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Clinical Utility of a Monoclonal Direct Fluorescent Reagent Specific for *Legionella pneumophila*: Comparative Study with Other Reagents

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Twenty-four lower respiratory tract samples taken from patients with culture-confirmed *Legionella pneumophila* infection were examined with three different direct immunofluorescent antisera to *L. pneumophila*, as were 29 samples from similar sources taken from patients without Legionnaires disease. The reagents studied were Genetic Systems Corp. (GS) monoclonal *L. pneumophila* conjugate, which reacts with all known serogroups of *L. pneumophila*, BioDx polyvalent *L. pneumophila* serogroups 1 through 6 conjugate, and Centers for Disease Control polyvalent pool A *L. pneumophila* serogroups 1 through 4 conjugate. The specimens had been frozen at −70°C for 0.5 to 5 years. Randomization was used in coding the samples, which were stained and read by an independent observer. All three conjugates correctly identified all positive and negative samples. No difference was noted among the conjugates in the absolute numbers of fluorescent *L. pneumophila* bacteria per sample. The GS conjugate had a much cleaner background than did the other two reagents. Mean staining intensity scores were 3.4, 3.9, and 3.7 for the GS, BioDx, and Centers for Disease Control conjugates, respectively. This study demonstrates that the diagnostic efficiency of all three conjugates is equivalent. Since the GS conjugate is easier to read, does not cross-react with non-*L. pneumophila* bacteria, and reacts with serogroups 1 through 10 of *L. pneumophila*, it appears to be preferable for use in diagnostic testing on nonhistopathologically processed specimens.

Direct immunofluorescence testing of lower-respiratory-tract samples and tissues is commonly used to diagnose Legionnaires disease (1, 3, 5, 11). This test has an estimated sensitivity of 25 to 70% and specificity in excess of 99%. Since there is no genus-specific conjugate available, multiple individual or pooled antisera must be used to detect multiple serogroups of *Legionella pneumophila* (10). Also, available reagents show significant reactions with non-*Legionella* bacteria, including some strains of *Pseudomonas* spp., *Bacteroides fragilis*, *Streptococcus pneumoniae*, *Flavobacterium-Xanthomonas* group, *Bacillus* sp., and *Escherichia coli* (2, 4, 6, 8, 9, 10; P. Edelstein, unpublished data). A newly produced conjugate, made by using a monoclonal antibody directed against an interior cellular protein, has been shown to react with serogroups 1 through 10 of *L. pneumophila* and not to react with bacteria which cross-react with other reagents (7; F. C. Tenover, P. H. Edelstein, L. C. Goldstein, J. C. Sturge, and J. J. Plorde, personal communication). We studied the use of this reagent in a retrospective blind study of known negative and positive respiratory tract samples.

MATERIALS AND METHODS

Clinical specimens. We picked 24 samples (from different patients) which had both a positive direct immunofluorescence examination and a positive culture for *L. pneumophila* (3). The serogroup distribution of the positive samples was as follows: serogroup 1, 18 samples; serogroup 2, 1 sample; serogroup 4, 3 samples; serogroups 1 and 4, 1 sample; and serogroup 9, 1 sample. Twenty-nine similar source samples which were negative on direct immunofluorescence testing and culture were used as negative controls (Table 1). All samples had been frozen without preservative at −70°C for 6 months to 5 years. The samples were randomized and then analyzed in order of their randomization. Preliminary studies showed that Genetics Systems Corp. (GS) conjugate staining of *L. pneumophila* in tissues fixed in Formalin for prolonged periods yielded variable results, and so no Formalin-fixed or paraffin-embedded samples were studied.

Conjugates. GS monoclonal conjugate (lot no. 0479-4) and BioDx (BD) polyvalent *L. pneumophila* serogroups 1 through 6 conjugate (lot no. G308) were compared with Centers for Disease Control (CDC) polyvalent *L. pneumophila* serogroups 1 through 4 pool A conjugate (lot no. 81-0138-9); all these are conjugated with fluorescein isothiocyanate. The GS conjugate used differs from that described previously (7) in that it has been reformulated so that detergent addition to the sample is not needed and so that it does not bind nonspecifically to *Staphylococcus aureus* cells.

Sample staining and reading. Samples were thawed at room temperature and smeared onto ethanol-cleaned glass slides labeled only with the random number. Slides with two 12-mm (inside diameter) Teflon rings (Cel-line, Newfield, N.J.) were used for the samples to be examined by the CDC and BD conjugates. Different slides, with a more hydrophobic surface but identical well size, were used for the samples to be examined by the GS conjugate (no. 100314; Carlson Scientific, Peotone, Ill.). Slides were air dried and gently heat fixed and stained as specified by the manufacturer. Staining and reading of any one set of sample slides was done on the same day for all three conjugates.

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TABLE 1. Types of samples examined

Source	No. positive[a]	No. negative[b]
Lung	7	10
Sputum	12	10
Transtracheal aspirate	1	2
Bronchial wash	0	6
Pleural fluid	0	1
Endotracheal aspirate	3	0
Heart valve	1	0

[a] Previously positive by both direct immunofluorescence and culture for *L. pneumophila* serogroups 1 through 4. One lung sample was positive for *L. pneumophila* serogroup 9 on both tests.
[b] Negative by above criteria.

A single observer read all the slides without knowledge of whether the sample had previously been found positive or negative. Slides were read by conjugate type, with all slides stained with one of the three conjugate types being read first; the order of reading was assigned on a rotating basis for each day's experiment. Only after the results of examination of all slides stained with a certain conjugate type were recorded were the other conjugate-stained slides examined.

Slides were examined by using a Leitz Dialux microscope equipped with an epifluorescence UV light system with a 100-W mercury bulb and a K2 prism cube (E. Leitz, Rockleigh, N.J.). Slides were read at ×500 magnification; positives were confirmed at ×625 magnification. Both wells of all slides were read. Appropriate negative and positive control slides were included. Slides were examined for 10 to 15 min before being classified as negative. Positive slides were ones which contained at least a single organism per smear which was typical of *L. pneumophila* in fluorescence and morphology. Semiquantitative and intensity scales were used to grade positive slides, with a 0 to 5 scale for numbers of fluorescent bacteria per well and a 0 to 4 scale for fluorescence intensity; the highest scale number represents the greatest possible number of organisms or brightest intensity, respectively.

RESULTS

All specimens were correctly identified by all three conjugates. No significant differences in numbers of fluorescent bacilli per slide were detected among the different conjugates, with mean semiquantitative scores of 3.7, 3.8, and 3.8 for the GS, BD, and CDC reagents, respectively; modal values were 5.0 for all three conjugates.

Each of the conjugates produced different staining of *L. pneumophila* cells. Mean fluorescence intensity scores for positive specimens were 3.4, 3.9, and 3.7 for the GS, BD, and CDC conjugates, respectively. Mode and range fluorescence intensity values, respectively, for the positive samples were 3.5 and 3.0 to 4.0 for the GS reagent, 4.0 and 3.0 to 4.0 for the BD reagent, and 4.0 and 2.0 to 4.0 for the CDC reagent. Regardless of serogroup, the GS conjugate stained all *L. pneumophila* bacteria uniformly. The lung sample containing serogroup 9 organisms, which was the only one examined for which specific antibodies were not included in the polyvalent reagents, stained 2 in intensity with the CDC conjugate, 3 with the GS conjugate, and 4 with the BD conjugate. Contrasted with the intensity of positively staining bacterial cells was the nonspecific background fluorescence intensity. The background of the majority of slides examined with the GS conjugate was very dark, with no fluorescence detected in leukocytes or tissue debris; slides occasionally contained green fluorescent debris. Nonspecific staining detected with the other conjugates, with both negative and positive specimens, was several orders of magnitude greater. This difference in background fluorescence made slide screening much easier and faster with the GS conjugate; overall the signal-to-noise ratio of the GS conjugate was judged to be the highest of the three conjugates. No nonspecifically staining organisms, such as diplococcal or blastospore-like forms, were observed with the GS conjugate, whereas they were occasionally seen with the other conjugates.

Another difference noted among the conjugates was that of cell wall staining. The GS conjugate almost always stained *L. pneumophila* cells diffusely, as opposed to the bright peripheral and absent central staining with the other two conjugates. As a result, most *L. pneumophila* cells stained with the GS conjugate had less distinct cell margins.

After the study was completed, an attempt was made to compare the time required to detect *L. pneumophila* cells in known positive slides by using different screening microscope magnifications. Screening at ×312 was rapid and accurate with the GS conjugate-stained slides, but not with the other conjugates because of confusing background fluorescence.

DISCUSSION

This study demonstrated that all three conjugates are equally sensitive and specific. Use of the highly specific GS conjugate will probably result in consistently fewer false-positive tests than will use of other reagents. Its exquisite specificity has been demonstrated in staining of bacterial colonies (7; Tenover et al., personal communication). It is our anecdotal experience that two sputum samples containing cross-reactive *Pseudomonas aeruginosa* strains were negative when stained with the GS conjugate but positive with the CDC pool A conjugate. The marked decrease in background fluorescence noted with the GS conjugate made it possible for us to correctly read GS-stained slides at lower magnification and with greater speed than with the other two conjugates.

An additional advantage of the GS conjugate is that it stains all 10 known serogroups of *L. pneumophila*, whereas the BD reagent stains only serogroups 1 to 6 and the CDC reagent stains only serogroups 1 to 4. Thus only a single reagent will be needed to screen for *L. pneumophila* in specimens, making the use of polyvalent pools unnecessary.

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