Correlation of *Chlamydia* and *Chlamydophila* spp. IgG and IgM Antibodies by Microimmunofluorescence With Antigen Detection Methods

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Correlation of serologic titers for *Chlamydia trachomatis* with other tests has been based on direct fluorescence antibody (DFA) testing and culture, but not on nucleic acid-based tests that are used for screening. We retrospectively reviewed the specificity of antibodies against *C. trachomatis*, *Chlamydia psittaci*, and *Chlamydophila pneumoniae* by microimmunofluorescence (MIF) when compared with DFA, culture, nucleic acid probe, and transcription-mediated amplification. Over a 6-year period, 226 cases had both MIF and one of these other methods performed for comparison. Agreement between *C. trachomatis* antigen or nucleic acid detection and MIF

results was 87% (197/226). *C. trachomatis* serology had a negative predictive value of 98%, and 10.6% of cases were positive by serology and negative by antigen testing. Of the 13 patients who had a positive *C. trachomatis* antigen or nucleic acid test result, 9 had IgG and/or IgM titers highest against *C. trachomatis*, 3 had IgG titers highest against *C. pneumoniae*, and 1 had undetectable titers for the three chlamydial species. Twenty-five patients had positive IgG and/or IgM titers to *C. trachomatis* but negative antigen test results. Serologic testing can increase the sensitivity of detecting *C. trachomatis* infections. J. Clin. Lab. Anal. 25:305–308, 2011. © 2011 Wiley-Liss, Inc.

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INTRODUCTION

Accurate serologic diagnosis of chlamydial infections in the clinical laboratory is challenged by the cross-reactivity of antibodies among the clinically relevant species, *Chlamydophila pneumoniae*, *C. psittaci*, and *Chlamydia trachomatis* (1–3). Microimmunofluorescence (MIF) antibody testing, the gold standard for chlamydial serology, uses genus- and species-specific antigens to measure antibody titers to these species individually. Correlation of MIF *C. trachomatis* titers with other tests has previously been based on direct fluorescence antibody (DFA) testing and culture, but no studies have been performed comparing MIF with nucleic acid amplification tests (NAAT) that are commonly used to screen for *C. trachomatis* (4–6). In this study we seek to assess the specificity of chlamydial serology results by MIF in comparison with a variety of antigen detection methods including NAAT.

MATERIALS AND METHODS

Laboratory results were retrospectively reviewed from January 2003 to October 2009 at ARUP Laboratories. Data were collected on patients who had both MIF serologic testing and one of the following tests performed within 6 weeks of MIF testing: DFA, culture, DNA probe, or transcription-mediated amplification (TMA) for *C. trachomatis*.

MIF testing was performed on serum using commercial antibodies following the manufacturer's protocol

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(Focus Diagnostics, Cypress, CA). An IgM titer $\geq 1:20$ and an IgG titer $\geq 1:64$ were considered positive. A two-fold or higher titer for one species compared with the others was considered to be positive for that species. If the highest titer was equal for two or more species, the result was considered nonspecific.

C. trachomatis DFA (Pathfinder; Bio-Rad Laboratories, Hercules, CA) was performed according to the manufacturer's protocol. For *C. trachomatis* culture, specimens were inoculated into buffalo green monkey or HEp2 shell vials, blind stained at 48–72 hr (Bartels Reagent; Trinity Biotech, Wicklow, EI), and if positive, confirmed by another fluorescent stain (MicoTrak; Trinity Biotech). The DNA probe (PACE 2; Gen-Probe, San Diego, CA) and TMA (Aptima; Gen-Probe) were performed following the manufacturer's protocols. Positive DNA probe results were confirmed by a probe competition assay kit and positive TMA results were confirmed with alternative target testing (Gen-Probe).

RESULTS

Two-hundred and twenty-six unique patients (99 male, 127 female, median age 29.5 yr) had MIF performed as well as DFA ($n = 11$), culture ($n = 9$), DNA probe ($n = 44$), or TMA ($n = 162$) for *C. trachomatis*. Specimen sources for nonserologic methods included 210 genitourinary tract, 2 rectal, 2 eye, 3 oropharynx, 7 respiratory, and 2 unknown. IgG and/or IgM titers were positive for *C. pneumoniae* in 35.8% (81/226), *C. psittaci* in 0%, and *C. trachomatis* in 14.6% (33/226).

Thirteen patients had a positive *C. trachomatis* antigen or nucleic acid test result, and all were from genitourinary sites (Table 1). Nine had IgG and/or IgM titers highest against *C. trachomatis*, three had IgG titers highest against *C. pneumoniae*, and one had undetectable titers for the three chlamydial species. Twenty-five patients had positive IgG and/or IgM titers to *C. trachomatis* but negative antigen test results from genitourinary ($n = 24$) or nasal ($n = 1$) sites (Table 2). Agreement between *C. trachomatis* antigen or nucleic acid detection and MIF results was 87% (197/226) (Table 3). The positive and negative predictive values of MIF was 26.4% (9/34) and 97.9% (188/192), respectively.

DISCUSSION

The prevalence of *C. trachomatis* is increasing in young women despite increased screening and awareness, highlighting the importance of accurate and sensitive diagnosis (7). Although NAAT is recommended for *C. trachomatis* screening, serologic testing may be useful in certain clinical situations to optimize the sensitivity of detecting infection. These may include patients previously treated with antibiotics and patients with upper genital tract infection (4,8,9). A specimen that is poorly collected or from a site that is relatively less sensitive (e.g., female urine) may also be supplemented by serologic testing if NAAT is negative despite high clinical suspicion of *C. trachomatis* infection.

The negative predictive value of *C. trachomatis* MIF was high (98%), which correlates with another serological

TABLE 1. Antibody Titer Values for All Patients Positive for *Chlamydia trachomatis* by a Direct Detection Method

Age ^a	Sex	Direct detection method ^b	Time elapsed until serology ^c	<i>C. trachomatis</i> IgG ^d	<i>C. trachomatis</i> IgM ^e	<i>C. pneumoniae</i> IgG	<i>C. pneumoniae</i> IgM	<i>C. psittaci</i> IgG	<i>C. psittaci</i> IgM
28	M	TMA	–4 days	<1:64	<1:20	<1:64	<1:20	<1:64	<1:20
21	F	TMA	+1 day	1:64	ND	<1:64	ND	<1:64	ND
28	M	TMA	0 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
25	F	TMA	0 days	ND	1:320	ND	<1:20	ND	<1:20
23	F	TMA	+37 days	<1:64	1:640	<1:64	<1:20	<1:64	<1:20
1 wk	F	TMA	0 days	1:1024	1:640	<1:64	<1:20	<1:64	<1:20
21	M	TMA	0 days	1:256	<1:20	1:128	<1:20	<1:64	<1:20
29	M	TMA	0 days	1:256	<1:20	1:512	<1:20	1:128	<1:20
21	F	Probe	–13 days	1:64	ND	<1:64	ND	<1:64	ND
23	F	Probe	0 days	1:128	1:160	1:64	<1:20	<1:64	1:20
20	F	Probe	+6 days	$\geq 1:1024$	<1:20	1:256	<1:20	1:256	<1:20
21	F	Probe	–42 days	<1:64	<1:20	1:256	<1:20	<1:64	<1:20
28	F	Culture	0 days	<1:64	<1:20	1:64	<1:20	<1:64	<1:20

ND, not done.

^aAge in years unless noted.

^bThe three direct detection methods included TMA, DNA probe, and culture. The specimen sources for the cases listed were all genitourinary. No DFA-positive specimens were found in this study.

^cTime between specimen collection for serologic testing relative to direct detection testing.

^dAll IgG antibody assays are positive if the titer value is greater than or equal to 1:64.

^eAll IgM antibody assays are positive if the titer value is greater than or equal to 1:20 and titers were twofold or greater than those for *C. pneumoniae* and *C. psittaci*.

TABLE 2. Antibody Titer Values for All Patients in Which *Chlamydia trachomatis* IgG or IgM Values were the Highest Titer Values and Direct Detection of *C. trachomatis* was Negative

Age ^a	Sex	Direct detection method ^b	Time elapsed until serology ^c	<i>C. trachomatis</i> IgG ^d	<i>C. trachomatis</i> IgM ^e	<i>C. pneumoniae</i> IgG	<i>C. pneumoniae</i> IgM	<i>C. psittaci</i> IgG	<i>C. psittaci</i> IgM
67	M	TMA	+1 day	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
56	M	TMA	+2 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
30	F	TMA	0 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
29	F	TMA	0 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
26	M	TMA	+1 day	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
24	F	TMA	0 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
64	M	TMA	-3 days	1:128	<1:20	<1:64	<1:20	<1:64	<1:20
61	F	TMA	-5 days	1:128	1:320	<1:64	<1:20	1:64	1:20
27	F	TMA	-15 days	1:256	<1:20	1:64	<1:20	1:128	<1:20
41	F	TMA	0 days	1:256	<1:20	1:128	<1:20	<1:64	<1:20
31	M	TMA	0 days	1:512	<1:20	1:64	<1:20	<1:64	<1:20
37	M	TMA	0 days	≥1:1024	<1:20	1:1024	<1:20	1:1024	<1:20
25	F	TMA	1 day	ND	1:20	ND	<1:20	ND	<1:20
30	F	TMA	0 days	<1:64	1:20	<1:64	<1:20	<1:64	<1:20
19	F	TMA	+2 days	<1:64	1:40	<1:64	<1:20	<1:64	<1:20
48	M	TMA	-4 days	ND	1:40	<1:64	<1:20	ND	<1:20
29	M	TMA	-4 days	<1:64	1:40	1:256	<1:20	<1:64	<1:20
23	F	Probe	0 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
42	F	Probe	+36 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
21	M	Probe	0 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
15	F	Probe	0 days	1:64	<1:20	<1:64	<1:20	<1:64	<1:20
35	F	Probe	+2 days	1:256	1:80	1:256	<1:20	1:128	<1:20
46	F	Probe	0 days	≥1:1024	<1:20	1:64	<1:20	1:128	<1:20
24	F	Probe	+1 day	ND	1:320	ND	<1:20	ND	<1:20
1 wk	M	DFA ^b	-1 day	ND	1:80	ND	<1:20	ND	<1:20

ND, not done.

^aAge in years unless noted.^bThe three direct detection methods included TMA, DNA probe, DFA, and culture. All specimen sources were genitourinary, except for the DFA specimen, which was from a nasal source.^cTime between specimen collection for serologic testing relative to direct detection testing.^dAll IgG antibody assays are positive if the titer value is greater than or equal to 1:64.^eAll IgM antibody assays are positive if the titer value is greater than or equal to 1:20 and titers were two-fold or greater than those for *C. pneumoniae* and *C. psittaci*.**TABLE 3. Summary of Results Comparing *Chlamydia trachomatis* IgG and IgM With Direct Detection**

	Direct detection ^a	
	Positive	Negative
<i>C. trachomatis</i> IgG and IgM ^b		
Positive	9	25
Negative	4	188

^aThe direct detection methods included TMA, DNA probe, DFA, and culture.^bResults for *C. trachomatis* IgG and IgM were considered positive if either the IgG titer was ≥1:64 or the IgM titer was ≥1:20 and titers were twofold or greater than those for *Chlamydia pneumoniae* and *Chlamydia psittaci*.

study that showed a high negative predictive value for *C. trachomatis* enzyme immunoassay (6). A negative serologic test result may therefore be helpful in excluding *C. trachomatis* infection, given that enough

time has elapsed since the time of exposure for an antibody response to develop. In the few cases in which the serology was negative when a direct detection method was positive, serology was drawn before or at the time of specimen collection for culture, DNA probe, or TMA, which may in part explain the discordance.

Not surprisingly, we found *C. trachomatis* antibody titers to be elevated when direct detection methods were negative in a large percentage (11%) of cases. Ten patients in particular had IgG titers to *C. trachomatis* of only 1:64, and it is difficult to interpret these results without clinical correlation. Nonetheless, the discordance between serology and direct detection could be explained by a number of reasons. Improper specimen collection for direct specimen testing, such as collection of specimens with suboptimal cellular material, is one possibility. Elevated antibody titers are also not specific for active infection, and may be seen in previously infected patients who have received treatment or are no

longer shedding organisms. Because most of these discordant cases had serology and direct detection testing drawn at or around the same time point (Table 2), this scenario is not unlikely. Culture and DFA are relatively insensitive for detection of *C. trachomatis* and may miss active infection in many cases.

Frequently, cross-reactivity between species antibodies or elevated titers to more than one species is observed. In this study, for example, one patient had a positive *C. trachomatis* IgM of 1:40, and an elevated *C. pneumoniae* IgG titer of 1:256 (Table 2). *C. trachomatis* IgM antibodies by MIF testing appear to be less heterologous than IgG antibodies, and are observed after primary but not repeat exposure to *C. trachomatis* (1). On the other hand, rheumatoid factor and Epstein Barr virus infections have been described to cause falsely elevated *C. trachomatis* IgM titers (10,11). Because the incidence of both *C. trachomatis* and *C. pneumoniae* is high, it is also possible that simultaneously elevated titers represent previous exposure to both pathogens. Interpretation of chlamydial antibody panels must be carried out carefully within the clinical context.

In most *C. trachomatis* antigen-positive or nucleic acid-positive patients, titers of *C. trachomatis* antibodies detected by MIF were higher than titers for *C. pneumoniae* or *C. psittaci*. However, as antibody levels of the latter two were still detectable, it is advisable for clinical laboratories to perform chlamydial antibodies by MIF as a panel, rather than offer them separately. This is especially true given the high seroprevalence of *C. pneumoniae* infection which was corroborated in this study. This study also demonstrates that it is important to test for both IgG and IgM antibodies to optimize the likelihood of detecting a serologic response.

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