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Article

Detection of Seroconversion and Persistence of *Chlamydia trachomatis* Antibodies in Five Different Serological Tests

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Abstract Microimmunofluorescence (MIF), a *Chlamydia trachomatis* species-specific enzyme immunoassay incorporating lipopolysaccharide-extracted *Chlamydia trachomatis* L2 elementary bodies, two different synthetic peptide-based species-specific tests, and a recombinant lipopolysaccharide genus-specific test were performed on multiple follow-up sera ($n = 104$ total) from 16 women with *Chlamydia trachomatis*-positive cervical swabs. These women included five with IgG seroconversions, five with *Chlamydia trachomatis* reinfections after initial therapy, and six with serologic follow-up of more than 6 years after antibiotic therapy. Of all the tests employed in this study, MIF IgG reverted earliest to negative titers, while MIF IgA was the least sensitive. The lipopolysaccharide-extracted elementary body enzyme immunoassay exhibited the closest correlation with the MIF test. The highest test sensitivity was observed in one of the synthetic peptide-based tests, which detected earliest seroconversions and longest IgG persistence. The other synthetic peptide-based test gave false-negative results in 2 of 16 women and did not detect seroconversion earlier than the MIF test. Seroconversion and persistence of genus-specific IgG – cross-reactivity with *Chlamydia pneumoniae* – against lipopolysaccharide were similar to species-specific IgG. A significant serologic response to reinfection was observed only in women with signs of pelvic inflammatory disease. Species-specific tests of high sensitivity and reproducibility are best suited for gynecological diagnostic purposes.

Introduction

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen worldwide [1] and is estimated to be responsible for two-thirds of tubal factor infertility cases [2]. Current infections are diagnosed by direct detection of *Chlamydia trachomatis* in urogenital specimens by DNA amplification assays or by less sensitive methods such as culture, direct immunofluorescence, or enzyme immunoassays (EIAs) [3]. Past *Chlamydia trachomatis* infections, e.g. in infertile women or women with ectopic pregnancy, are diagnosed by detection of species-specific IgG, the finding of which suggests tubal damage caused by *Chlamydia trachomatis* [4].

The species-specific microimmunofluorescence (MIF) test has been accepted as the gold standard of chlamydial serological tests [5]; however, it remains laborious and is performed in only a few research laboratories. In addition, the reading of MIF tests is subjective, resulting in inter-laboratory variations in MIF results [6]. A variety of commercially available serological tests have been introduced in routine laboratories, among which are three *Chlamydia trachomatis* species-

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specific tests (ImmunoComb Chlamydia bivalent, Orgenics, Israel; *Chlamydia trachomatis* EIA, Labsystems, Finland; SeroCT EIA, Savyon, Israel) and one genus-specific test (rElisa; Medac, Germany). Genus-specific serological tests allow no differentiation between *Chlamydia pneumoniae* and *Chlamydia trachomatis* and cannot be recommended for gynecological diagnostic purposes [7] due to the 50–70% prevalence of *Chlamydia pneumoniae* antibodies in the general population [8].

Direct detection of *Chlamydia trachomatis* in women with clinically suspected genital infection may give false-negative results due to low numbers of elementary bodies present in urogenital specimens [9, 10]. Therefore, serological tests for chlamydiae have been used by many gynecologists as a "back-up" to direct detection assays for exclusion (negative IgG) or confirmation (positive IgG) of a genital chlamydial infection in symptomatic women. Positive chlamydial IgA titers have been interpreted as an indicator of a current infection with *Chlamydia trachomatis*, and some gynecologists believe that persistent follow-up titers after antibiotic treatment indicate treatment failure. However, these assumptions remain speculative. Few studies have been published on the serological follow-up of patients with *Chlamydia trachomatis* genital infections [11, 12], and data comparing different serological tests during follow-up are lacking.

The purpose of this study was to verify the assumptions mentioned above by evaluating the IgG/IgA sensitivity of five different serological tests during *Chlamydia trachomatis* seroconversion, reinfection, and long-term follow-up. The results demonstrate clear differences in test sensitivities and provide some insights into the usefulness and limits of serological tests for gynecological purposes.

Materials and Methods

Study Population. A database containing some 10,000 patients from the Freiburg University gynecological outpatient clinic who had been tested for *Chlamydia trachomatis* between 1988 and 1998 was searched for *Chlamydia trachomatis* antigen-positive women, from whom multiple follow-up sera and cervical specimens had been tested. Among these patients were found five women who seroconverted, five women with documented reinfections during the follow-up period, and six women who had been followed-up serologically for more than 6 years. All of these 16 women (18–30 years in age), and their sexual partners, had been treated with effective antibiotics after *Chlamydia trachomatis* antigen was detected. The sera (a total of 104) of these women were thawed from –20 °C and re-examined in the MIF test, the ImmunoComb Chlamydia bivalent, the *Chlamydia trachomatis* EIA, the SeroCT EIA, and the rElisa according to the respective manufacturer's instructions. The sera of each woman were tested simultaneously in one test kit to avoid intertest variability.

Chlamydia trachomatis Antigen Detection. Cervical swabs had been tested by direct fluorescence (MicroTrak; Syva, USA) and/or cell culture.

Chlamydia trachomatis Species-Specific Serology. The MIF test was performed according to Wang and Grayston [5]. The MIF antigen consists of three serovar pools of formalin-fixed *Chlamydia trachomatis* elementary bodies (Washington Research Foundation, Seattle, USA). Fluorescein-isothiocyanate-conjugated (FITC) anti-human IgG from Behring (Germany) and anti-human IgA from Medac (Germany) were used. The serovar pool yielding the highest titer was determined, and IgG titers of $\geq 1:16$ and IgA titers of $\geq 1:8$ were interpreted as positive.

The ImmunoComb Chlamydia bivalent (Orgenics, Israel) is a solid-phase EIA incorporating LPS-extracted *Chlamydia trachomatis* L2 and *Chlamydia pneumoniae* elementary bodies at distinct sites [4]. *Chlamydia trachomatis* species-specific IgG titers of $\geq 1:16$ and IgA titers of $\geq 1:8$ were interpreted as positive.

The *Chlamydia trachomatis* EIA (Labsystems, Finland) is based on four synthetic peptides derived from the variable domain IV of the major outer membrane protein (MOMP) of *Chlamydia trachomatis* serotypes C, G, E, and L2 [13]. IgG and IgA cutoff values ranged between 0.4 and 0.45, and maximum absorbance was 3.0.

The SeroCT EIA (Savyon, Israel) antigen consists of a mixture of MOMP-derived synthetic peptides from different *Chlamydia trachomatis* serovars. Cutoff values ranged between 0.4 and 0.5, and maximum absorbance was 3.0.

Genus-Specific Chlamydial Serology. Recombinant chlamydial genus-specific lipopolysaccharide [14] is used as antigen in the rElisa EIA (Medac, Germany). IgG titers of $\geq 1:100$ and IgA titers of $\geq 1:50$ were interpreted as positive.

Chlamydia pneumoniae Species-Specific Serology. Chlamydia pneumoniae species-specific IgG was tested in all sera by MIF, ImmunoComb Chlamydia bivalent, and SeroCP (synthetic *Chlamydia pneumoniae* peptide test; Savyon, Israel).

Results

Seroconversion. All sera of the five women (4–6 follow-up sera per patient) with *Chlamydia trachomatis* species-specific seroconversions were MIF IgG (and IgA) negative at the time of initial serological testing (Table 1). Four of these women (2 with cervicitis, 2 screened 1 week after delivery) had *Chlamydia trachomatis*-positive swabs at their initial visit, all of whom were *Chlamydia trachomatis* EIA IgG positive at the time of initial serological testing; three of these four women had concomitant positive *Chlamydia trachomatis* EIA IgA titers (IgA was nonreactive in 1 woman). Two of these four women were rElisa IgG positive at initial serological testing, although all four remained negative in the MIF test, the ImmunoComb, and the SeroCT IgG until seroconversion was observed in sera collected at follow-up visits 1–3 months later. Titers of one of these four women are shown in Table 2.

The fifth woman was serologically negative in all tests 8 months before she became pregnant. IgG seroconversion was observed 10 months later (no swab collected) in all tests except for the SeroCT, which remained nonreactive throughout the observation period of 30 months (Table 1). One week after delivery, all positive

Table 1 Results of MIF, ImmunoComb Chlamydia bivalent, *Chlamydia trachomatis* EIA (Ct-EIA), SeroCT, and rElisa for five women's sera obtained at first visits and at multiple follow-up visits. IgG and IgA test results of these five women were categorized according to when seroconversions occurred in each test, i.e. as "earlier than MIF IgG", as "concomitantly with MIF IgG", or as "nonreactive" (negative titers in all follow-up sera drawn from one woman)

Time of seroconversion	No. of patients who seroconverted									
	MIF		Immunocomb		Ct-EIA		SeroCT		rElisa	
	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA
Earlier than MIF IgG	0	0	0	0	4	3	0	0	2	1
Concomitantly with MIF IgG	5	0	5	3	1	1	4	2	3	2
Nonreactive	0	5	0	2	0	1	1	3	0	2

Table 2 Titers (MIF, ImmunoComb, rElisa) and absorbances (Ct-EIA, SeroCT) of IgG and IgA for sera from a 21-year-old woman who presented with cervicitis and irregular bleeding at day 0 (direct immunofluorescence [DFA] positive). She and her male partner were treated with doxycycline 200 mg/day from day 15 to 24. *Chlamydia pneumoniae* IgG remained negative throughout the 5-year follow-up period

Day	Result of DFA/ culture	Seroconversion in a patient with cervicitis									
		MIF		ImmunoComb		Ct-EIA		SeroCT		rElisa	
		IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA
0	pos./n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14	pos./pos.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21	neg./neg.	neg.	neg.	neg	neg.	1.0	0.5	neg.	neg.	neg.	neg.
95	neg./neg.	1:32	neg.	1:64	neg.	≥3.0	2.9	2.6	1.5	1:200	neg.
664	neg./n.d.	1:16	neg.	1:64	1:8	≥3.0	≥3.0	2.3	1.1	1:200	neg.
1,290	neg./n.d.	1:32	neg.	1:64	neg.	≥3.0	≥3.0	2.1	0.8	1:200	1:50
1,736	neg./n.d.	1:32	neg.	1:64	neg.	≥3.0	≥3.0	2.0	0.8	1:200	1:50

pos., positive; neg., negative; n.d., not done

IgG titers had risen significantly during the past 7 months, and she had a *Chlamydia trachomatis*-positive cervical swab.

In two women, seroconversion in the MIF, the ImmunoComb, and the SeroCT IgG tests occurred after the initiation of antibiotic treatment. The *Chlamydia trachomatis* EIA was the most sensitive IgA test, but IgG was generally more sensitive than IgA (Table 1). In all five women, the MIF IgA remained negative (nonreactive) throughout the observation period.

Both MIF tests (IgG and IgA), the ImmunoComb, and the SeroCP *Chlamydia pneumoniae* IgG remained negative or unchanged in three women, whereas seroconversion to low titers – concomitant with seroconversion in the MIF *Chlamydia trachomatis* IgG – was observed in all three tests in the other two women.

Reinfections. Five women who had *Chlamydia trachomatis*-positive cervical swabs on their first clinical visits were treated immediately, together with their sexual partners. Reinfections were diagnosed by positive cervical swabs within 1–4 years after their first clinical presentation. Three of these five women had had a documented partner change before reinfection. Six to 12 follow-up sera were collected per patient over a time period of 5–7 years (Table 3).

A significant IgG titer increase in all tests at the time of documented reinfection was observed in two of the five women. IgA was less sensitive than IgG (Table 3). One of these two women had pelviscopically verified pelvic inflammatory disease (PID), and the other had clinical signs and a pelvic examination that were highly suggestive of PID.

No significant titer change was observed in the other three women when reinfection was diagnosed by a positive cervical swab. These women presented with cervicitis but had no clinical signs of PID. One of these three women exhibited a significant IgG titer increase in all tests (except the rElisa) 1 month after initiation of antibiotic treatment with doxycycline, whereas the other two women showed no titer increase after antibiotic treatment.

Chlamydia pneumoniae IgG titers (MIF, ImmunoComb, SeroCP) remained negative in two and unchanged at constant positive titers in another two of the five women. One of the women with PID had a *Chlamydia pneumoniae* IgG seroconversion to low

Table 3 Results of MIF, ImmunoComb Chlamydia bivalent, *Chlamydia trachomatis* EIA (Ct-EIA), SeroCT, and rElisa for follow-up sera (obtained 5–7 years after treatment) from five women with *Chlamydia trachomatis* reinfections diagnosed by positive cervical swabs. IgG and IgA test results of each woman were categorized as either a "significant titer increase" or as "no significant titer change" at the time of reinfection. IgG and IgA tests with negative titers in all follow-up sera were categorized as "nonreactive"

Response to reinfection	No. of patients									
	MIF		ImmunoComb		Ct-EIA		SeroCT		rElisa	
	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA
Significant titer increase[a]	2	1	2	2	2	1	2	1	2	1
No significant titer change	3	0	3	3	3	4	3	1	3	2
Nonreactive	0	4	0	0	0	0	0	3	0	2

[a] ≥4-fold titer increase (MIF, ImmunoComb, lipopolysaccharide) or ≥100% increase of EIA absorbance (Ct-EIA, SeroCT) at the time of reinfection

Table 4 Results of MIF, ImmunoComb Chlamydia bivalent, *Chlamydia trachomatis* EIA (Ct-EIA), SeroCT, and rElisa for follow-up sera from six effectively treated *Chlamydia trachomatis*-positive women. The titer results of each test from the time of the initial clinical visit, up to the final follow-up visit, were categorized as either "no change", "significant decrease", "reversion to negative", or "nonreactive" (negative titers in all follow-up sera)

Initial vs. final titer	No. of patients									
	MIF		ImmunoComb		Ct-EIA		SeroCT		rElisa	
	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA
No change	0	0	2	1	3	0	1	0	2	1
Significant decrease[a]	0	0	2	2	1	5	3	0	3	0
Reversion to negative[b]	6	0	2	2	1	1	1	2	1	2
Nonreactive	0	6	0	1	1	0	1	4	0	3

[a] ≥4-fold titer decrease (MIF, ImmunoComb, lipopolysaccharide) or ≥50% decrease of EIA absorbance (Ct-EIA, SeroCT)
[b] Positive titer at the first clinical visit, negative titer at the last follow-up visit

titers in all three tests that subsided to negative within 1 month.

Serological Follow-Up After Antibiotic Therapy. All of the six women (3 with PID, 3 with cervicitis) who had been followed-up serologically for more than 6 years (6–10 follow-up sera per patient) reverted to negative in the MIF IgG (Table 4), whereas only one woman reverted to negative IgG in the *Chlamydia trachomatis* EIA. IgG titers decreased more slowly in the other tests than in the MIF IgG, but faster than in the *Chlamydia trachomatis* EIA IgG. No titer increase was seen in any test during the follow-up period.

MIF IgA was completely insensitive and remained negative (nonreactive) in all follow-up sera. SeroCT IgA and rElisa IgA were also rather insensitive. The *Chlamydia trachomatis* EIA IgA was the most sensitive, and titers declined more rapidly in this test than in the *Chlamydia trachomatis* EIA IgG.

Interestingly, one of the six women had an initial MIF IgG titer of 1:128 but was nonreactive in both SeroCT IgG and IgA. This woman was also nonreactive in the *Chlamydia trachomatis* EIA IgG, whereas the *Chlamydia trachomatis* EIA IgA showed near maximum absorbance in all sera drawn from this patient.

Chlamydia pneumoniae IgG titers (MIF, ImmunoComb bivalent, SeroCP) remained consistently positive at constant titers in all follow-up sera from three women and consistently negative in all follow-up sera drawn from the other three women.

Discussion

The sensitivity of a serological test for the diagnosis of a primary current infection with *Chlamydia trachomatis* depends on the time lag between infection and IgG seroconversion. The serologic diagnosis of *Chlamydia trachomatis* reinfections after effective therapy requires at least a significant IgG titer increase. Seroconversions and reinfections are ideal for the evaluation of serological tests but are rarely observed in genital infections with *Chlamydia trachomatis*.

Seroconversion in the *Chlamydia trachomatis* EIA IgG occurred earlier than in the other tests (Table 1), but the time lag between primary genital infection with

Chlamydia trachomatis and seroconversion cannot be determined from our data. However, our findings suggest that IgG titers increase over several weeks until they reach their maximum, since – in four of the five women who seroconverted – *Chlamydia trachomatis* EIA IgG absorbance levels increased from around 1 in the first positive serum to ≥3 in the follow-up serum 1–3 months later.

Serological responses during reinfections were variable (Table 3) and appeared to depend on the "invasiveness" of *Chlamydia trachomatis*, since only women with PID exhibited a significant IgG titer increase. In any case, *Chlamydia trachomatis* serology is not reliable in the diagnosis of reinfections and cannot replace direct antigen detection. This result may not be very surprising, since – before treatment of both partners – reinfections occur regularly during sexual intercourse without increasing IgG titers that have reached their steady-state.

Interestingly, antibiotic treatment may be followed by a significant rise in IgG and IgA titers, possibly reflecting an immune stimulus by treatment-induced release of reticulate body antigens. Titer persistence after effective antibiotic treatment correlated well with the sensitivity of the serological test employed (Table 4). Apparently, *Chlamydia trachomatis* IgG may persist for a long time without any sign of ongoing infection. Puolakkainen et al. [11], who used an indirect immunofluorescence test based on cellular inclusions of *Chlamydia trachomatis* L2, found constant IgG titers over 3–6 years in 43% of 70 women treated with antibiotics for PID.

According to our data, the sensitivity of IgA was unsatisfactory in all tests except the *Chlamydia trachomatis* EIA. Negative MIF IgA titers remained negative when tested with four different commercially available anti-IgA-FITC conjugates. IgA titers tended to decrease more rapidly than IgG titers but still persisted for years after effective treatment (defined as negative cervical swabs at all follow-up visits after treatment). This finding suggests that positive IgA titers do not indicate current *Chlamydia trachomatis* infections, but *Chlamydia trachomatis* antigen persistence below detection levels cannot be ruled out by our data.

Chlamydia pneumoniae IgG (MIF, ImmunoComb, SeroCP) was positive and persisted at constant titers in 7 of the 16 women in this study. Six women remained *Chlamydia pneumoniae* IgG negative throughout the observation period. Three transient *Chlamydia pneumoniae* IgG seroconversions to low titers – in both MIF assays, the ImmunoComb, and the SeroCP – were observed concomitantly with *Chlamydia trachomatis* IgG seroconversions in two women and a significant *Chlamydia trachomatis* IgG titer increase in one woman with PID, suggesting some cross-reaction – or an unspecific general immune response – during the initial phase of primary or secondary stimulation of the immune system by *Chlamydia trachomatis*. MIF, the ImmunoComb, and the SeroCP appeared to be equally species-specific.

Chlamydia trachomatis IgG titers declined more rapidly in the MIF than in the ImmunoComb Chlamydia bivalent, and MIF IgA was surprisingly insensitive. This might be a consequence of subjective reading of the MIF test, as pointed out by Peeling et al. [6] in relation to *Chlamydia pneumoniae* MIF titers. In contrast to the ImmunoComb Chlamydia bivalent, MIF elementary bodies contain LPS, which may complicate MIF interpretation and conceal MOMP fluorescence.

The ImmunoComb Chlamydia bivalent exhibited the highest intertest variability, whereas the intertest reproducibility of both synthetic peptide tests was excellent. However, certain synthetic peptides (*Chlamydia trachomatis* EIA) appear to be more sensitive antigens for IgG and IgA detection than elementary bodies, other synthetic peptides (SeroCT), or genus-specific LPS. The increased sensitivity of the *Chlamydia trachomatis* EIA results in earlier seroconversion (Table 1) and higher IgG prevalence rates in *Chlamydia trachomatis*-antigen positive women. Among 120 women with positive cervical swabs, 107 (89%) had positive *Chlamydia trachomatis* species-specific IgG in the *Chlamydia trachomatis* EIA versus 90 (75%) in the ImmunoComb Chlamydia bivalent (data not published). The increased sensitivity of the *Chlamydia trachomatis* EIA in comparison with the other four serologic tests results in a higher negative predictive value of this test. The specificity of this test must be demonstrated by studies in low-prevalence groups.

SeroCT IgG and IgA titers remained negative (nonreactive) in all follow-up sera from 2 of the 16 women in this study, suggesting that this test does not cover all *Chlamydia trachomatis* serotypes. *Chlamydia trachomatis* EIA IgG was also nonreactive in one of these two women, with maximum concomitant IgA absorbance levels observed in all sera of this patient. Positive *Chlamydia trachomatis* EIA IgA with negative concomitant IgG was also observed by Närvänen et al. [13], and might reflect a test artifact: nonreactivity of the patient's polyclonal *Chlamydia trachomatis* IgG with the 12 amino acid test peptide (no IgG clones produced against this peptide).

Seroconversion and persistence of IgG against genus-specific LPS were similar to species-specific IgG against MOMP. According to our data, there is no indication for the use of the genus-specific rElisa instead of species-specific tests.

The results of our study underline the notion of Black [3] that serologic testing of a single serum sample does

not allow any differentiation between a previous or current *Chlamydia trachomatis* genital infection. Negative predictive values and titer persistence vary between different serological tests and depend on the test sensitivity. As shown by our data, *Chlamydia trachomatis* species-specific antibodies develop with some time lag (probably weeks or months) after a genital infection with *Chlamydia trachomatis*. IgG seroconversion occurs earlier or concomitantly with IgA seroconversion. After seroconversion, IgG and IgA titers reach a maximum within some weeks and may persist for years or decrease slowly, depending on the sensitivity of the serological test. Persistence of titers appears not to be dependent on the presence of detectable *Chlamydia trachomatis* antigen but rather on the reactivity of an individual's immune system. Thus, the only information *Chlamydia trachomatis* species-specific serological tests can provide is the discrimination between ever-infected and never-infected individuals. False-negative results during the initial phase of the infection and in patients with low persisting titers can be minimized by using the most sensitive *Chlamydia trachomatis* species-specific serological tests, e.g. the *Chlamydia trachomatis* EIA. Serological tests with poor reproducibility will be inaccurate in discriminating ever-infected from never-infected individuals in patients with low titers (intertest results varying between negative and low positive titers). Genus-specific tests cannot discriminate between an infection with *Chlamydia trachomatis* or *Chlamydia pneumoniae*. Thus, species-specific tests of high sensitivity and reproducibility are best suited for gynecological diagnostic purposes, i.e. for discriminating ever-infected from never-infected individuals. However, even the most sensitive serological test cannot exclude an early infection, since it takes at least a few weeks to develop an antibody response after a *Chlamydia trachomatis* infection of the urogenital tract.

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