JOURNAL OF CLINICAL MICROBIOLOGY, May 2002, p. 1603–1609 Vol. 40, No. 5
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.5.1603–1609.2002

Comparison of Eleven Commercial Tests for *Chlamydia pneumoniae*-Specific Immunoglobulin G in Asymptomatic Healthy Individuals

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Received 14 November 2001/Returned for modification 2 February 2002/Accepted 13 February 2002

The seroprevalence of anti-*Chlamydia pneumoniae*-specific immunoglobulin G (IgG) antibodies is high in the adult population. Experience is required to perform a microimmunofluorescence test (MIF), the current "gold standard" for serological diagnosis, and the assay still lacks standardization. Partially automated enzyme-linked immunosorbent assays (ELISAs) and enzyme immunoassays (EIAs), which are more standardized and for which the reading of results is less subjective, have been developed. The different commercially available serological tests differ in their sensitivities and specificities, depending primarily on the antigen used. Therefore, we evaluated 11 different tests (10 were species specific, 1 was genus specific) for IgG antibodies using serum samples of 80 apparently healthy volunteers. The interpretation of the results was based on the results of the gold standard, MIF: a sample was judged positive if it was positive by at least three of the four different MIFs. Based on this internal standard, we found that 71% of the samples were positive, while 8% were false positive by some tests. The correlations between the results of the different MIFs ranged from 83 to 99%, and the correlations between the results of the MIFs and the different ELISAs and EIAs ranged from 78 to 98%. Comparison of the IgG titers measured by MIF showed good agreement (r = 0.76 to 0.91). This analysis revealed that some ELISAs and EIAs fail to detect low IgG titers. The specificities of the species-specific tests varied from 95 to 100%, and the sensitivities varied from 58 to 100%. These results indicate that serological assays for the detection of anti-*C. pneumoniae*-specific IgG vary greatly in their sensitivities and specificities. MIF must still be considered the best method for the detection of IgG in apparently healthy subjects, but the sensitivities and specificities of new ELISAs approximate those of MIFs.

Chlamydia pneumoniae represents an important respiratory pathogen and is believed to be responsible for about 10% of the cases of community-acquired pneumonia (2, 14, 17, 25). It was discovered in 1965 in Taiwan (20) and was classified in 1989 as a new species of the genus *Chlamydia* (19). It occurs worldwide, and about half of the adult population has antibodies against *C. pneumoniae* (18). Since 70% of infections are subclinical or asymptomatic (18), many seropositive individuals are not aware that they have been infected with *C. pneumoniae*. The high prevalence of antibodies against *C. pneumoniae* suggests that infections often recur (2, 18). On the other hand, there is evidence that *C. pneumoniae* often establishes persistent infections (13, 16, 23). In vivo, persistence has been observed in blood monocytes (5, 6, 30), where *Chlamydia* circumvents the endocytic pathway and survives within a nonacidic vacuole in the host cell (3). The development of persistence in vitro is characterized by a reduction of the intracellular growth rate of *C. pneumoniae* and by the appearance of morphologically aberrant reticulate bodies (27). Persistent *C. pneumoniae* has been shown to be metabolically active (1, 10, 32), but the period of persistence as well as the influence on the antibody titer of the host is unknown. There is emerging evidence that persistent *C. pneumoniae* might be implicated in the pathogenesis of several chronic and sometimes destructive diseases of the lung and the nervous system and atherosclerosis (13, 29, 41–43, 48, 52).

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Testing for a putative link between a previous *C. pneumoniae* infection and the prevalence of a chronic disease, particularly atherosclerosis, is difficult. Provision of direct proof of persistent *C. pneumoniae* infection by PCR is still not routine (4, 7, 31, 45); therefore, serological analysis represents the current routine method for the diagnosis of *C. pneumoniae* infection. However, findings reported on the basis of seroepidemiology have been controversial (11, 12, 42). The diagnostic method used must be accurate for a study to be valid, and the different accuracies of the diagnostic methods used might explain the opposing findings. The "gold standard" at the moment is the microimmunofluorescence assay (MIF). It was primarily developed for *C. trachomatis* by Wang and coauthors, but it was later adapted for use in serodiagnosis of *C. pneumoniae* infections (21, 49). The MIF uses formalin-fixed chlamydia elementary bodies (EBs) to quantitatively detect specific antibodies. However, the informative value of the results of MIF strongly depends on the antigen preparation and the experience of the individual performing the assay (37). Furthermore, there is still a lack of standardization of the performance of the MIF, and the specificity of MIF has been questioned (22, 26, 35). Partially automated enzyme-linked immunosorbent assays (ELISAs) and enzyme immunoassays (EIA), which are more standardized and for which the reading of results is less subjective, were therefore developed for routine diagnosis. The aim of this study was to evaluate a broad variety of commercial species-specific MIFs, ELISAs, and EIAs, as well as the genus-specific rELISA, for detection of anti-*C. pneumoniae*-specific immunoglobulin G (IgG) within the healthy population. Since the rELISA has been reported to be cross-reactive with antibodies against parvovirus and *Myco-*

plasma pneumoniae in some cases (39), we also included serological assays for these pathogens. Samples which could not be clearly classified on the basis of the gold standard assay, MIF, were further characterized by immunoblotting, which is not a routine clinical laboratory test for *Chlamydia* antibodies but which allows antibodies against separate chlamydial antigens to be distinguished. Furthermore, for characterization of the sera, we also determined the seroprevalence of anti-*C. pneumoniae*-specific IgM and IgA in our study group of asymptomatic, healthy volunteers.

MATERIALS AND METHODS

Serum donors. Sera were collected from 80 healthy volunteers at the University of Konstanz between February and April 2000. The mean age of the volunteers was 30 years (age range, 22 to 57 years), and they showed no signs of acute infection, confirmed by their differential blood cell count and anamnesis. Of the 80 volunteers, 50 were male and 30 female.

Serological studies. Chlamydia-specific IgG antibodies were determined by four different ELISAs, three EIAs, and four MIFs. The ELISAs and EIAs evaluated were the Elegance Chlamydia pneumoniae IgG EIA (r-biopharm, Darmstadt, Germany), Immunocomb Chlamydia Bivalent IgG EIA (Innogenetics, Heiden, Germany), SeroCP and SeroCP Quant (Quant) (Savyon Diagnostic, Ashdod, Israel, purchased from Hain Diagnostika, Nehren, Germany), Chlamydia pneumoniae IgG EIA (Labsystems, Helsinki, Finland, purchased from Merlin, Bornheim, Germany [referred to as the Labsystems EIA]), Vircell Chlamydia Pneumoniae IgG ELISA (Viva Diagnostica, Köln-Hürth, Germany), and the Chlamydia IgG rELISA (Medac, Wedel, Germany). Chlamydia-specific IgA antibodies were determined by the Quant ELISA (Savyon Diagnostic, purchased from Hain Diagnostika).

The tests were performed according to the instructions of the manufacturers. All tests except the Immunocomb EIA are solid-phase assays performed on microtiter plates; the Immunocomb EIA uses combs. The teeth of the combs are specifically spotted with an antigen from *C. pneumoniae* and an additional antigen from *C. trachomatis*, and the test therefore differentially detects antibodies against both species. The assays differ mainly in the antigen preparations used for detection of the anti-*Chlamydia* antibodies. The SeroCP and Quant ELISAs use complete lipopolysaccharide (LPS)-containing *C. pneumoniae* antigens. The Elegance, Immunocomb, Labsystems, and Vircell assays use preparations of purified *C. pneumoniae*-specific antigens which are free of cross-reactive LPS. However, the manufacturers do not specify the precise compositions of the antigens. Only the rELISA uses a chemically defined, chlamydia-specific recombinant LPS fragment which is claimed to be genus specific (9). The MIFs evaluated were the Chlamydia pneumoniae IgG/M IFT MIF (Labsystems, purchased from Merlin [referred to as the Labsystems MIF]), the Chlamydia pneumoniae IgG MIF (MRL Diagnostics, Los Angeles, Calif., purchased from Genzyme Virotech, Rüsselsheim, Germany [referred to as the MRL MIF]), the SeroFIA MIF (Savyon, purchased from Hain), and the Vircell Chlamydia pneumoniae MIF (Viva Diagnostica).

The tests were performed and analyzed as recommended by the manufacturers. All tests are solid-phase immunofluorescence assays performed on glass slides; and all assays use purified formalin-treated EBs of *C. pneumoniae*, *C. trachomatis*, and *C. psittaci* as antigens. The immunological activities of *C. pneumoniae* and *C. trachomatis* LPSs are reduced in ways not specified by the manufacturers in order to render the tests species specific. The MIFs from Labsystems, MRL, and Savyon use EBs from *C. pneumoniae* isolate TW-183 (Taiwan), while the MIF from Viva Diagnostica uses EBs from isolate 2032 (New York, N.Y.). The MIF from Labsystems was also used for determination of anti-*C. pneumoniae*-specific IgM after removal of the cross-reactive rheumatoid factor with the GullSORB reagent (Meridian Diagnostics, Bad Homburg, Germany) to reduce false-positive reactions.

For detection of anti-*C. trachomatis*-specific IgG antibodies, the Chlamydia trachomatis IgG EIA from Labsystems was used in addition to the MIFs mentioned above. All MIFs were performed and analyzed blindly and in parallel by the same two persons.

IgG antibodies against parvovirus B19 were detected by an immunofluorescence assay (Biotrin, Sinsheim-Reihen, Germany). An immunoassay (Serodia-MYCO II; Fujirebio Inc., Tokyo, Japan) was used for detection of anti-*M. pneumoniae*-specific IgG.

Immunoblotting. *C. pneumoniae* HK92, a local isolate from a patient with chronic pharyngitis, was used as the antigen for the immunoblot assay. This strain is propagated in BGM cells in serum-free SF-3 medium (Cytogen, Lohmar, Germany) without antibiotics. The cell culture was tested on a regular basis by DNA staining and PCR to exclude *Mycoplasma* infection. The infected cells were harvested mechanically and lysed by sonication. The EBs were prepared by density gradient centrifugation. The EBs were diluted at a concentration of 5×10^8 and inactivated at 75°C for 15 min. They were mixed with an equal volume of Laemmli sample buffer and heated to 95°C for 10 min. The samples were electrophoresed in 12 and 10% polyacrylamide gels with sodium dodecyl sulfate. Transfer of the fractionated proteins to nitrocellulose was carried out with a semidry transblot system (Bio-Rad, Munich, Germany). The blots were blocked with 1% powdered milk in phosphate-buffered saline containing 0.05% Tween. The blots were sequentially incubated with a 1:100 dilution of serum samples and with a 1:5,000 dilution of an alkaline phosphatase-conjugated goat anti-human IgG Fab fragment (Dianova, Hamburg, Germany). The incubation time for each antibody was 1 h at room temperature. The blots were developed with Sigma Fast (5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium) (40).

Statistics. Statistical analysis was performed with Prism software (version 3.0; GraphPad Software, San Diego, Calif.). Correlation of the IgG titers that were determined by the different tests was done by nonparametric correlation (Spearman).

RESULTS

Seroprevalence in healthy individuals determined by 11 different tests. Serum samples of 80 healthy volunteers were tested for antibodies against *C. pneumoniae* by 11 different tests: 4 MIFs, 3 EIAs, and 4 ELISAs, of which 1 was an rELISA that detects anti-LPS antibodies and that therefore cannot distinguish between the different *Chlamydia* species. The results of the different tests are presented in Table 1. The seroprevalence according to the results of the different tests ranged from 43 to 73% (Table 1, bottom row). Interpretation of the results was done on the basis of the current gold standard, MIF (51). Since the results of the four different MIFs were not homogeneous, we defined a positive sample as one that was positive by at least three of the four MIFs. Based on this internal standard, we found that 57 (71%) samples were positive and 23 (29%) samples were negative, of which 6 samples (8%) also gave some false-positive results. These six samples where further investigated by immunoblotting, which showed the reaction of human serum antibodies to 11 *C. pneumoniae*-specific proteins (proteins of 28, 32, 35, 39.5, 45, 49, 52, 60, 69, 75, and 110 kDa). Control samples which were positive by MIF showed a strong reaction to more than six of these proteins, while MIF-negative samples showed no or only a weak reaction to a smaller number of proteins (up to five). Among the six samples with questionable results, the four samples that were negative by all MIFs did not react with any protein. The one sample that was positive by one MIF reacted weakly with one protein, and the sample that was positive by two MIFs reacted weakly with five proteins. All samples were therefore judged to be negative. Investigation of the 80 serum samples for IgA antibodies revealed that 40 (50%) were positive (data not shown). All IgA-positive samples were also positive for IgG antibodies. IgM antibodies were not found in any of the samples.

The Medac rELISA for the anti-*Chlamydia* LPS fragment was positive for 30 (53%) of the 57 positive samples and 4 (17%) of the 23 negative samples. Thus, in total, 34 samples (43%) were positive. The cross-reactivity of the rELISA with parvovirus and *M. pneumoniae* might account for false-positive results (39). Therefore, the four samples negative for *C. pneumoniae*-specific IgG antibodies but positive by rELISA were

TABLE 1. Seroprevalence of anti-*C. pneumoniae* antibodies by the different tests

Sample group and no. of samples	ELISA and EIA result[a]						MIF result[a]				No. of samples positive by rELISA
	Elegance	Immunocomb	Vircell	SeroCP	Quant	Labsystems	Vircell	MRL	SeroFIA	Labsystems	
Positive samples											
34	+	+	+	+	+	+	+	+	+	+	21
5	−	+	+	+	+	+	+	+	+	+	3
1	+	−	+	+	+	+	+	+	+	+	1
4	+	+	+	+	+	+	−	+	+	+	1
1	−	−	+	+	+	+	+	+	+	+	1
1	−	+	−	+	+	+	+	+	+	+	
1	−	+	−	+	+	−	+	+	+	+	
1	−	−	−	+	+	+	+	+	+	+	
1	−	−	+	+	+	+	−	+	+	+	1
1	+	−	−	+	+	+	−	+	+	+	
1	−	+	+	−	+	+	−	+	+	+	
1	−	−	+	+	+	+	−	+	+	+	1
1	−	−	+	+	+	−	−	+	+	+	1
1	−	−	−	+	+	+	−	+	+	+	
1	−	−	+	+	−	−	+	+	−	+	
1	−	−	−	+	+	−	−	+	+	+	
1	−	−	−	+	−	−	−	+	+	+	
Negative samples											
1	−	−	±	−	−	−	−	−	±	±	
1[b,c]	−	±	−	−	±	−	−	−	±	−	1
1[b]	−	−	−	−	−	±	−	−	−	−	1
1	−	−	−	−	−	±	−	−	−	−	
1	−	−	−	−	−	±	−	−	−	−	
1	−	−	−	±	−	−	−	−	−	−	
17	−	−	−	−	−	−	−	−	−	−	2
% Positive samples	50	59	64	70	70	69	56	71	73	73	43

[a] +, positive result; −, negative result; ±, false-positive result.
[b] The sample tested positive for parvovirus (the same subset of samples was analyzed).
[c] The sample was positive for *C. trachomatis* and *C. psittaci*.

tested for antiparvovirus-specific and anti-*M. pneumoniae*-specific IgG. All samples were negative for *M. pneumoniae*, and two of the four samples were positive for parvovirus (Table 1).

Furthermore, the analysis of the four MIFs, the Labsystems EIA, and the Immunocomb EIA revealed that of the 80 samples tested, 9 (11%) were positive for *C. trachomatis* (Table 2). In six cases, infections with both *C. pneumoniae* and *C. trachomatis* were detected. Four samples were positive for *C. psittaci* but also showed the presence of anti-LPS antibodies (Table 2). The sample which was positive for *C. trachomatis* and *C. psittaci* (Table 2) was false positive for *C. pneumoniae* IgG three times (Tables 1 and 2).

Correlation of the results of the 11 different tests. The overall correlation of the results of the 11 different tests is shown in Table 3. There were very high correlations (≥96%) between the results of the different MIFs except for the Vircell MIF, whose results showed a weaker correlation (82%) with those of the other MIFs. The best correlation (99%) was found between the results of the MIF from Labsystems and those of the MIF from MRL. The agreement between the results of the MRL, SeroFIA, and Labsystems MIFs and those of the Vircell, SeroCP, Quant, and Labsystems ELISAs and EIAs ranged from 89 to 98%. A poor correlation was found for the results of the Elegance EIA (78 to 79%) in comparison to those of the SeroCP ELISA, as well as those of the MIFs from Labsystems, MRL, and SeroFIA. The overall agreement within the ELISA-EIA group was between 88 and 94% for the Labsystems, Quant, SeroCP, and Vircell assays but only 79 to 85% if the EIAs from Elegance and Immunocomb are considered. The results of the rELISA showed the best correlation with those of the Elegance EIA and the Quant and Vircell MIFs (65 to 68%). The agreement of the results of the rELISA with those of the other tests ranged between 54 and 63%.

Correlation of IgG titers. The 80 serum samples were serially diluted to determine the IgG titers by the MIFs and the Quant ELISA, the only ELISA which allows determination of titers, similar to the outputs of the MIFs. The correlations between the defined titers and those determined by the differ-

TABLE 2. Presence of anti-*C. pneumoniae*-, anti-*C. trachomatis*-, and anti-*C. psittaci*-specific IgG antibodies in serum of 80 healthy donors

Antibody	No. of samples:	
	Total[a]	With anti-LPS antibodies[b]
C. pneumoniae IgG	43	25
C. trachomatis IgG	2	
C. psittaci IgG		
C. pneumoniae and *C. trachomatis* IgG	5	2
C. pneumoniae and *C. psittaci* IgG	2	2
C. pneumoniae, *C. trachomatis*, and *C. psittaci* IgG	1	1
C. trachomatis and *C. psittaci* IgG[c]	1	1

[a] Determined by all MIFs and the Immunocomb E and Labsystems EIAs.
[b] The number of these samples also positive by the rELISA (Medac).
[c] The sample was false positive for *C. pneumoniae* three times.

TABLE 3. Concordance of individual tests detecting anti-*C. pneumoniae* antibodies

Assay	% Concordance										
	ELISAs and EIAs						MIF				
	Elegance	Immunocomb	Vircell	SeroCP	Quant	Labsystems	Vircell	MRL	SeroFIA	Labsystems	rELISA
Elegance EIA		86	84	79	80	81	83	79	78	78	65
Immunocomb EIA			88	85	89	85	86	85	86	84	63
Vircell ELISA				88	90	88	84	93	89	93	61
SeroCP ELISA					94	86	84	96	93	96	61
Quant ELISA						91	84	96	98	95	65
Labsystems EIA							81	90	89	89	63
Vircell MIF								84	80	83	68
MRL MIF									96	99	61
SeroFIA MIF										98	63
Labsystems MIF											60
rELISA											

ent MIFs and the Quant ELISA are shown in Table 4 (Spearman correlation). The correlation was best for the titers of the MIFs from MRL and Labsystems ($r = 0.91$), followed by the correlation of the titers determined by the MRL and Labsystems MIFs and the titers determined by the SeroFIA MIF and the Quant ELISA ($r = 0.81$ to 0.89). The greatest divergence was found for the titers determined by the Vircell MIF ($r = 0.65$ to 0.79).

Having shown that the two MIFs from Labsystems and MRL led to comparable IgG titers for the 80 serum samples, the Labsystems MIF was chosen for use for further evaluation of the results of the seven different ELISAs and EIAs. Table 5 shows that all 14 (100%) samples which had high IgG titers (1:512) by the Labsystems MIF were positive by the seven ELISAs and EIAs. A total of 92 to 97% of the samples with medium IgG titers (1:128) were also found to be positive by the Labsystems EIA and the SeroCP, Quant, and Vircell ELISAs, whereas only 81 and 64% of the samples were found to be positive by the Immunocomb and Elegance EIAs, respectively. Among the samples with low IgG titers (1:16 or 1:32), 100% were positive by the SeroCP ELISA, 75% were positive by the Quant ELISA, and 50% were positive by the Labsystems EIA and the Vircell ELISA. The results are worse for the Elegance and Immunocomb EIAs, which found only 38% of the samples with low titers to be positive. The rELISA found 79% of the samples with high IgG titers, 47% of the samples with medium titers, and 25% of the low titer samples to be positive.

Sensitivity and specificity. On the basis of our internal gold standard and the results of immunoblotting analysis, we determined the specificities and sensitivities of the different tests (Table 6). The specificities of all ELISAs and EIAs and of all MIFs for the detection of anti-*C. pneumoniae*-specific IgG in the sera of healthy donors was between 95 and 100% for all assays except the rELISA, for which the specificity was 88%. The sensitivities were 100% for the MIFs from MRL and Labsystems, 96% for the SeroCP ELISA and the SeroFIA MIF, and 92% for the Quant ELISA. The Labsystems EIA and the Vircell ELISA achieved sensitivities of 74 and 76%, respectively. The sensitivities of the Elegance EIA, the Immunocomb EIA, the rELISA, and the Vircell MIF were ≤66%.

DISCUSSION

This study was designed to evaluate the validities of 11 different commercially available tests for the detection of anti-*C. pneumoniae*-specific IgG antibodies in serum from 80 healthy volunteers. It is not a diagnostic study but is an assay comparison. Four different versions of the MIF, which is the current gold standard for the serodiagnosis of *Chlamydia* infections, were included in the study, as were seven ELISAs and EIAs, which differed in particular in the antigen preparations used. Since the results of the different MIFs were discrepant, we defined an internal standard, in which we considered positivity by at least three of the four MIFs to be a true-positive result. Six samples that had false-positive results by some assays were further investigated by immunoblotting, which classified all these samples as negative. For these six samples, the results of the rELISA were not considered, since it also detects antibodies against *C. trachomatis* or *C. psittaci*. Taken together, we found that 57 (71%) of the 80 samples were positive and that 23 (29%) of the 80 samples were negative for *C. pneumoniae*-specific IgG antibodies. This is in line with the known high prevalence of anti-*C. pneumoniae* antibodies in adults (18). Forty (50%) of the 80 serum samples were positive for IgA antibodies. All these samples also had IgG antibodies, and in total, 70% of the IgG-positive samples were IgA positive as well. Schumacher et al. have also been reported a lower prevalence of IgA than of IgG in healthy controls (44). Therefore, detection of IgG seems to be the more sensitive method for determination of the seroprevalence of anti-*C. pneumoniae* antibodies in a healthy population.

TABLE 4. Spearman correlation of IgG titer determinations by different MIFs

Assay	Correlation				
	Labsystems MIF	MRL MIF	SeroFIA MIF	Quant ELISA	Vircell MIF
Labsystems MIF	1	0.91	0.82	0.85	0.76
MRL MIF		1	0.81	0.89	0.79
SeroFIA MIF			1	0.87	0.65
Quant ELISA				1	0.76
Vircell MIF					1

TABLE 5. Evaluation of the results of the different ELISAs and EIAs in relation to the IgG titers determined by MIF

Assay	No. of samples positive by each test	No. of positive samples negative by MIF	% of samples with IgG titers of:		
			1:32 (n = 8)	1:128 (n = 36)	1:512 (n = 14)
Labsystems MIF[a]	58	Not applicable	100	100	100
Elegance EIA	40	0	38	64	100
Immunocomb EIA	47	1	38	81	100
Vircell ELISA	51	0	50	92	100
Quant ELISA	56	1	75	97	100
SeroCP ELISA	57	1	100	94	100
Labsystems EIA	55	3	50	94	100
rELISA	34	4	25	47	79

[a] The MIF used for classification of samples.

The levels of agreement between the results of the Labsystems, MRL, and SeroFIA MIFs and those of the Quant, SeroCP, Labsystems, and Vircell ELISAs or EIAs for the detection of IgG in the 80 samples were high (89 to 99%). However, the results of the Immunocomb and Elegance EIAs as well as those of the Vircell MIF showed lower correlations (78 to 86%); and among the 57 IgG-positive samples, only 34 (61%) were found to be positive by all species-specific tests. The IgG titers determined by the Labsystems, MRL, and SeroFIA MIFs correlated well (r_{ho} = 0.8 to 0.9), and therefore, the results of one MIF (Labsystems) were chosen and the positive and negative results of all ELISAs and EIAs were evaluated on the basis of the IgG titers determined by that MIF (Table 5). This analysis demonstrates that samples with high IgG titers were detected without problems by all tests, while lower IgG titers led to at least one and often more false-negative results by all tests. This might be explained by the different cutoff levels of the tests, which arbitrarily determine the sensitivity. Since anti-*C. pneumoniae*-specific IgG titers are often medium or low in people whose infections have passed, tests like the Elegance EIA and the Immunocomb EIA do not appear to be useful for investigation of the seroprevalence of *C. pneumoniae* in healthy populations.

By the rELISA, only 34 (43%) samples were found to be positive, and 4 of these samples were not judged to be positive on the basis of the internal standard. The chlamydial LPS, on which the rELISA is based, is chemically related to the LPSs of enterobacterial core-deficient rough mutants, and the respective antisera are serologically cross-reactive (8). These rough mutants rarely occur in nature, and the rELISA is therefore considered genus specific (9). Nevertheless, cross-reactivity with *M. pneumoniae* and parvovirus has been reported (39). Of the four samples with false-positive results by the rELISA, none was positive for *M. pneumoniae* and two were positive for parvovirus; one of the latter two samples was also positive for *C. trachomatis* and *C. psittaci* (Tables 1 and 2). Notably, the latter sample was also false positive for *C. pneumoniae* three times by other ELISAs. Until now, no sample with only anti-LPS antibodies and no concurrent antiprotein antibodies has been described. Therefore, the false-positive rELISA results for three of the four samples are due to unspecific reactions. Of the four samples with a positive reaction for *C. psittaci*, all had anti-LPS antibodies, suggesting genus-specific cross-reactivity rather than an infection with *C. psittaci*.

Anti-LPS antibodies occur early during infection with *Chlamydia*, but their levels also decline faster than those of anti-protein antibodies (39, 47). We found 30 (54%) of the true-positive samples to be positive by rELISA. In addition, it has been found that rELISA detects a lower prevalence of *Chlamydia*-specific IgG antibodies than MIF does in patients with chronic obstructive pulmonary disease and coronary heart disease (44, 46). Most of the samples which were found to be positive by the rELISA had high antibody titers by MIF, which might indicate a very recent infection. Whether the rELISA represents a suitable method for the detection of *C. pneumoniae* infection is controversial: some studies show that rELISA is a sensitive method for the detection of acute infections (39, 46, 47). In contrast, a poor correlation between the detection of *C. pneumoniae* infection by culture of the organism and the detection of the organism by serology by rELISA in children has been reported (28). In line with our findings, the rELISA does not seem to be the appropriate test for determination of the seroprevalence of *Chlamydia* in a population (46).

The specificities of the different tests for the detection *of C. pneumoniae* IgG determined in this study were high; i.e., there were only a few false-positive results. The sensitivities of the tests varied a lot, probably depending on the kind and the quality of the antigen preparation used. The sensitivities of all MIFs except the Vircell MIF were high (96 to 100%). The number of false-negative results obtained by the Vircell MIF also shows that the qualities of the commercially available MIFs are different. The Vircell MIF is the only MIF which does not use EBs from *C. pneumoniae* isolate TW-183 as the

TABLE 6. Specificities and sensitivities of the different tests for anti-*C. pneumoniae* IgG determination

Assay	Specificity (%)	Sensitivity (%)
ELISA or EIA		
Elegance	100	58
Immunocomb	98	65
Vircell	98	76
SeroCP	98	96
Quant	98	92
Labsystems	95	74
rELISA	88	42
MIF		
Vircell	100	66
MRL	100	100
SeroFIA	97	96
Labsystems	98	100

antigen; it uses EBs from isolate 2023 as the antigen, which could be an explanation for the false-negative results. One study that tested the reactions of eight *C. pneumoniae* isolates, including isolates TW-183 and 2023, to *C. pneumoniae*-specific monoclonal antibodies showed that all antigens react similarly (50). Therefore, it is more likely that the different MIF results are due to the purity and the concentration of the antigen preparation instead of to isolate specificity. The recent work of Peeling et al. (37), in which they compared the performances of 14 laboratories for determination of the IgM and IgG titers in 22 samples by MIF, showed that the agreement of the results is good for samples with high IgG titers but poor for samples with low IgG and IgM titers or samples that are negative for IgG and IgM. In that study, the best results (100% agreement) compared to the results obtained with the reference standard were obtained with the MIF from MRL, followed by the MIF from Labsystems and three MIFs prepared in-house (90% agreement). However, for seven laboratories, the correlation of the MIF results with those of the reference standard were only between 50 and 80%, demonstrating the lack of standardization of the performance of MIFs (37). Another study reported on the failure of MIF to detect anti-*C. pneumoniae* antibodies in children with acute episodes of wheezing: among the culture-positive children, 58% had no detectable antibodies to *C. pneumoniae* when they were monitored for up to 5 months (15). On the basis of the 96 to 100% sensitivities of the MIFs determined in our study, some commercial MIFs seem to be appropriate for use for the serological diagnosis of past *C. pneumoniae* infections in adults if they are performed by experienced individuals. However, the protocol is time-intensive and problematic for use for routine diagnosis. Furthermore, the chlamydiae which are used for EB preparation are grown under cell culture conditions and might express proteins different from those expressed during human infection. Therefore, even a test sensitivity of 100% does not exclude the possibility that anti-*C. pneumoniae* antibodies may be present in human serum.

Among the partially automated and less subjective ELISAs and EIAs, the SeroCP and Quant assays showed the best sensitivities (96 and 92%, respectively), followed by the Vircell ELISA and the Labsystems EIA, which each had a sensitivity of about 75%. Considering that the prevalence of *C. pneumoniae* antibodies is ≥70% in people older than 50 years, a test sensitivity of only 90% already leads to many false-negative results. In addition, antigens prepared from *C. pneumoniae* do not necessarily comprise the complete set of antigenic surface proteins, or the prepared antigens might not be in the natural conformation. In both cases, the tests fail to detect the respective subsets of antibodies which are directed against *C. pneumoniae*, therefore leading to false-negative results. A recent study compared five serological tests for the diagnosis of acute infections caused by *C. pneumoniae*. The MIF and EIA from Labsystems, as well as the SeroCP ELISA and the rELISA, were included in the evaluation (38). Compared to the results of MIF, the specificities and sensitivities of the Labsystems EIA, the SeroCP ELISA, and the rELISA ranged from 89 to 95%. Compared to the results of our study, the previous results indicate that the sensitivities of ELISAs and EIAs are higher than that of the MIF if acute-phase sera are used, which is probably due to the higher antibody titers in these sera.

In our study, 18% of the samples showed high IgG titers (1:512, as determined by the MIF from Labsystems), but the individuals who provided those samples had no signs of acute infection. Several other studies have also shown that high anti-*C. pneumoniae*-specific IgG titers are common in healthy individuals (24, 33, 34, 38). For the detection of an increase in the IgG titer, analysis of paired serum specimens in the same test run is necessary. Usually, anti-*C. pneumoniae*-specific IgG titers tend to decline after 3 years (36), and the long-lasting presence of IgG could be a marker of a persistent infection because the continuous exposure of the host's immune system to *C. pneumoniae* might result in repetitive stimulation of anti-LPS IgG, as well as high IgG titers. The ongoing discussion about the impact of persistent *C. pneumoniae* infection on chronic and degenerative diseases (especially atherosclerosis) prompts both epidemiological and therapeutic studies with asymptomatic healthy individuals. As long as no measure of persistence is available, these studies must be based on serodiagnosis. The large number of samples required for epidemiological studies favors the use of automated ELISAs, whose results must be validated against those of MIF. Therapeutic studies require the use of both suitable inclusion criteria, i.e., adequate test sensitivities and specificities, and monitoring parameters, i.e., titer quantification. The results of this study indicate that serological assays vary greatly in terms of their sensitivities and specificities, and our study might give guidance in this regard and illustrate the impact of the choice of test.

ACKNOWLEDGMENTS

We are grateful for the excellent technical assistance of A. Haas and I. Seuffert. We thank S. von Aulock for help with the manuscript. We are indebted to U. Brunner and his team for the introduction to the performance of MIF and continuous advice.

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