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Microenzyme-Linked Immunosorbent Assay for Detection of Immunoglobulin G and Immunoglobulin M Antibodies to *Legionella pneumophila*

E. M. ELDER,[1]† A. BROWN,[1,2]* J. S. REMINGTON,[3] J. SHONNARD,[1] AND Y. NAOT[3]

Laboratory Service, Veterans Administration Medical Center, Pittsburgh, Pennsylvania 15240[1]; Research Service, William Jennings Bryan Dorn Veterans' Hospital, Columbia, South Carolina 29201[2]; and Palo Alto Research Foundation and Stanford University Medical Center, Palo Alto, California 94301[3]*

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The microenzyme-linked immunosorbent assay (ELISA) for the detection of immunoglobulin M and G (IgM, IgG) antibodies to *Legionella pneumophila* serogroup 1 antigens was evaluated. IgM antibodies were measured by both double-sandwich and single-sandwich techniques. These assays were compared with the previously standardized indirect immunofluorescence test in four groups of subjects: (i) pneumonia patients with culture-proven Legionnaires disease with serogroup 1 isolates, (ii) pneumonia patients with serogroup 1 organisms detected by direct immunofluorescence testing of respiratory secretions but without culture confirmation, (iii) pneumonia patients with negative culture and direct immunofluorescence tests, and (iv) healthy hospital employees. In addition, the sensitivity and specificity of the IgG ELISA were evaluated with larger groups of controls and Legionnaires disease patients. The ELISA was more sensitive than the indirect immunofluorescence test. However, it detected antibody rises in pneumonia patients without culture or direct immunofluorescence evidence of *L. pneumophila* serogroup 1 infection, thereby suggesting that the specificity of the ELISA was slightly lower than that of the indirect immunofluorescence test. The double-sandwich ELISA was a sensitive method for detecting IgM antibodies and, as previously reported, appeared to be free from interference by rheumatoid factor. IgM anti-*Legionella* antibodies detected by the ELISA appeared earlier and were less persistent than IgG antibodies. In addition, the IgM ELISA was useful in detecting antibodies in necropsy serum samples obtained from patients dying acutely of Legionnaires disease. The data presented show that the ELISA is a reliable method for the detection of specific anti-*Legionella* antibodies.

The indirect immunofluorescent-antibody (IFA) test is the standard method for detecting antibody to the *Legionellaceae* (9, 18, 20, 22, 24, 30, 33, 34, 36). An enzyme-linked immunosorbent assay (ELISA), one of several other tests for detecting these antibodies, has also been described (12, 13) but has not received widespread use. When few serum samples are to be tested, the ELISA is too complex and time consuming, but with many samples it becomes cost effective and time efficient since samples can be tested in batches and the results can be quickly screened visually. In addition, the ELISA can be automated, the endpoint can be determined spectrophotometrically, and the test is more sensitive than the IFA test (38). The advantage of the IFA test is its relative simplicity; its disadvantages include its limited sensitivity compared with the ELISA, its subjective endpoint standardization, the cumbersomeness of determining endpoints by fluorescent microscopy when many serum samples are tested, and the expense of a fluorescent microscope.

The class of specific immunoglobulin produced in response to *Legionella* antigens has been studied by IFA testing (19, 24, 34). In these studies, slightly more patients with Legionnaires disease (LD) were seropositive with the immunoglobulin M (IgM) IFA test than with the IgG IFA test. The kinetic response of IgM either was similar to that of IgG or IgM tended to appear slightly earlier in the illness (24). From these studies it was recommended that a polyvalent anti-immunoglobulin reagent be used in the IFA test for maximum efficiency (34).

In this paper, our experience with the ELISA to detect IgM and IgG antibody to *Legionella pneumophila* is described. Because rheumatoid factors (RF) are reported to interfere with the

† Present address: Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

IgM assay, a double-sandwich (DS) technique was adopted which has been free of RF interference in assays for antibody to *Toxoplasma gondii* and hepatitis A virus (6, 25–27). Four groups of subjects were studied with single-sandwich (SS) and DS ELISAs and with the IFA test: (i) patients with pneumonia whose respiratory cultures were positive for *L. pneumophila*; (ii) pneumonia patients with negative cultures but with positive secretions by the direct immunofluorescent-antibody (DFA) test; (iii) patients with pneumonia but whose culture and DFA tests were negative for *L. pneumophila*; and (iv) healthy volunteers among hospital employees. A larger group of serum samples from controls and LD patients was studied by the IgG ELISA alone. Our results demonstrate that the ELISA is a reliable method of anti-*Legionella* antibody detection and suggest that the use of separate ELISAs for IgM and IgG antibody will detect the maximum number of patients with *L. pneumophila* pneumonia.

MATERIALS AND METHODS

Sample selection. Since February 1979, the diagnosis of LD was made for approximately 60 patients with pneumonia at the Pittsburgh Veterans Administration Medical Center. The criteria for this diagnosis were clinical and radiological evidence of pneumonia combined with a culture positive for *L. pneumophila*, a respiratory tract or pleural fluid specimen positive for *L. pneumophila* serogroup 1 by DFA testing, or a fourfold rise in antibody titer to ≥1:128 by the IFA test. Paired acute and convalescent serum specimens were available from 37 of these patients (92 sera).

In addition, the ELISA was used to test 2,664 serum samples obtained from unselected patients on hospital admission, 129 samples from healthy hospital employees, and acute and convalescent serum samples obtained from 47 patients with episodes of pneumonia but without DFA, IFA, or culture evidence of LD.

Selected serum samples were screened for the presence of RF by latex agglutination (RA test; Hyland Diagnostics, Costa Mesa, Calif.). All assays were performed in a blinded fashion, without knowledge of the identity of the patients or the results of earlier testing.

Diagnostic tests for LD. Respiratory tract cultures (specimens of transtracheal aspirate, sputum, and bronchial washing), pleural fluids, and lung tissue obtained by biopsy or necropsy were cultured on charcoal yeast extract buffered with *N*-(2-acetamido)-2-aminoethanesulfonic acid (14, 28) with and without dyes (bromcresol purple, bromthymol blue) (32) or antibiotics (7) in addition to other standard media. Typical colonies with appropriate growth characteristics (15) were confirmed by DFA staining or slide agglutination (5, 35) and in some instances by fatty acid analysis (23). Antiserum for the slide agglutination test was prepared as previously described (16).

IFA tests were performed at the Veterans Administration Reference Laboratory for Selected Serologic Studies, Lexington, Ky., with reagents from and by the procedures recommended by the Centers for Disease Control, Atlanta, Ga. (33, 34, 36). Selected samples were sent to the Centers for Disease Control for confirmation. The titer recorded is the highest serum dilution giving at least 1+ staining of the antigen. At least a fourfold rise in titer to a convalescent titer of 1:128 or greater was considered a seroconversion.

The DFA test was performed with reagents obtained from and by procedures developed at the Centers for Disease Control (4, 5). Positive and negative controls were included in every run. To be considered positive, a specimen had to contain strongly fluorescing bacilli with typical morphology. For sputum, the presence of five organisms was considered positive as suggested in the Centers for Disease Control protocol accompanying the reagents (revised May 1982), but only 4 of the 21 samples considered to be positive by DFA testing contained fewer than 25 organisms, and all samples positive by the DFA test from patients without culture or serological confirmation of infection contained at least 25 organisms per oil immersion field. All organisms recovered from samples positive by the DFA test were tested with DFA for cross-reactivity if *L. pneumophila* was not concomitantly cultured. No cross-reacting organisms were recovered from our population.

Antigens. Bacterial antigens were prepared from *L. pneumophila* serogroup 1 (Knoxville strain) cultured aerobically on charcoal yeast extract agar for 3 days at 37°C. The bacteria were collected by adding 3 ml of sterile phosphate-buffered saline (PBS), pH 7.2, to each plate and then gently scraping the growth off the agar with a pipette. To prepare soluble antigens, harvested organisms were heated to 100°C for 1 h in flowing steam. These heat-killed bacterial suspensions were centrifuged at 2,000 × *g* for 15 min, and the bacterial pellets, after two washings with PBS, were suspended in PBS. These suspensions (ca. 0.5 ml of packed cells per 10 ml of PBS) were stored at 4°C for 10 days to allow the release of soluble antigens (12, 13). The bacterial cells were then removed by centrifugation, and the supernatant was stored at 4°C as the soluble antigen stock solution. Bacterial sonicates were prepared from 0.3 g (wet weight) of washed bacteria suspended in 20 ml of distilled water. These suspensions were then sonicated with 10 bursts of 30 s each in a Branson sonifier at full power at 4°C in a biological safety cabinet. After centrifugation at 2,000 × *g* for 15 min, the supernatants were used as the bacterial sonicate antigen in the studies. Protein determinations were performed with the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, Calif.) or by the method of Lowry et al. (21) with bovine serum albumin (BSA) as a standard.

Optimal dilutions of antigens used in the SS and DS ELISAs were determined by block titration. The dilutions giving the best resolution between positive and negative control serum samples were selected.

Antibodies to human IgM. For the DS ELISA for IgM, the IgG fraction of rabbit anti-human IgM (μ chain-specific) antibodies were obtained from Cappel Laboratories, Inc. (Cochranville, Pa.). The specificity of this antibody preparation for human IgM and the optimum dilution to be used in the DS ELISA for IgM were determined as recently described (27).

Enzyme-conjugated F(ab')$_2$ fragment of IgG antibodies to *L. pneumophila*. Rabbits were immunized with *L. pneumophila* serogroup 1 (Knoxville 1 strain) as previ-

TABLE 1. Distribution of IgG ELISA titers for *L. pneumophila* serogroup 1

Titer	Patients on admission[a]		Healthy hospital employees[b]		Pneumonia patients not suffering from LD[c]	
	No.	%	No.	%	No.	%
≤40	2,187	82.1	81	62.8	27	79.4
80	382	14.3	39	30.2	6	17.6
160	80	3.0	7	5.4	1	2.9
320	7	0.3	2	1.6		
640	5	0.2				
1,280	2	0.1				
2,560	1	0.04				

[a] Serum was obtained from patients at the time of admission to the hospital between January 1980 and June 1981.

[b] Serum was obtained from 129 healthy members of the hospital staff who had IFA titers ≤1:64.

[c] Convalescent-phase serum was obtained from 34 pneumonia patients without evidence of LD from culture or the DFA test and with negative (<1:64) IFA titers. Etiological diagnoses include *H. influenzae* (8 patients), *S. pneumoniae* (6), *T. micdadei* (5), *P. aeruginosa* (4), unidentified virus (4), *K. pneumoniae* (2), *E. coli* (2), *S. aureus* (1), *Mycoplasma* sp. (1), and *Aspergillus* sp. (1). Serum samples from an additional 13 patients with documented aspiration penumonia were tested and had titers of ≤1:80.

ously described (16). The IgG fraction was isolated by chromatography of the rabbit antiserum on Sepharose-protein A. The isolated IgG was then digested with pepsin (Worthington Diagnostics, Freehold, N.J.), and the $F(ab')_2$ fragment was separated from the Fc fragment and undigested IgG on a Sepharose-protein A column (1). These $F(ab')_2$ fragments were conjugated with alkaline phosphatase type VII from calf intestine (Sigma Chemical Co., St. Louis, Mo.) by the one-step method with glutaraldehyde as previously described (11, 38). The optimal dilutions of enzyme-conjugated antibodies were determined by block titration (12, 13).

Enzyme-conjugated antibodies to human immunoglobulin (unfractionated), IgG, and IgM. Horseradish peroxidase-conjugated rabbit anti-human immunoglobulin, IgG, and IgM reagents were obtained from DAKO-Immunochemicals, Ltd., Copenhagen, Denmark. The optimal dilution of conjugate was also determined by block titration.

Detection of antibody by the SS ELISA. The SS ELISA for detecting antibody to *L. pneumophila* was performed as previously described by Farshy and Feeley (12) and Farshy et al. (13) with some modification. Wells of polystyrene microtitration plates (U bottom) (Dynatech Laboratories, Inc., Alexandria, Va.) were coated by incubation for 18 h at 37°C with 100 μl of the soluble antigen (4.25 μg of protein per ml) or bacterial sonicate (6.25 μg of protein per ml) in PBS, pH 7.2, and then were stored at 4°C until use. Before use, the plates were washed with PBS containing 0.05% Tween 20 (PBS-T). A 100-μl sample of an initial 1:80 dilution of serum made in PBS-T containing 0.05% BSA was added to the first row of antigen-containing wells, and serial twofold dilutions were made. After incubation for 1 h at 37°C, the plates were washed, and 100 μl of a working dilution of horseradish peroxidase-conjugated rabbit anti-human unfractionated immunoglobulin (IgG + IgM + IgA), IgG, or IgM in PBS-T–BSA was added. After a second incubation, the plates were washed, and 100 μl of substrate solution containing 0.01% *o*-phenylenediamine and 0.003% hydrogen peroxide was added. The plates were reincubated at 37°C for 50 min. The reaction was then stopped by adding 8 N H_2SO_4. Serum titer endpoints were read visually (for color) by comparison with the first dilution of the negative control.

IgM detection by DS ELISA. The DS ELISA for IgM was performed essentially as previously described for the detection of IgM antibodies to hepatitis A antigens (6) and for the detection of IgM antibodies in acute and congenital toxoplasmosis (25, 26, 27). Briefly, the wells of flexible polyvinyl microtiter plates with U-shaped wells (Dynatech Laboratories, Inc.) were coated by overnight incubation at 4°C with 100 μl of rabbit IgG antibodies to human IgM diluted in 0.1 M carbonate buffer, pH 9.8. The plates were washed in PBS-T and then were postcoated with 1% BSA in PBS-T at 37°C for 1 h. After additional washing, 150 μl of PBS was added to each well, and serial fourfold dilutions of the serum samples were made in the wells, beginning with a dilution of 1:4. Plates were incubated for 1 h at 37°C and then were washed with PBS-T. A 100-μl sample of *L. pneumophila* soluble antigens (6.25 μg of protein per ml) or sonicated antigens (12.5 μg of protein per ml) in PBS was added to each well, and the plates were again incubated for 1 h at 37°C. After washing, 100 μl of the alkaline phosphatase-conjugated $F(ab')_2$ fragment prepared from the IgG of rabbits immunized with serogroup 1 *L. pneumophila* diluted in PBS-T containing 4% BSA was added to each well. After incubation at 37°C for 1 h and washing, 100 μl of 1% *p*-nitrophenylphosphate disodium (Sigma Chemical Co.) in 0.05 M carbonate buffer containing 0.001 M $MgCl_2$ was added to each well. The enzyme-substrate reaction was allowed to proceed at 37°C for 1 h, and the absorbance of the enzymatic product (at 405 nm) was determined directly in the wells with an automated micro-ELISA reader (Dynatech Laboratories, Inc.). Positive and negative control serum samples were included in each plate. The reported titer for the DS ELISA for IgM is the highest serum dilution that resulted in an absorbance at least twice that of the average of eight wells containing the negative control serum.

Control serum samples for DS and SS ELISAs. A serum sample obtained from a previously diagnosed

TABLE 2. Comparison of ELISA and IFA tests for the detection of antibodies to *L. pneumophila* serogroup 1 in patients with LD confirmed by culture or by the DFA test

Patient group	No. of patients	No. of seroconversions[a]	
		IFA test	ELISA[b]
Positive culture only	13	6 (46.2)	12 (92.3)
Positive culture and DFA test	6	5 (83.3)	5 (83.3)
Positive DFA test only	15	2 (13.3)	7 (46.7)
All groups	34	13 (38.2)	24 (70.6)

[a] Numbers in parentheses show percentages of whole group.
[b] Determined with immunoglobulin conjugate against human IgG, IgM, and IgA.

patient 35 days after the clinical onset of LD served as positive control in the ELISAs. This serum sample was positive in the IFA test (1:1,024) and IgG ELISA (1:320). The diagnosis of LD was made in this patient by finding both positive respiratory secretions (by DFA testing) and a positive culture for *L. pneumophila* serogroup 1. The negative control was a serum sample obtained from an uninfected individual; this sample was negative (<1:64) in the IFA test and negative (<1:20) in the IgG ELISA.

RESULTS

A total of 2,932 serum samples were tested for antibody to *L. pneumophila* serogroup 1 antigens by the IFA test and the IgG ELISA. These included 2,664 serum samples obtained from patients on admission to the Pittsburgh Veterans Administration Medical Center over an 18-month period, 129 samples from healthy hospital employees, 47 samples of convalescent-phase serum from patients with non-LD pneumonia, and 92 acute and convalescent serum samples from 37 patients with LD pneumonia documented by culture isolation of *L. pneumophila*, by DFA-positive clinical samples, or by demonstration of a fourfold antibody titer rise by the IFA test. All the serum samples from the patients with LD were also tested for IgM by the SS ELISA. In addition, serum samples obtained from 57 of these individuals representing four groups were tested for antibody to *L. pneumophila* serogroup 1 antigens by seven different procedures, including the SS ELISA for IgG or total immunoglobulin (IgG, IgM, and IgA) with a soluble antigen preparation, the SS and DS ELISAs for IgM with both soluble and sonicated bacterial antigens, and the standard IFA procedure for total immunoglobulin.

To determine the specificity of the ELISA, 34 patients with pneumonia and with negative culture or DFA tests for *L. pneumophila* were studied. Presumed or proven etiologies included *Haemophilus influenzae* (8 patients), *Streptococcus pneumoniae* (6), *Tatlockia micdadei* (5), *Pseudomonas aeruginosa* (4), unidentified virus (4), *Escherichia coli* (2), *Klebsiella pneumoniae* (2), *Staphylococcus aureus* (1), *Mycoplasma* sp. (1), and *Aspergillus* sp. (1). In 33 of these patients, no anti-*Legionella* antibody was detected by any of the serological tests used. One patient with a diagnosis of pneumococcal pneumonia (based on sputum culture) and a negative RF had IgM antibodies to *L. pneumophila* serogroup 1 detectable by both the SS and the DS ELISAs 14 days after the onset of pneumonia. Two other patients in this group had significant IgM and IgG antibody rises to *L. pneumophila* serogroup 1 antigens detected by all ELISAs but not by the IFA test. In addition, no antibody was detected in a group of 13 patients with documented aspiration pneumonia, and serum samples obtained from 120 of 129 healthy hospital

TABLE 3. Comparison of results with anti-human IgM-specific, IgG-specific, and IgG + IgA + IgM-specific conjugates in ELISAs of convalescent serum samples from 37 cases of LD[a]

Basis of diagnosis	No. positive by ELISA/no. tested	No. positive with conjugate specific for[b]:			
		Immunoglobulin	IgM	IgG	IgM or IgG
Positive culture only[c]	12/13	12	12	10	12
Positive culture and DFA test[c]	5/6	5	5	5	5
Positive DFA test only[c]	9/15	7	4	8	9
Positive IFA test only	3/3	3	3	3	3

[a] Diagnosis based on positive culture of DFA test for *L. pneumophila* serogroup 1 or on serogroup 1 seroconversion demonstrated by IFA test.
[b] Percentages tested positive with different conjugates: Immunoglobulin, 73.0; IgM, 64.9; IgG, 70.3; IgM or IgG, 78.4
[c] These may also have seroconverted by IFA test (see Table 2).

TABLE 4. Representative antibody titers in 8 of the 13 LD cases proven by culture

Patient	Day after onset	ELISA: DS for IgM—sonicate[a]	ELISA: SS for IgM: Soluble[a]	ELISA: SS for IgM: Sonicate[a]	ELISA: IgG	ELISA: Immunoglobulin	IFA test	RF	*T. micdadei*[b]
1	9	<1:4	1:320	1:320	1:80	1:320	1:64	–	
	19	1:4,096	1:2,560	1:1,280	1:2,560	1:1,280	1:128	–	
	40	1:1,020	1:1,280	1:320	1:1,280	1:640	1:256	–	
	65	<1:4	1:640	1:320	1:640	1:320	1:256	–	
2	4	<1:4	<1:80	<1:80	<1:80	<1:80	<1:64	–	
	6	<1:4	1:80	1:160	1:80	<1:80	<1:64	–	
	11	1:256	1:320	1:320	1:320	1:320	1:128	–	
	20	1:16,384	1:10,240	1:5,120	1:10,240	1:10,240	1:256	–	
	40	1:16,384	1:5,120	1:5,120	1:1,280	1:5,120	1:256	–	
	63	1:4,096	<1:1,280	1:2,560	1:1,280	1:1,280	1:256	–	
3	0	<1:4	<1:80	1:80	<1:80	<1:80	<1:64	–	
	8	1:4,096	1:1,280	1:5,120	1:160	1:320	1:128	–	
	13	1:4,096	1:5,120	1:5,120	1:160	1:640	1:128	–	
	20	1:16,384	1:10,240	1:5,120	1:160	1:2,560	1:256	–	
	35	1:16,384	≥1:40,960	1:10,240	1:160	1:10,240	1:160	–	
	38	≥1:65,536	≥1:40,960	1:20,480	1:640	1:10,240	1:256	–	
4	11	<1:4	1:80	<1:80	1:80	1:80	<1:64	–	
	15	<1:4	1:80	<1:80	1:80	1:80	<1:64	–	
	39	1:4,096	1:320	1:320	1:80	1:320	<1:64	–	
5	13	<1:4	1:80	1:160	<1:80	<1:80	<1:64	–	
	35	1:4,096	1:80	1:160	1:80	1:80	1:64	–	
	67	<1:4	<1:80	1:80	1:80	<1:80	<1:64	–	
6	3	<1:4	1:320	1:160	1:80	1:160	<1:64	+	
	10	<1:4	1:320	1:160	<1:80	1:80	<1:64	+	
	25	<1:4	1:1,280	1:2,560	1:160	1:320	<1:64	–	
	40	1:4,096	1:5,120	1:1,280	1:1,280	1:1,280	1:182	NT[c]	
7	0	<1:4	<1:80	1:80	<1:80	<1:80	<1:64	–	
	6	<1:4	<1:80	1:80	1:80	<1:80	<1:64	+	
	15	<1:4	1:80	1:160	1:160	1:80	<1:64	+	
	32	<1:4	1:320	1:320	1:320	1:640	<1:64	+	
	41	<1:4	1:320	1:320	1:640	1:640	<1:64	NT	
8	0	<1:4	<1:80	1:80	<1:80	<1:80	<1:64		<1:80
	10	<1:4	<1:80	1:80	<1:80	<1:80	<1:64		<1:80
	16	<1:4	<1:80	1:80	<1:80	<1:80	<1:64		640
	66	<1:4	<1:80	1:80	<1:80	<1:80	<1:64		640
	115	<1:4	<1:80	1:80	<1:80	<1:80	<1:64		640

[a] ELISAs with sonicate or saline-soluble antigens as shown.
[b] Titers for IgG antibodies to saline-extracted *T. micdadei* antigens as detected by SS ELISA.
[c] NT, Not tested.

employees lacked antibody to *L. pneumophila*. Table 1 shows the distribution of titers in these individuals and in a large group of patients on admission. Approximately 97% of all titers in these groups were 1:80 or less.

Table 2 shows the number of seroconversions detected by IFA or by ELISA for unfractionated immunoglobulins in a group of 34 pneumonia patients with evidence of LD by culture or by the DFA test. Seroconversion was detected in 24 (70.6%) by the ELISA and in 13 (38.2%) by the IFA test. In the group of 19 patients from whom *L. pneumophila* was isolated, 17 (89.5%) had significant titer rises detected by ELISA, but only 11 (57.9%) showed significant rises by IFA. In the group of 15 patients with evidence of LD by the DFA test only but not by culture, the seroconversion rate was much lower.

The sensitivity and immunoglobulin specificity of ELISA antibody detection in convalescent-phase serum samples from 37 LD patients are shown in Table 3. These patients include the

TABLE 5. Presence of IgM and IgG antibodies to *L. pneumophila* serogroup 1 after onset of LD pneumonia

Week of illness	Serum samples tested[a]	Positive samples by ELISA[b]	
		IgM	IgG
1	13	7 (53.8)	1 (7.7)
2	11	10 (90.9)	6 (54.5)
3–8	27	27 (100)	27 (100)
9–16	15	9 (60.0)	14 (93.3)
17–24	7	3 (42.9)	6 (85.7)
25–36	6	1 (16.7)	4 (66.7)

[a] A total of 79 serial serum samples from 21 patients with positive immunoglobulin ELISAs were tested.
[b] Positive serum samples were those with titers ≥1:160. Numbers in parentheses show percentages of the total tested.

34 shown in Table 2 in addition to 3 in whom the diagnosis was made by IFA seroconversion after an episode of pneumonia, without a positive culture or DFA test. All three of the patients who had antibody rises detected by the IFA test also had antibody detected by the unfractionated immunoglobulin, IgM, or IgG ELISA. For all groups, antibody was detected with approximately equal frequency by each ELISA test. The detection rates of antibody and seroconversion were slightly greater if both the IgM and the IgG ELISAs were performed. For two LD patients with positive cultures, antibody to *L. pneumophila* serogroup 1 could not be detected in several convalescent-phase specimens by any of the serological tests. One of these patients had a convalescent rise in antibody levels to *T. micdadei* antigens; the other had as a complicating disease multiple myeloma and so may not have been able to respond to these antigens.

A total of 49 serum samples from 13 patients with LD confirmed by the isolation of serogroup 1 *L. pneumophila* were tested for the presence of antibody to serogroup-specific antigen by six ELISA methods and by the standard IFA test. One technique, the DS ELISA for IgM with a soluble antigen, was abandoned early since it was not as sensitive as the other IgM ELISA detection systems. A total of 12 of the 13 patients seroconverted to serogroup 1 antigens; 11 seroconverted or had high titers in four or more of the tests. Six patients were positive in all serological tests. One LD patient had no serological response to serogroup 1 *L. pneumophila* antigens in any of the tests but did, as previously noted, seroconvert when tested with *T. micdadei* (Pittsburgh pneumonia agent) antigens. RF was detected in the serum samples of two patients who were positive for IgM by the SS ELISA but not by the DS ELISA. One serum sample with a positive RF test was positive for the presence of IgM antibodies by both the DS and the SS ELISAs. The other serum samples were negative for RF. Three patients had positive IgM and IgG titers by the ELISA but were negative by the IFA test.

The antibody titers obtained in 8 of the 13 cases proven by culture are shown in Table 4 to point out the differences in their antibody responses. Patients 1 and 2 had IgM and IgG responses detected by all tests; the titers rose in the second or third week and began to fall in the second month. Patients 3, 4, and 5 had primarily an IgM response. The early apparent IgM response in patient 6 and the IgM response detected by the SS ELISA in patient 7 were presumably due to the presence of RF in their serum samples since they were negative when tested with the more specific DS ELISA. Patient 8 was the only patient with culture-proven LD who did not develop detectable antibody to *L. pneumophila* antigens but did, however, have a detectable rise in antibody titer to *T. micdadei* antigens.

All serum samples tested from patients who developed antibodies to serogroup 1 *L. pneumophila* were positive 3 to 8 weeks after the onset of their illnesses (Table 5). IgM antibodies were detected by the ELISA in 7 out of 13 samples during the first week and in 10 out of 11 samples during the second. Three of seven samples obtained from patients 17 to 24 weeks after the onset of an LD pneumonia episode were still positive for IgM antibodies. In contrast to IgM antibodies, IgG antibodies were detected in only 6 of 11 serum samples tested during the second week, but a greater number of samples had persistent IgG titer elevations for over 6 months.

Figure 1 shows the distribution of titers in acute and convalescent serum specimens from the three groups of patients with pneumonia: those with cultures positive for serogroup 1 *L. pneumophila* (three were also positive by the DFA test), those with a positive DFA test but negative culture, and those with neither a positive DFA test nor a positive culture. In some cases the absence of a positive culture or DFA test may have been due to submission of an inadequate or inappropriately timed specimen.

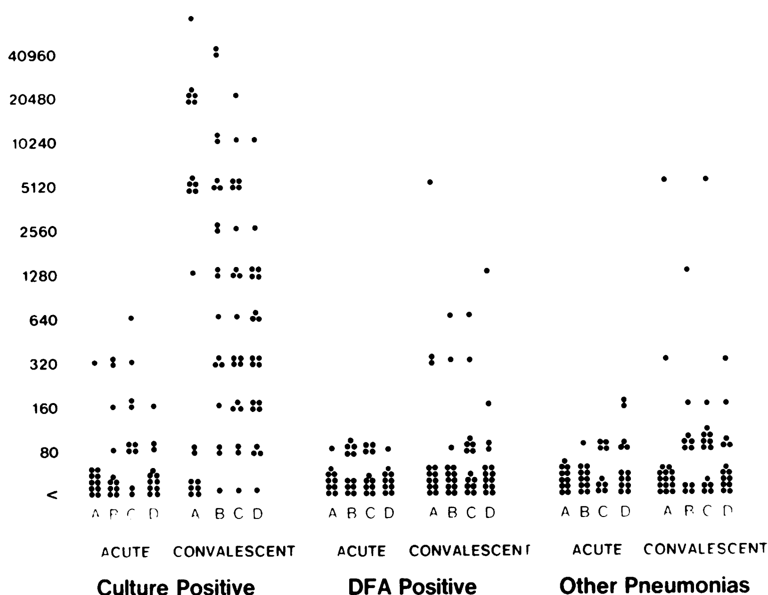

FIG. 1. Distribution of titers to *L. pneumophila* serogroup 1 antigens. Test groups are defined in the text. (A) DS ELISA for IgM with a bacterial sonicate antigen. (B) SS ELISA for IgM with soluble antigens. (C) SS ELISA for IgM with a bacterial sonicate antigen. (D) SS ELISA for IgG with soluble antigens.

Titers are shown for the DS ELISA for IgM with bacterial sonicate antigen, SS ELISA for IgM with soluble antigen, SS ELISA for IgM with a bacterial sonicate antigen, and the SS ELISA for IgG with soluble antigen. To simplify the figure, the data from the other tests are not shown; but the distribution of titers with these tests was similar. The distribution of acute- and convalescent-phase titers in the DFA-positive, culture-negative pneumonia group was similar to that seen in the group with a negative DFA test and culture. Some 4 of the 13 DFA-positive pneumonia patients had serological evidence of recent or past infection with serogroup 1 *L. pneumophila*, compared with 3 of 21 in the group with a negative DFA test and culture (chi square = 0.52; not significant).

The titers of serum samples obtained at necropsy can be seen on Table 6. The necropsy serum specimens obtained from all four patients dying of LD confirmed by culture tests had significant IgM antibody levels detected by the ELISA. In this group with detectable IgM antibodies, the titers obtained with the immunoglobulin conjugate which detected IgG, IgM, and IgA were at least one dilution lower than the titers obtained when IgM was measured alone. None of the four patients with LD confirmed by antemortem respiratory secretions or postmortem lung tissue that were positive by DFA tests, but without culture confirmation, had detectable antibody levels in their postmortem serum samples. None of the postmortem samples had a significant IgG titer by the ELISA or a significant titer by the IFA test.

DISCUSSION

This study evaluated the ELISA as a diagnostic test for the detection of antibodies to serogroup 1 *L. pneumophila*. The ELISA was used to detect IgM, IgG, or IgG, IgM, and IgA combined (the latter is referred to as the immunoglobulin ELISA). Based on the analysis of serum samples from unselected patients on hospital admission and from healthy hospital employees, a titer of 1:160 or greater was considered to be our threshold as it excluded approximately 97% of the titers found in these populations. A seroconversion was therefore defined as at least a fourfold rise in antibody level to a titer ≥1:160. The slightly greater proportion of titers of ≥1:80 in healthy hospital employees may be due to the exposure of these employees to *L. pneumophila*, which was widely present in the hospital environment in Pittsburgh (31). The relative sensitivity of the immunoglobulin ELISA was 70%, compared with a relative sensitivity of 38% for the IFA test in our studies. If only culture-proven LD cases were considered, 17 of 19 patients had fourfold or greater rises in antibody titer by the immunoglobulin ELISA (89.5%) as compared with only 11 of 19 by the IFA test (57.5%). The time course and relative prevalence of the IgM and IgG responses were similar to those previously reported (24). Because early antigenic exposure

TABLE 6. Detection of *L. pneumophila* serogroup 1 antibody by ELISA in necropsy serum samples from eight cases of LD confirmed by the DFA test or by culture

Patient	Confirming test		Days after onset	Antibody titer			
				ELISA			IFA
	DFA	Culture		IgM	IgG	Immunoglobulin	
1	+	+	6	640	<80	160	<64
2	−	+	6	320	80	160	64
3	−	+	5	640	80	320	64
4	−	+	5	160	<80	80	<64
5	+	−	5	<80	<80	<80	<64
6	+	−	5	<80	<80	<80	<64
7	+	−	6	<80	<80	<80	<64
8	+	−	7	<80	<80	<80	<64

occurs to intact microorganisms, a bacterial sonicate, which contains dispersed particulate surface components, was used as the antigen in IgM assays tested in parallel with the soluble antigen prepared as described by Farshy and Feeley (12) and Farshy et al. (13). The DS ELISA for IgM with soluble antigen was less sensitive than the other IgM ELISAs (data not shown) and was abandoned early in the study. The sensitivity of the SS ELISA with either antigen preparation was, on the other hand, comparable. The IFA test seemed to be resistant to the influence of RF as noted by Wilkinson et al. (33); however, as previously demonstrated (25), the SS ELISA when used to detect IgM or total immunoglobulin was not. Another problem of assays which simultaneously detect IgG and IgM is that IgG binding, which occurs more rapidly, may interfere with IgM binding to the antigen. Indeed, immunoglobulin ELISA levels were often lower than IgM ELISA levels. The DS ELISA avoids this potential problem. Therefore, although the immunoglobulin ELISA was the most sensitive single assay in our experience and is recommended for screening patient serum samples for anti-*Legionella* antibodies, titers should be determined definitively by a combination of the DS ELISA for IgM with a bacterial sonicate as the antigen and the SS ELISA for IgG with the previously described soluble antigen extract (12, 13). Since these procedures can be automated in a reference laboratory, the triple test system is feasible for routine use. Although our sample was small, our data with necropsy serum samples suggest that the IgM ELISA is the best procedure for detecting anti-*Legionella* antibodies in patients dying acutely of LD pneumonia. The patient with culture-proven LD who had no significant rise in antibody levels except to *T. micdadei* antigens may have suffered dual infection with *L. pneumophila* and *T. micdadei*; a similar case confirmed by culture has been reported (3).

The convalescent titers of the group of pneumonia patients with a positive DFA test but without culture confirmation were not significantly different from the titers of a group of pneumonia patients with no evidence of LD by the DFA test or culture (Fig. 1). The DFA test was considerably less sensitive than the other tests; only 6 of 19 patients with a positive culture tests were also positive by the DFA test. The association between seroconversion by any of the serological tests and culture positivity was great (chi square = 11.9; $P < 0.001$); there was, however, poor correlation of DFA test results with either culture or seroconversion (chi square = 0.63 and 0.01, respectively). The specificity of immunological tests for the detection of *Legionella* antigens or anti-*Legionella* antibodies may be compromised by the presence of antigens shared with *Chlamydia* spp. (19), *Pseudomonas* spp., (17), and certain anaerobic organisms (8). Although this may also influence antibody detection systems, our data raise serious questions about the specificity and sensitivity of the DFA procedures as presently performed on clinical specimens (primarily sputum). The experience of others with DFA tests has been variable (2, 9, 10, 29, 37), although not as poor as ours.

In summary, the ELISA proved to be an economical, sensitive, and specific method for the detection of anti-*Legionella* antibodies when many samples were tested. For screening purposes, serum samples from patients may be tested in duplicate at dilutions of 1:80 and 1:160 with a detection system which recognizes both bound IgG and IgM. Positive serum samples should then be titered out both with the DS ELISA for IgM with a bacterial sonicate antigen and with the SS ELISA for IgG with the previously described soluble antigen; this procedure will maximize sensitivity and specificity. These assays have also been successfully used to detect antibody to the other serogroups of *L. pneumophila*, *T. micdadei*, and *Fluoribacter* spp. (data not shown). Serum specimens may be screened on plates coated with a pool of two or

three antigens without significant interference (data not shown). We currently regard the ELISA as the method of choice for detecting antibodies for this group of organisms.

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