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Evaluation of a Solid-Phase Immunofluorescence Assay for Detection of Antibodies to *Legionella pneumophila*

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A semiautomated solid-phase immunofluorescence technique (FIAX) was compared with the standard indirect immunofluorescence assay (IFA) for the determination of antibody levels to *Legionella pneumophila* serogroup 1 in paired human serum samples. The FIAX method was in agreement with the IFA test for 91.8% of the serum pairs but gave evidence of a recent *Legionella* infection significantly fewer times than did the IFA. These results suggest that the FIAX technique may eventually be a useful alternative test for measuring *Legionella* antibodies. However, further study will be required to determine its efficacy in providing a serodiagnosis of legionellosis.

The standard method for measuring an immune response in legionellosis cases is the indirect immunofluorescence assay (IFA; 9). Several alternative tests have been described for the serodiagnosis of this disease (3, 4), but so far, their disadvantages outweigh their advantages for laboratories with relatively large workloads. The main disadvantages of IFA are the requirement of a fluorescence microscope and the fact that considerable experience is required to visually interpret fluorescence intensity reproducibly.

An alternative to IFA that has been developed for the serodiagnosis of several diseases is the fluorescence immunoassay (FIAX) system of International Diagnostic Technology, Santa Clara, Calif. This semiautomated solid-phase immunofluorescence test, which substitutes a fluorometer for the fluorescence microscope, has been used for the diagnosis of herpesvirus (1), rubella (2), *Toxoplasma* (5), and cytomegalovirus (1) infections. Recently, Vogel et al. (6) have shown that a FIAX test with *Legionella pneumophila* antigen can detect antibodies produced by guinea pigs that are immunized with *L. pneumophila* cells and Freund adjuvant. They have suggested that the test may also be useful for the detection of human antibodies. A major advantage of FIAX is that it provides a quantitative measure of fluorescence intensity, thus obviating the need for an experienced fluorescence microscopist for test interpretation.

The system consists of a flattened, plastic stick (Surface Technique for Immuno Quantitation, STiQ) with polymeric (cellulose acetate-nitrate) disks back to back at one end. Antigen is allowed to adsorb on one surface whereas the other surface is exposed to buffer only and thus serves as a control. The stick is incubated in one dilution (predetermined experimentally when the test is standardized) of the patient's serum, washed, incubated in fluorescein conjugate, washed again, and inserted into a fluorometer. The amount of fluorescence is directly proportional to the amount of antibody present in the sample. Standard curves are prepared with known positive samples (calibrators).

Our study was undertaken to compare the FIAX method and the standard IFA for measuring antibody levels in serum from patients with legionellosis.

A total of 392 human serum specimens, 196 acute- and convalescent-phase pairs, that were submitted to the Special Immunology Laboratory, Center for Infectious Diseases, Centers for Disease Control, from patients with suspected legionellosis were tested by IFA and FIAX for *L. pneumophila* serogroup 1 antibodies. IFA tests were performed as previously described (9) with a heat-killed, whole-cell antigen, strain Philadelphia 1, in 0.5% buffered normal yolk sac. Preparation of the FIAX antigen was based on the method of Vogel et al. (6). The Knoxville 1 strain *L. pneumophila* serogroup 1 was killed by suspending the growth from four charcoal-yeast extract agar slants in 1.0% Formalin in 0.1 M phosphate-buffered saline for 24 h. The cells were then washed twice in 0.15 M sterile saline by centrifugation and were stored in 0.15 M sterile saline. The cells were adjusted to an optical density of 0.30 at 420 nm in saline (Coleman Junior Spectrophotometer). The antigen suspension was then sonicated for 2 min (60 to 70% of maximum setting on a Biosonik IV, VWR Scientific, San Francisco, Calif.) in an ice bath. STiQ samplers were prepared by applying

TABLE 1. Comparison of FIAX and IFA with paired sera from suspected cases of legionellosis

IFA	FIAX	
	+[a]	–
+[b]	55[c]	13
–	3	125

[a] Greater than or equal to fourfold rise in titer to at least 100.
[b] Greater than or equal to fourfold rise in a titer to at least 138.
[c] Number of paired sera showing the indicated test results. Agreement, 91.8% (180/196). A positive test result was obtained by IFA significantly more often than by FIAX (χ^2 = 5.06; P = 0.024).

25 μl of the sonicated cell suspension to one surface and allowing it to air dry. The opposite side served as a control. STiQ samplers were used on the same day that they were prepared.

Calibrators with IFA titers of 512, 256, 128, and 64 were prepared by serially diluting a single, high-titered human serum specimen in filtered normal human serum. The fluorescence signals (ΔFS) obtained for 10 replicate FIAX assays of each calibrator were plotted versus IFA titers on log-log paper because titers are log-normally distributed when doubling dilutions are used. A best-fit straight line was then drawn and was used to determine the FIAX titer for each calibrator from mean ΔFS values. These FIAX titers were 494, 275, 129, and 63. Alternatively, calibrator FIAX titers could be determined from the IFA titers and the ΔFS values with a least-squares linear regression program and a TI-55 calculator.

Serum samples were diluted 1:40 in 0.01 M Tris-buffered saline containing 0.25% Tween 20, pH 8.0 (TBS). One STiQ sampler was placed antigen-surface down into a test tube (12 by 75 mm) containing 0.6 ml of diluted serum for 30 min at room temperature (at each incubation and wash step the tubes were shaken at approximately 200 oscillations per min). The samplers were then washed in TBS for 10 min. Next, the samplers were incubated in a 1:250 dilution in TBS of fluorescein isothiocyanate-labeled sheep anti-human immunoglobulins G, M, and A (Wellcome Research Laboratories, Beckenham, England) for 30 min. After a final 10-min wash in TBS to remove nonspecific fluorescence, the fluorometer was calibrated with the known serum samples. A FIAX standard curve was prepared for each assay by plotting calibrator FIAX titers versus the mean of duplicate ΔFS determinations. The FIAX titer of each unknown sample was then determined by interpolation from the curve.

A positive IFA test was defined as a greater than or equal to fourfold increase in titer to at least 128 from the acute- to the convalescent-phase sera (9). A positive FIAX test was defined as a greater than or equal to fourfold increase in titer to at least 100. The latter value was chosen because FIAX titers are based on a continuum of measurements of fluorescence intensity, whereas IFA titers are determined from doubling dilution values only. Since 128 is used as the cutoff level for IFA, the arithmetic mean of 64 and 128 was estimated as the FIAX cutoff titer. The mean, 96, was rounded to 100 for convenience with no effect on subsequent analysis of the data.

A total of 180 (91.8%) pairs of sera agreed in and 125 were negative (Table 1). Of the 13 pairs that were negative by FIAX and positive by IFA, 6 were barely positive by IFA (fourfold rise to 128) and were negative by only one twofold dilution factor with FIAX. Conversely, of the three pairs with positive FIAX and negative IFA values, one had a positive FIAX result and negative IFA test result by one doubling factor. A one twofold dilution factor difference is considered within the limits of experimental error for IFA. IFA detected a positive test result significantly more often than did FIAX (χ^2 = 5.06; P = 0.024, McNemar test for paired observations). However, when titers of the total number of sera were compared irrespective of interpretive criteria, 88.0% of the FIAX titers were within ±1 dilution factor of the corresponding IFA titers and 96.7% were within ±2 dilution

TABLE 2. Precision of FIAX anti-*Legionella* antibody titer determinations

Calibrator (IFA titer)	Within assay			Between assays		
	FIAX[a] titer	CV (%)[b]	Range of titers	FIAX[c] titer	CV (%)	Range of titers
1 (512)	460 ± 25	5.3	412–508	494 ± 31	6.3	433–555
2 (256)	293 ± 37	12.6	221–365	275 ± 28	10.2	220–330
3 (128)	138 ± 11	8.2	116–160	129 ± 15	11.8	99–159
4 (64)	59 ± 6	7.8	50–68	63 ± 5	8.3	53–73

[a] Mean titer ± standard deviation from 10 replicate sample determinations within the same assay run.
[b] CV, (standard deviation/mean) × 100.
[c] Mean titer ± standard deviation from 20 replicate sample determinations in separate assay runs.

factors. Precision data are given in Table 2. The coefficient of variation (CV) values were always <13%.

This study provided evidence that the FIAX and IFA tests measure comparable levels of antibody to *L. pneumophila* serogroup 1 antigen if titers that varied no more than one twofold dilution factor were considered in agreement. However, significantly fewer seroconversions could be interpreted as positive with FIAX values than with IFA titers. Of the 16 discrepant results, 7 nevertheless agreed within one doubling factor, which is usually considered within the limits of experimental error. Repeat test results of all discrepant values (not included in the present analysis) agree with the original test results. The discrepant titers we observed may have been the result of our use of antigens prepared from different strains: Philadelphia 1 for IFA and Knoxville 1 for FIAX. IFA antigens prepared from different strains can also cause discrepant IFA titers (unpublished observation). We used the Knoxville 1 antigen because it was used successfully by Vogel et al. in a feasibility study with guinea pig antisera (6). Further studies are required to determine the optimal strain for preparing FIAX antigens.

Also required before the value of FIAX as a practical serodiagnostic test for *Legionella* infection can be determined is an evaluation of *Legionella* antigens other than *L. pneumophila* serogroup 1. The genus now includes 8 species with 14 serogroups, and the number will most likely continue to increase. Multiple antigens are required for optimal test sensitivity since the immune response to legionellosis can be serogroup specific or common antigen reactive (7, 8, 9). It is unlikely that polyvalent FIAX antigens can be used because the fluorometer cannot distinguish differences in fluorescence intensity among cells in a single antigen preparation. Therefore, sera with only serogroup-specific antibodies could give deceptively low FIAX values since cells of heterologous serogroups would be unstained. In IFA, the number of cells fluorescing has minimal influence on the titer determination within a broad range, as compared with FIAX. However, FIAX may eventually prove to be useful with monovalent antigens as a nonmicroscopic technique for laboratories performing small numbers of serological tests for legionellosis. Its major advantages are reproducibility (CV, ≤12.6%) and the fact that it provides a quantitative, objective assessment of antibody titer. Its major disadvantage is that larger volumes of reagents are required than are required for IFA, approximately twice the amount of antigen and five times the volume of conjugate.

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