	99	99
IHNMOMP-PCR	73	44	0	0	483	388	7	5	91	90	100	100	100	100	99	99	99	99
IHRT-PCR	76	50	0	0	485	386	2	1	97	98	100	100	100	100	100	100	100	100
Roche PCR	78	50	9	7	475	380	1	0	99	100	98	98	90	88	100	100	98	98

storage, and half the quantity of total DNA (5 µl instead of 10 µl) in the amplification reaction.

Eleven and eight per cent of Amplicor PCR and IHNCP-PCR positive results, respectively, were designated false according to the criterion used in this study. This finding was in line with a published study that reported 15 % of the Amplicor PCR positive results as false (Van Dyck *et al.*, 2001). Due to the psychosexual and medico-legal effects of false positive results for a sexually transmitted infection, the accuracy of a positive result is paramount. Hence the Chlamydia Forum of the Health Protection Agency, UK, recommends repeat testing for all positive results generated by a NAAT for the diagnosis of *C. trachomatis* infection (Health Protection Agency, 2005b), while the Centers for Disease Control and Prevention, USA, state that repeat testing should be performed on all positive samples from populations with a low prevalence of *C. trachomatis* infection (Centers for Disease Control and Prevention, 2002). If all positives generated by Amplicor PCR had to be repeated, it would increase the workload by up to 15 %, resulting in increased costs and turnaround time. In IHRT-PCR, two different *C. trachomatis*-specific targets are amplified in the same reaction, hence automatically confirming each positive and negative result. Repeat testing was needed for only 2 % of samples that contained very low levels of *C. trachomatis* DNA.

In conclusion, IHRT-PCR demonstrated its superiority over Amplicor PCR for the diagnosis of *C. trachomatis* infection, due to its comparable sensitivity, higher specificity and ability to confirm results in a single reaction. At this point, IHRT-PCR is semi-automated. Further work is under way to fully automate the assay for high workload.

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