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Evaluation of Chlamydia Immunoglobulin M (IgM), IgG, and IgA rELISAs Medac for Diagnosis of *Chlamydia pneumoniae* Infection

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***Chlamydia pneumoniae* is an important pathogen responsible for a variety of respiratory diseases in humans. Cell culture remains the most specific method for *C. pneumoniae* diagnosis, but it is labor-intensive and time-consuming. Thus, serology, particularly microimmunofluorescence (MIF) testing, is frequently utilized. However, the MIF test has a significant subjective component. We evaluated a new serological test: Chlamydia Immunoglobulin M (IgG), IgA, and IgM rELISAs Medac, based on a recombinant *Chlamydia*-specific lipopolysaccharide (LPS) fragment, for the diagnosis of *C. pneumoniae* infection. The results of this study demonstrated that the use of rELISAs Medac with single sera does not appear to be sensitive or specific for diagnosis of *C. pneumoniae* infection compared to culture. In children, sensitivities of the rELISAs compared to culture did not exceed 34.2%, and the specificities ranged from 68.4% (IgG) to 91.2% (IgA). In adults, the sensitivities of the rELISAs were slightly higher, up to 77.8% (IgA or IgG), but the specificities ranged from a very low 20.8% for IgA or IgG to 81.1% for IgM. When multiple sera were tested, the results of the rELISAs Medac correlated with culture results in five of eight (62.5%) patients. However, this offers only a retrospective diagnosis, which makes it difficult to manage these patients prospectively.**

Chlamydia pneumoniae has been shown to be an important and common respiratory pathogen in humans (4, 5). However, diagnosis of *C. pneumoniae* infections has been difficult for many investigators. Cell culture remains the most specific method, but it is labor-intensive and time-consuming, and there are few facilities where culture is available. Because of the perceived difficulty in performing *C. pneumoniae* culture, serology, particularly the microimmunofluorescence (MIF) method, has been the most frequently used diagnostic technique. However, the MIF test is also labor-intensive, requires a highly experienced reader, and in addition has a significant subjective component. The results of recent studies have demonstrated that many children with culture-documented *C. pneumoniae* infection do not develop any MIF antibodies (1, 3). Several studies in adults (7–9, 11) also found that sensitivity and specificity of MIF were lower than generally appreciated. In this study we evaluated a new serological test, Chlamydia Immunoglobulin M (IgM), IgG, and IgA rELISAs Medac, for diagnosis of *C. pneumoniae* infection in patients with known culture statuses.

MATERIALS AND METHODS

Study population. We examined acute-phase sera from previously cultured children and cultured adults. Patients included symptomatic individuals with asthma, bronchitis, cystic fibrosis, pneumonia, and upper respiratory infections and also asymptomatic individuals, including healthy controls. Multiple sera were also available from five culture-positive symptomatic children and 3 culture-positive symptomatic adults.

ELISAs. The Medac rELISAs were performed according to the instructions of the manufacturer. The test is an enzyme immunoassay (ELISA) based on the use of a recombinant, exclusively *Chlamydia* genus-specific fragment of the chlamydial lipopolysaccharide (LPS) (2). The advantages of this test, suggested by the manufacturer, include the use of noninfectious and reproducible antigen, short performance time, and objective reading. The absorbance values were measured in a spectrophotometer at 405 to 410 nm. Antibody titers were calculated according to formulas provided by the manufacturer. A titer of ≥50 was considered positive for IgA and IgM, and a titer of ≥100 was considered positive for IgG. The manufacturer also recommends that IgG be evaluated in combination with IgA results. Briefly, of the eight possible combinations, if IgG and IgA are positive, this indicates acute infection; if the IgG is positive and the IgA is negative, this indicates past infection. If the IgG is negative and the IgA is positive, the manufacturer states, this may indicate the early stage of an active infection, and retesting in 10 to 14 days is recommended. Sensitivities and specificities of the rELISAs were evaluated in comparison to culture.

Culture of *C. pneumoniae*. Isolation of *C. pneumoniae* from nasopharyngeal swab specimens was performed with cycloheximide-treated HEp-2 cells grown in 96-well microtiter plates as previously described (14). Culture conformation was performed with fluorescein-conjugated genus-specific (Kallestad, Chaska, Minn.) and species-specific (Washington Research Foundation) monoclonal antibodies for the presence of chlamydial inclusions.

RESULTS

Acute-phase sera, obtained from 95 children, 1 to 17 years of age (mean age, 7.8, and standard deviation [SD], 3.8 years), and 62 adults, 18 to 78 years of age (mean age, 41.9, and SD, 15.7 years), were examined by the Medac rELISAs. Acute-phase sera were collected 1 to 4 days after the onset of respiratory symptoms. Patient distribution by clinical diagnosis is shown in Table 1.

Single sera. The results of determination of anti-*Chlamydia* LPS antibodies in acute-phase sera from patients with different culture statuses are presented in Table 2. The prevalence of IgG and IgM antibodies in the culture-positive and -negative children was essentially the same. Though IgA was present in only 8.8% of the culture-negative children, compared to 18.4% of the culture-positive children, this was not statistically different ($P = 0.0967$, the Fisher exact test). The combination of positive IgA and IgG was also present less frequently (8.8%) in

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TABLE 1. Distribution of patients by clinical diagnosis

Clinical diagnosis	No. of patients[a]			
	Children (n = 95)		Adults (n = 62)	
	Sympt.	Asympt.	Sympt.	Asympt.
Asthma	25		14	
Bronchitis	1		1	
Pneumonia	58		5	
Upper respiratory infection			7	
Cystic fibrosis	1	5	9	10
HIV[b]		1		
Coronary artery disease				7
Healthy		4		9
Total	85	10	36	26

[a] Sympt., symptomatic; Asympt., asymptomatic.
[b] HIV, human immunodeficiency virus.

culture-negative than in culture-positive (15.8%) children, but this difference was also not statistically significant ($P = 0.1480$).

The prevalence of anti-LPS antibodies in culture-positive adults was higher than that observed in children (Table 2). There was no difference in the proportion of culture-positive and culture-negative adults who had anti-LPS IgG. IgA and IgM were more frequently detected in culture-negative adults than in culture-positive adults.

The results of rELISAs with children and adults with respect to respiratory symptoms are analyzed in Table 3. The presence of anti-LPS antibodies was essentially the same in symptomatic, culture-positive and asymptomatic, culture-negative children. In contrast, among asymptomatic, culture-negative children, 22% had IgG antibody, but none had any detectable IgA or IgM.

As shown in Table 3, the proportions of symptomatic adults who had anti-LPS IgG and IgA antibodies were similar regardless of their culture status. Asymptomatic adult patients also had IgG, IgA, and IgM anti-LPS antibodies in approximately the same percentage as the culture-positive symptomatic adults.

There were no significant differences between symptomatic culture-positive and culture-negative patients and symptomatic and asymptomatic patients, both adults and children (P values ranged from 0.1609 to 0.4144 by the Fisher exact test). The one exception was the difference in the presence of IgA between the symptomatic culture-positive and -negative children (18.9 versus 10.4%, $P = 0.0018$, the Fisher exact test).

Multiple sera. We tested multiple sera from five symptomatic, culture-positive children. Consecutive sera were collected on dates shown in Table 4. Two children had pneumonia (patients 1 and 5), and three had asthma. Two children, patients 2 and 3, failed to develop any detectable antibody despite 6 to 8 weeks of follow-up. An 11-year-old girl with pneumonia (patient 1) had stable IgG titers but was negative for IgA and IgM, which is suggestive of past infection. Patients 4 and 5 were positive for IgG, IgA, and IgM antibodies, which indicates active infection according to the manufacturer's interpretation.

The results of rELISAs with multiple sera from three culture-positive symptomatic adults were consistent with active infection (Table 4). Patients 6 and 8 had stable IgG and IgA titers, which, the manufacturer states, is indicative of active infection. Patient 7 seroconverted IgG from negative to positive with an IgG titer of 1:100.

Sensitivities and specificities. The sensitivities and specificities of Medac rELISAs on single acute-phase sera compared to culture are shown in Table 5. We found no difference in sensitivity and specificity when using the combined IgG and IgA results, as suggested by the manufacturer.

TABLE 2. Results of rELISAs Medac compared to those by culture in diagnosis of *C. pneumoniae* infection

rELISA Medac result(s)	No. of patients (%)[a]			
	Children (n = 95)		Adults (n = 62)	
	Culture pos. (n = 38)	Culture neg. (n = 57)	Culture pos. (n = 9)	Culture neg. (n = 53)
IgA	7 (18.4)	5 (8.8)	3 (33.3)	27 (50.9)
IgG	12 (31.6)	18 (31.6)	6 (66.7)	30 (56.6)
IgM	5 (13.2)	8 (14.0)	1 (11.1)	10 (18.9)
IgA or IgG	13 (34.2)	18 (31.6)	7 (77.8)	42 (79.2)
IgA and IgG	6 (15.8)	5 (8.8)	2 (22.2)	15 (28.3)

[a] Pos., positive; neg., negative.

DISCUSSION

The MIF test remains the most frequently used method for serologic diagnosis of *C. pneumoniae* infection. However, the MIF assay fails to detect antibodies in the majority of children with culture-documented *C. pneumoniae* infections (1, 3). The immunodominant antigen in the MIF test is probably the major outer membrane protein (MOMP). When sera from these culture-positive, MIF-negative children were examined by immunoblotting, almost all sera reacted with several *C. pneumoniae* proteins (13). However, less than 30% of them reacted with MOMP. Peterson et al. (12), in a recent article in which they described utilizing a mouse model, assessed the immunogenicity of synthetic peptides representing the *C. pneumoniae* MOMP variable domains. They demonstrated that these peptides were not able to stimulate a humoral neutralizing response. In contrast, monoclonal antibodies recognizing the MOMP and polyclonal sera raised to the variable domains of the MOMP of *C. trachomatis* have been shown to have neutralizing activities in vivo and in vitro.

The MIF test is mainly available in research laboratories and is difficult to perform. There is a significant subjective component in the reading of this assay. There is obviously a need for a reliable and simple serologic test. The Chlamydia rELISAs Medac are based on the use of a recombinant chlamydial LPS and are intended for the detection of serum anti-LPS IgG, IgA, and IgM antibodies in patients with chlamydial infections. The theoretical advantages of these tests over MIF and culture include reproducibility, objective analysis, and short performance time. However, this test cannot differentiate between chlamydial species due to the genus-specific nature of chlamydial LPS.

The results of this study demonstrate that the use of rELISAs Medac with single sera does not appear to be sufficiently sensitive or specific for diagnosis of *C. pneumoniae* infection compared to culture. In children, sensitivities of the rELISAs did not exceed 34.2%, and the specificities ranged from 68.4 to 91.2%. In adults the sensitivities of the rELISAs were slightly higher, but the specificities were low.

This is the only study in which the results of the rELISAs Medac were compared to those by isolation of *C. pneumoniae* in culture. There are several published reports on the use of the rELISAs Medac for the diagnosis of *C. trachomatis* (15, 17), *C. psittaci* (5), and *C. pneumoniae* (16) infections. In all of these studies the rELISA Medac was compared only to other serological tests, usually the MIF test. High sensitivities and specificities compared to those of MIF, ELISA, or complement

TABLE 3. Prevalence of anti-LPS antibodies by rELISAs Medac in symptomatic and asymptomatic patients by culture status

Patients (total no.)	No. (%) of patients rELISAs Medac positive for:			
	IgA	IgG	IgM	IgA and IgG
Children				
Symptomatic				
Culture positive (37)	7 (18.9)	11 (29.7)	5 (13.5)	6 (16.2)
Culture negative (48)	5 (10.4)	15 (33.3)	8 (16.7)	5 (10.4)
Asymptomatic, culture negative[a] (9)	0	2 (22.2)	0	0
Adults				
Symptomatic				
Culture positive (9)	3 (33.3)	6 (66.7)	1 (11.1)	2 (22.2)
Culture negative (27)	15 (55.6)	19 (70.4)	6 (22.2)	8 (29.6)
Asymptomatic, culture negative (26)	12 (46.2)	11 (42.3)	4 (15.4)	5 (19.2)

[a] One asymptomatic culture-positive child is omitted.

fixation test were reported (6, 15–17). These authors claimed that rELISA was a useful method for the diagnosis of chlamydial infection and for assessing the effect of treatment. However, microbiological evidence of chlamydial infection was not proven with culture or PCR. Efficacy of treatment cannot really be monitored without proven eradication of the microorganism.

A recent study by Numazaki et al. (10) describes what is probably a more specific ELISA that utilizes *C. pneumoniae* outer membrane complex as antigen. They found an "excellent" correlation between MIF and ELISA with Japanese children. However, up to 75 and 64% of 342 children without any evidence of respiratory symptoms in the control group had IgA and IgM antibody to *C. pneumoniae* by MIF or ELISA.

TABLE 4. Results of testing multiple sera in culture-positive, symptomatic children and adults

Patient no., age (yr), sex	Diagnosis	Date (mo/day/yr)	Culture	rELISA Medac titer[a]		
				IgA	IgG	IgM
1, 11, F	Pneumonia	2/02/93	+	<50	100	<50
		2/16/93	+	<50	100	<50
2, 13, M	Asthma	5/21/93	+	<50	<100	<50
		7/01/93	−	<50	<100	<50
3, 13, M	Asthma	1/15/92	+	<50	<100	<50
		1/27/92	−	<50	<100	<50
		2/27/92	−	<50	<100	<50
4, 16, F	Asthma	12/01/91	+	100	>400	100
		1/17/92	+	100	>400	100
5, 12, M	Pneumonia	12/16/94	+	>200	200	>200
		2/01/95	−	50	200	<50
6, 32, F	Cystic fibrosis	5/12/92	+	100	200	<50
		8/05/93	−	50	200	<50
		9/20/93	+	50	200	50
7, 41, F	Cystic fibrosis	2/24/93	+	<50	<100	<50
		3/17/93	−	<50	100	<50
8, 44, F	Bronchitis	4/02/93	+	50	400	<50
		8/10/93	+	50	400	<50

[a] Titers were calculated from A_{405}: >50, positive for IgA and IgM; >100, positive for IgG.

TABLE 5. Performance of rELISAs Medac versus that of cell culture for diagnosis of *C. pneumoniae* infection

rELISA Medac result(s)	Children (n = 95)		Adults (n = 62)	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
IgA	18.4	91.2	33.3	49.1
IgG	31.6	68.4	66.7	43.4
IgM	13.2	86.0	11.1	81.1
IgA or IgG	34.2	68.4	77.8	20.8
IgA and IgG	15.8	91.2	22.2	71.7

The chlamydial LPS is a genus-specific antigen shared by the four recognized chlamydial species. Infection with any of them may induce a response to the cross-reactive antigenic determinant (9, 11). In adults, infection with other *Chlamydia* species might explain the high prevalence of anti-LPS antibodies of different isotypes seen in asymptomatic, culture-negative adults. In contrast, only 22% of asymptomatic, culture-negative children had anti-LPS IgG.

When acute- and convalescent-phase sera were tested, the results of the rELISAs Medac correlated with culture results in five of eight (62.5%) patients; however, the numbers were limited. It should be mentioned that the manufacturer recommends retesting of sera after 10 to 14 days, except when both IgG and IgA were positive with acute-phase sera, because "in cases of fresh acute chlamydial infections the serological antibody results may be negative." However, there are no clinical or culture data available that support this recommendation. Grayston et al. (5) have suggested that one may need more than 2 months to demonstrate seroconversion with MIF. However, serology with paired sera provides only a retrospective diagnosis, which makes it difficult to manage these patients prospectively. Obviously, detection of *C. pneumoniae* by culture or a DNA amplification method, such as PCR, would be preferable. It is hoped that a standardized, commercially available assay will be available in the future.

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