Low Correlation of Serology with Detection of *Chlamydia trachomatis* by Ligase Chain Reaction and Antigen EIA

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Summary

The aim of the present study was to evaluate the diagnostic accuracy of serology by using new assays for the detection of genus and species-specific IgG, IgM, IgA and secretory IgA antibody in female sex workers. Cervical swabs and first void urine (FVU) from 314 female sex workers were submitted to nucleic acid amplification by ligase chain reaction (LCx, Abbott). Concomittantly, blood samples were tested for the presence of IgG, IgM and IgA antibodies using a genus-specific assay (rELISA, Medac) and species-specific test (SeroCT, Orgenics). *Chlamydia trachomatis* infection was detected in a total of 30 (9.6%) female sex workers by LCR. With rELISA, seroprevalences for IgG, IgM, and IgA antibody to *Chlamydia* were 88.9%, 19.1% and 62.7%, respectively. IgG and IgA antibody prevalences against *C. trachomatis* (SeroCT) were 65.0% and 23.9%, respectively. In comparison to the positive LCR results obtained from cervical swab and/or FVU, the sensitivity of rELISA for *Chlamydia* IgG, IgA and IgM detection was 93.3%, 83.3% and 16.7%, respectively. With SeroCT, the sensitivity for *C. trachomatis*-specific IgG and IgA detection was 86.7% and 33.3%, respectively. The specificities of both serologic tests in comparison to LCR were very low. In conclusion, the correlation of serology with active *C. trachomatis* infection of the lower genital tract is very low. According to our results, serologic testing for *Chlamydia* can exclude active infection of the lower genital tract with a high reliability (≥ 95%). However, detection of *C. trachomatis* can only be reliably achieved by nucleic acid amplification assays.

Key Words

Ligase chain reaction · *Chlamydia trachomatis*-specific antibody · Lower genital tract infection · Upper genital tract infection · Female sex workers

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Introduction

Chlamydia trachomatis is the most common sexually transmitted pathogen in industrialized countries [1]. The clinical manifestations of *C. trachomatis* infections are urethritis and epididymitis in men and cervicitis, salpingitis and pelvic inflammatory disease in women. Asymptomatic infections are relatively frequent. A major complication of chronic *C. trachomatis* infection is infertility due to tubal obstruction.

The real challenge in laboratory diagnosis of *C. trachomatis* infection is accurate sample prelevement and sensitive detection of the infectious agent. The isolation in cell culture, although relatively sensitive is work intensive, requires experienced technicians and remains restricted to laboratories with cell culture facilities. The detection of chlamydial antigens by direct fluorescent antibody assay (DFA) or enzyme-linked immunosorbent assay (EIA) permits a screening of high sample numbers, however, the sensitivity of both assays is relatively low and EIAs might yield false-positive results [2].

Diagnosis of chlamydial infection has been improved by the introduction of nucleic acid amplification techniques (polymerase chain reaction [PCR], ligase chain reaction [LCR] and transcription-mediated amplification [TMA]) which permit a highly sensitive detection of specific target DNA sequences through amplification. Numerous evaluations have shown that amplified DNA techniques are markedly more sensitive than the antigen detection methods [3–9]. Furthermore they permit a noninvasive laboratory diagnosis in male and female patients, since DNA sequences may be detected from first void urine (FVU) samples, thus permitting an adequate therapy monitoring.

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Although serology cannot substitute *C. trachomatis* DNA or antigen detection, it is considered as a useful indicator for the provisional diagnosis of chlamydial infection. Detection of specific antibodies is of great value in complications associated with chronic infection, i. e., infertility and pelvic inflammatory disease. A major problem of *Chlamydia* serology is that most of the commercially available EIAs are not species-specific and show extensive cross reactivity with *Chlamydia pneumoniae*. The microimmunofluorescence (MIF) test which uses purified elementary bodies permits the differentiation of *C. trachomatis* and *C. pneumoniae* antibodies. However, MIF is difficult to interpret and is relatively insensitive [10].

In the present study, *C. trachomatis* detection in cervical swabs and FVU with LCR was compared to serologic testing for IgG, IgA and IgM antibody against *Chlamydia*. The objective was to test the hypothesis whether serologic testing for *C. trachomatis* is suited for screening of infections of the lower and upper genital tract in a high-risk population.

Patients and Methods

Patient Population

Female sex workers (n = 314) were examined at the counseling and investigation center for sexually transmitted diseases board of health (Stadtgesundheitsamt Frankfurt). A total of 199 female sex workers reported suffering from urogenital symptoms. Resolved *Treponema pallidum* infections (TPHA ≥ 1/80 confirmed by IFA-Abs., VDRL negative) were observed in 48 (15.3%) of the female sex workers.

Specimen Collection

From all the female prostitutes, swabs were collected from the endocervix for LCR and antigen EIA. If excessive cervical secretion was present, it was cleaned with a large cotton swab before the test swabs were collected. FVU samples (25–40 ml) were collected in sterile screw-cap plastic jars and stored at 4 °C for a maximum of 24 h. Samples were stored frozen at –20 °C prior to examination at the Institute of Medical Virology. Serum samples were taken at the same time as cervical swabs and FVU. *C. trachomatis* IgM determination was performed in 257 serum samples. Detection of secretory IgA was performed in 202 of the 314 female sex workers.

Ligase Chain Reaction (LCR)

LCR (LCx *C. trachomatis* assay, Abbott Diagnostics, North Chicago, IL, USA) was performed and interpreted according to the manufacturer's recommendations.

Antibody Detection

***Chlamydia* IgG, IgM and IgA rELISA.** Chlamydia IgG, IgM and IgA rELISA (Medac, Hamburg, Germany) includes a chemically pure structure of recombinant lipopolysaccharide (LPS) which contains a genus-specific epitope of *Chlamydia* spp. pathogenic for humans. Prior to IgM determination, rheumatoid factor absorption was performed. The rELISA was performed and interpreted according to the manufacturer's recommendations. Index values calculated by dividing the signal of the sample through the cut-off value were converted in titers. IgG, IgM and IgA titers ≥ 1/50 were considered positive. According to the manufacturers' recommendations, active *Chlamydia* infection should be considered in the presence of IgG titer ≥ 1/800 and or IgA titer ≥ 1/200.

Determination of secretory IgA was performed from cervical swabs used for Micro Trak II Chlamydia EIA (Syva, Palo Alto, USA). Sample prelevement was performed in accordance with the manufacturer's recommendations. Cervical swabs were eluted in 1 ml of Micro Trak II Chlamydia EIA sample buffer for secretory IgA detection and diluted 1/5 in *Chlamydia* IgG, IgM and IgA rELISA sample buffer, in accordance with the manufacturers' recommendations. A sample was considered *C. trachomatis* sIgA-positive if it was reactive in a dilution of 1/5.

SeroCT-IgG/IgA-specific *C. trachomatis*. SeroCT-IgG/IgA-specific *C. trachomatis* (Savyon Diagnostics LTD, Kiryat, Minrav, Ashdod, Israel) is a microtiter-based ELISA using a mixture of species-specific peptides of the major outer membrane protein (MOMP) of *C. trachomatis* for the detection of specific IgG and IgA antibody. Samples with index values (signal sample/mean value of negative control X 2) greater than 1.1 were considered positive. Index values ranging between 1.0 and 1.1 were interpreted as borderline.

SeroCP-IgG/IgA/IgM-specific *C. pneumoniae*. SeroCP-IgG/IgA/IgM-specific *C. pneumoniae* (Savyon Diagnostics LTD) uses as antigen *C. pneumoniae* strain TWAR 183 purified elementary antibodies (not species-specific). Test procedure and interpretation were identical to those of SeroCT.

Statistical Analysis

For calculation of sensitivity, specificity, and positive and negative predictive values an expanded gold standard defined as LCR cervical swab and/or FVU was considered. Cases with positive LCR results were defined as active infections of the lower genital tract.

Results

C. trachomatis infection (active infection of the lower genital tract) was detected in a total of 30 (9.6%) female sex workers by LCR (Table 1). In 20 female sex workers *C. trachomatis* DNA could be amplified from both cervical swabs and FVU. Five of the 25 female sex workers with a positive LCR result from cervical swab were negative on FVU. Conversely, 5 FVU LCR-positive female sex workers were negative for *C. trachomatis* when tested on cervical swab (Table 1). Urogenital symptoms were present in 22 (73.3%) of LCR-positive women. Among the LCR-negative female sex workers 177 (62.3%) had urogenital symptoms.

With rELISA, seroprevalences for IgG (cut-off titer 1/50), IgM, and IgA antibody (cut-off titer 1/50) to *Chlamydia* were 88.9%, 19.1% and 62.7%, respectively. IgG and IgA antibody prevalences against SeroCT were 65.0% and 23.9%, respectively. With SeroCP, IgG, IgM and IgA antibody to *C. pneumoniae* were detected in 60.5%, 33.8% and 52.9%, respectively. Dual IgG antibody reactivity against *C. trachomatis* and *C. pneumoniae* was observed in 137 (43.6%) of the female sex workers with SeroCP/SeroCT. Dual IgA antibody reactivity was present in 58 (18.5%) of the cases. A total of 66 (21.0%) of the female sex workers were seropositive for *C. trachomatis* IgG only. Conversely,

52 (16.6%) of the women were only reactive for *C. pneumoniae* IgG antibody with SeroCP.

Of the 30 individuals with positive LCR cervical swabs and/or positive LCR on FVU, 28 (93.3%) and 26 (86.7%) presented IgG antibody against *Chlamydia* with rELISA and SeroCT, respectively. Among the 284 LCR-negative female sex workers, 251 (88.4%) and 178 (62.7%) were IgG-positive with rELISA and SeroCT, respectively. IgG antibody against *C. pneumoniae* (SeroCP) was observed in 20 (66.7%) LCR-positive and 170 (59.9%) LCR-negative individuals. *Chlamydia*-specific IgA reactivity was present in 25 (83.3%) samples with rELISA (cut-off titer 1/50) and in 10 (33.3%) LCR-positive female sex workers with SeroCT. In analogy to the findings for IgG seroprevalence, high numbers of LCR-negative female sex workers were IgA-positive (n = 172 for rELISA [cut-off titer 1/50] and n = 65 for SeroCT). IgM antibody against *Chlamydia* was detected with rELISA in only 16.7% LCR-positive and 19.4% negative female sex workers.

In comparison to the combination of positive LCR results from cervical swab and/or FVU, the sensitivity of rELISA for *Chlamydia* IgG (cut-off titer 1/50), IgA (cut-off titer 1/50) and IgM detection was 93.3%, 83.3% and 16.7%, respectively (Table 1). With SeroCT, the sensitivity for *C. trachomatis*-specific IgG and IgA detection was 86.3% and 33.3%, respectively. The specificities of both serologic tests in comparison to LCR were very low. For rELISA, the specificity for IgG (cut-off titer 1/50), IgA (cut-off titer 1/50) and IgM detection was 11.6%, 39.4% and 80.6%, respectively. SeroCT showed a specificity of 37.3% for IgG and 77.1% for IgA detection (Table 1). While positive predictive values for serologic assays were very low (0–15.6%), high negative predictive values were observed for rELISA IgG (cut-off 1/50) (94.3%), rELISA IgA (cut-off 1/50) (95.7%), and SeroCT IgG (96.4%) (Table 1).

Alternatively, by using higher cut-off titers for the interpretation of rELISA IgG (1/800) and IgA (1/200) results, lower values for sensitivity (IgG: 33.3%, IgA: 23.3%) but higher values for specificity (IgG: 79.6%, IgA: 86.8%) were observed (Table 1). The correlation with active infection, as detected with LCR was, however, very weak (Table 1).

Secretory IgA was detected in none of the LCR-positive cervical swabs with rELISA but was present in eight LCR-negative samples (Table 1).

Table 1
Results of serologic assays in comparison to the expanded gold standard (LCR FVU and/or LCR cervical swab positive).

Assay	Result	No. of samples		Sensitivity	Specificity	PPV[a]	NPV[b]
		LCR-positive	LCR-negative	(%)	(%)	(%)	(%)
rELISA secretory IgA	Positive	0	8	0	95.6	0	89.7
	Negative	20	174				
rELISA IgG (cut-off titer 1/50)	Positive	28	251	93.3	11.6	10.0	94.3
	Negative	2	33				
rELISA IgG (cut-off titer 1/800)	Positive	10	58	33.3	79.6	14.7	91.9
	Negative	20	226				
rELISA IgM	Positive	5	55	16.7	60.6	8.3	90.2
	Negative	25	172				
rELISA IgA (cut-off titer 1/50)	Positive	25	178	83.3	39.4	12.7	95.7
	Negative	5	106				
rELISA IgA (cut-off titer 1/200)	Positive	7	38	23.3	86.8	15.6	91.5
	Negative	23	246				
SeroCT IgG	Positive	26	178	86.7	37.3	12.7	96.4
	Negative	4	106				
SeroCT IgA	Positive	10	65	33.3	77.1	13.3	91.6
	Negative	20	219				
SeroCP IgG	Positive	20	170	66.7	40.1	10.5	91.9
	Negative	10	114				
SeroCP IgA	Positive	21	145	53.3	68.3	15.1	93.3
	Negative	9	139				
SeroCP IgM	Positive	16	90	70.0	48.9	12.7	93.9
	Negative	14	194				

[a] PPV: positive predictive value, [b] NPV: negative predictive value

Discussion

The results of the present study confirm that serology is not adequate for diagnosis of lower tract infections since the correlation with *C. trachomatis* plasmid DNA detection with LCR was relatively low even with a *C. trachomatis*-specific assay. According to a previous study of *Schachter* et al. [11], positive predictive values for all the serological markers were under 20%, including detection of secretory IgA on cervical secretions. IgG detection showed a very low specificity. By raising the cut-off value, the specificity of rELISA IgG was substantially increased, however, the sensitivity was very poor, corresponding to that of SeroCT IgA (Table 1). *Douvier* et al. [12] observed a low correlation (62%) between antigen detection and serology in a population consulting an STD center.

While chlamydial serology cannot be substituted for agent detection in diagnosing these infections, the negative predictive values of rELISA IgG/IgA and SeroCT IgG were relatively high. In consequence, serologic testing for *Chlamydia* excludes an active infection of the lower female genital tract in comparison to the expanded gold standard (LCR cervical swab and/or FVU positive) with a high probability (Table 1).

In patients with upper genital tract infection, i. e., pelvic inflammatory disease, *C. trachomatis* infection is detected more often using serology in comparison with PCR or tissue culture [13]. High antichlamydial titers are associated with tubal factor infertility and ectopic pregnancy [14, 15]. Seropositivity is also correlated with the number of sexual partners [16]. The high seroprevalence rate in our collective of female sex workers reflects the high number of sexual partners and may probably also be related to upper genital tract infections. In this context, *C. trachomatis* species-specific ELISA would be an appropriate screening assay, since it appears to be more sensitive than MIF IgA and shows a strong correlation with positive cervical smears in obstetric patients [10]. In sterility patients, the tube status is predicted by species-specific ELISA with 74% positive predictive value and 87% sensitivity [17].

With rELISA, a 23.6% higher *Chlamydia* IgG seropositivity was observed in comparison to SeroCT. This higher prevalence rate is for the major part attributable to cross-reactivity, since 16.6% of the female sex workers presented only IgG reactivity to *C. pneumoniae*. Antibodies to *C. pneumoniae* (and *Chlamydia psittaci*) account for up to half of all C*hlamydia* IgG-positive cases attending genitourinary clinics [18, 19]. The SeroCT is expected to be less cross-reactive with *C. pneumoniae* than LPS-based immunoassays [10, 17]. Furthermore, the specificity of recombinant LPS EIAs may be low [19]. On the other hand, LPS EIA may be currently the preferred tool for diagnosing acute respiratory *Chlamydia* infections in routine clinical practice [20].

The value of anti-*C. trachomatis* endocervical immunoglobulin IgA determination for diagnosis of a current infection remains controversial. In our study, no secretory IgA was detected in LCR-positive individuals. According to our results, *Tejls* et al. [21] did not find cervical antibodies useful in the diagnosis of a *C. trachomatis* infection in a low-prevalence population. The elution of a cervical swab in 1 ml sample buffer and a further dilution of 1 : 5 results in a dilution that might be too high for detecting secretory antibodies, furthermore the collection of mucus might not be sufficient to detect endocervical IgA. In contrast, *Witkin* et al. [22] observed a sensitivity of 95.7% for a rapid ELISA (IgA Rapid SeroTest, Savyon Diagnostics) for the detection of *C. trachomatis* in the endocervices of 167 inner-city pregnant women. *McComb* et al. [23], using a detection assay that did not differentiate between IgA and IgG antibodies, demonstrated that the presence of anti-chlamydial immunoglobulins in the cervix, but not in the circulation, correlated with chlamydial isolation from the cervix. The presence of secretory IgA in LCR-negative women (Table 1) can be explained by the possible presence of LCR inhibitors, recently cleared infection with persistence of IgA, upper genital tract infection or contamination with blood leading to a positive antibody test.

In order to achieve a high diagnostic sensitivity in women, both FVU and endocervical swabs should be examined, since infection may not necessarily be detected in either localization, even with highly sensitive nucleic acid amplification techniques. According to previous studies [24–26], discrepancies in amplification techniques from FVU and cervical swabs and/or antigen detection were observed in 16 of the female sex workers. A relatively low detection rate of chlamydial infection in FVU was observed in comparison to the cervix. The presence of polymerase or transcriptase inhibitors in urine and/or generally lower concentrations of the infectious agent in urine may lead to false-negative results in amplification techniques. Therefore it is recommended to perform nucleic acid amplification techniques (NAT) on cervical swabs and if possible on FVU for *Chlamydia* screening. Vulval swabs also represent an effective alternative noninvasive sample type for *C. trachomatis* detection in women by NAT [27]. Compared with urine, the processing of vulval swabs is uncomplicated and does not appear to be influenced by inhibition problems or by a small load of elementary bodies [28]. However, no single NAT test detects all infected individuals with a single sampling type.

The rate of appearance of unidentified inhibiting substances in urine probably varies according to specimen type, storage and transport conditions, and may also be different according to gender and study population. In asymptomatic pregnant and nonpregnant military recruits, there was no evidence for the presence of inhibitors in FVU [29]. In contrast, *Mahony* et al. [30] reported a prevalence of 2.6% LCR inhibitors in urine specimens from pregnant and nonpregnant women. Nitrites were associated with LCR amplification inhibition.

Inhibitors of Taq polymerase have been found which disappear on storage and can be concentrated by centrifugation [31, 32]. Several approaches have been proposed to

circumvent this problem, such as dilution of FVU samples [9], spiking with *C. trachomatis*, heat treatment and addition of an internal standard, freeze/thawing and overnight storage. With the new Amplicor combination PCR for *C. trachomatis* and *Neisseria gonorhoeae* which has an internal DNA control amplification and detection assay, the prevalence of inhibitors to amplification in clinical samples can be evaluated. It is therefore of great importance that manufacturers include an internal control in the amplification procedure in order to exclude false-negative results.

Since serological testing for *Chlamdia* shows a high negative predictive value for lower genital tract infection and a high correlation with upper genital tract infection, antibody testing with type-specific antigens may be of use for screening in low prevalence areas since the costs for reagents are lower than for NAT. NAT could be used as second line test for the detection and treatment follow-up of individuals with lower tract genital infection. In high-risk collectives, screening with NAT is more efficient since infectious carriers are directly identified and a high percentage of antibody-positive individuals are not suffering from active infection.

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