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Comparison of strand displacement and ligase chain amplification for detection of *Chlamydia trachomatis* in urogenital specimens

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Running head: Diagnosis of *Chlamydia trachomatis* by LCx and ProbeTec

Two amplification tests for diagnosis of *Chlamydia trachomatis*, ligase chain reaction (LCx; Abbott Laboratories) and strand displacement (ProbeTec: Becton Dickinson) were compared in samples from 1,183 patients at STD clinics. The overall prevalence of positive outcome was 8.0%, and agreement between the two assays was 98.8%. For endocervical, urethral and male urine samples, the agreement was 99.3%, 99.4% and 97.7%, respectively. For 10 discrepant samples, alternative amplification assays suggested that LCx and ProbeTec gave erroneous results in 6 and 4 cases, respectively. Inhibition of amplification was found in three urine specimens (0.25%). The results with the two assays were thus closely similar.

Keywords *Chlamydia trachomatis*; sexually transmitted infections; DNA amplification

INTRODUCTION

Chlamydia trachomatis continues to account for the most common bacterial cause of sexually transmitted disease in the Western World (1). Untreated, acute chlamydial infections, such as cervicitis and urethritis, may be complicated by pelvic inflammatory disease and infertility, chronic abdominal pain, and reactive arthritis (2). Since both symptomatic and asymptomatic cases may be at equal risk of these complications a high preventive role of early laboratory diagnosis as well as screening programs and contact tracing is well recognized (3).

In Sweden, following a decline during the previous decade, an almost doubled incidence of *C.trachomatis* infections since a few years has been noted, from 13,800 cases in 1997 to 26,800 cases in 2003, viz. from 160 to 300 cases per 100,000 inhabitants (Swedish Institute for Infectious Disease Control; www.smittskyddsinstitutet.se). Altered sexual behaviour may largely account for these trends. However, since in our country, *C.trachomatis* infections were made notifiable by law in 1988, a substantial reduction of cases recorded until mid 90ies was conceivably related to enhanced contact tracing. On the other hand, increasing use of newer diagnostic techniques, in particular nucleic acid amplification (NAA) procedures, may have contributed to the higher incidence found during the last few years.

A higher sensitivity of NAA assays compared to culture for the laboratory diagnosis of *C. trachomatis* has already been widely documented (4, 5). Furthermore, by NAA test diagnosis of *C. trachomatis* infections in urine or vaginal specimens may appear almost as acceptable as urethral and endocervical samples (6-8), though reduced sensitivity for female urine compared to endocervical swab has been reported (9, 10).

In the present study the performance of two NAA commercial methods, ligase chain reaction (LCR) earlier in use in our laboratory, and a more recently developed technique, strand displacement (SDA) was compared. Both rely on the detection of a *C.trachomatis* specific cryptic plasmid. Though both procedures are highly convenient from practical point, one advantage with SDA is a shorter processing time than with LCR. Our evaluation, comprised both urethral, endocervical and urine specimens, and those with discrepant results were further examined by independent assays at other laboratories.

METHODS

Two venereological clinics (Lund and Helsingborg) and four clinics for adolescents participated in the study, which took place during the time May - October 1999. From each patient, duplicate samples from one location only were obtained following the instructions of the respective manufacturer. The order of sampling was changed with two weeks´ interval. In total, 1183 consecutive patients were included, accounting for 675 endocervical, 347 male urine and 161 male urethral samples; both patients with symptoms of genital tract infection and asymptomatic cases were included but not separately studied, and the laboratory did not have any information on presence of symptoms. The urine sample was first void, taken in one tube then divided into two.

Analysis for chlamydial DNA by LCR (LCx *Chlamydia trachomatis* assay; Abbott Laboratories, North Chicago, Ill.) and SDA (ProbeTec Chlamydia assay, Becton Dickinson Bioscience Europe) was performed following the respective manufacturer´s guide-lines; LCx was generally done within 2 days after sampling, whereas the ProbeTec assay was performed after 2 - 6 weeks during which samples were kept frozen at –20°C. For both methods, a repeat positive outcome was required for the sample to be considered as positive. In the case one positive and one negative result was recorded the sample was judged as negative and not further examined; however, no such results were recorded in the study.

Specimens yielding divergent outcome by the two methods were stored frozen for later testing at the Department of Clinical Microbiology, University Hospital of Malmö (Dr. Kenneth Persson), and the State Serum Institute in Copenhagen (Dr. Jorgen Skov Jensen), respectively. In the Malmö laboratory, analysis was done by a commercial PCR assay (Cobas Amplicor *Chlamydia trachomatis* Test; Roche Diagnostic Systems Inc., Branchburg, N.J.), targeting the same cryptic plasmid as the two assays under investigation, whereas in the Copenhagen laboratory, an in-house PCR assay, targeting 16S ribosomal RNA and including an internal amplification control, was used. Both aliquots of the discrepant cervical and urethral samples were referred to either laboratory.

RESULTS

Among all 1,183 specimens, the overall agreement between LCx and ProbeTec was 98.8%, and the prevalence of positive outcome by either of the methods was 8.0%. The corresponding figures for the 675 endocervical samples were 99.3% and 6.1%, for urethral samples 99.4% and 9.3% and for urine samples 97.7% and 11.2%, respectively (Table 1).

In total, the samples from 10 patients gave discrepant outcome and were further tested by two other amplification assays at other laboratories. As with the whole material, no clinical data were used in these evaluations. Four samples were endocervical, one was urethral, and five were urine. It was considered that LCx gave falsely positive outcome in five cases as compared to one only by ProbeTec; on the other hand, falsely negative outcome might have occurred in three cases by the ProbeTec as compared to one case by the LCx. Specimen no 4 was considered positive since it was reactive in both the LCx and Amplicor assays. (Table 2).

DISCUSSION

The present study was aimed at comparing two commercial NAA assays for the laboratory detection of *C.trachomatis* in clinical samples. As mentioned, the superiority of NAA tests over culture has emerged from a number of studies. Thus, the sensitivity of culture compared to LCR, one of the tests used in our study, was reported to be no more than 80 % for endocervical and considerably less for urethral samples (11,12). Furthermore, culture for detection of *C.trachomatis* in urine is known to be inadequate (13).

In our investigation, comprising almost 1,200 consecutive patients, a satisfactory level of agreement, 98.8%, between LCR and SDA was obtained indicating closely similar performance of the two NAA assays. Notably, the agreement among endocervical and urethral specimens, requiring separate sampling for the two assays, was even higher than among urine specimens. One major reason for the high agreement observed might be that both the LCR and SDA assays are based on detection of a common target, a cryptic, multicopy plasmid, specific to *C.trachomatis* (14). Theoretically, this could imply a problem of specificity in that falsely positive outcomes might be undetected; however, an optimal specificity of the LCR test has been amply documented in other studies suggesting such error to be less relevant (15).

The analysis of discrepant samples, based on two independent NAA assays, indicated a falsely negative outcome by LCR in one and by SDA in three cases. One of the latter and two additional samples showed non-specific inhibition according to the manufacturer´s definition.

Since inhibition control was included in the two independent NAA assays, the conclusion that LCR yielded false positive was less likely. On the other hand, LCR was considered to yield falsely positive outcome in five of the samples. It may be underlined that all these samples were only weakly reactive by the ProbeTec or the LCx assay. We believe that repeated defrosting (3 times) of the samples included in the discrepant analysis did not account for negative outcomes since results were in agreement with the initial ProbeTec outcome. In general, the rate of non-specific inhibition, limited to these three samples, was comparatively low which was in agreement with other reports (16,17).

In conclusion, the two NAA assays LCx and ProbeTec performed almost similarly in the detection of *C.trachomatis* in urethral, endocervical and urine samples. These results agree with a report from UK [10] showing high levels of sensitivity and specificity for both SDA and LCR on endocervical and urine specimens. Efficient contact tracing combined with the use of NAA diagnostic methods may be anticipated to reduce the pool of asymptomatic carriers and decrease the incidence of chlamydial genital infections. Furthermore, reduction of costs associated with tubal infertility, including in vitro fertilisation, make an important argument for the use of DNA amplification technology in the diagnosis of *C.trachomatis*.

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substances associated with inhibition and removal of inhibitory activity. *J Clin Microbiol* 1998;36:3122-6.

Table 1 Comparison of LCx and ProbeTec for detection of *Chlamydia trachomatis* in different specimen types

Specimen type	Total	No positive	Prevalence* (%)	Agreement (%)
Endocervix	675	41	6.1	99.3
Urethra	161	15	9.3	99.4
Urine (male)	347	39	11.2	99.7
Total	1,183	95	8.0	98.8

* similar figures for LCx and ProbeTec

Table 2 Analysis of discrepant samples and interpretation

Specimen/type	ProbeTec	LCx	Copenhagen* (inhouse)	Malmö* (Amplicor)	Final judgement
1/urethra	-	+	n.d./+	-/+	Positive
2/endocervix	+	-	-/-	-/-	Negative
3/endocervix	-	+	-/-	-/-	Negative
4/endocervix	-	+	-/-	-/+	Positive
5/endocervix	-	+	-/-	-/	Negative
6/urine	- inhib.	+	-	-	Negative
7/urine	- inhib.	+	-	-	Negative
8/urine	-	+	-	-	Negative
9/urine	- inhib.	+	+	-	Positive
10/urine	+	-	n.d.	+	Positive

* Sample for ProbeTec/LCx, respectively.

Inhib.: non-specific inhibition as defined by the ProbeTec guidelines.

N.d.: not done