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# Comparison of Cross-Staining Reactions by *Pseudomonas* spp. and Fluorescein-Labeled Polyclonal and Monoclonal Antibodies Directed against *Legionella pneumophila*

FRED C. TENOVER,[1,2]* PAUL H. EDELSTEIN,[3,4] LYNN C. GOLDSTEIN,[5] JERRILYN C. STURGE,[5] AND JAMES J. PLORDE[1,2]

*Seattle Veterans Administration Medical Center, Seattle, Washington 98108[1]*; Department of Laboratory Medicine, University of Washington, Seattle, Washington 98195[2]; Genetic Systems Corporation, Seattle, Washington 98121[5]; Medical and Research Services, Wadsworth Veterans Administration Medical Center, Los Angeles, California 90073[4]; and Department of Medicine, University of California, Los Angeles, California 90024[3]*

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**Commercially prepared polyclonal antisera to *Legionella pneumophila* are known to cross-react with organisms of the genus *Pseudomonas*. To determine whether a commercially available monoclonal antibody reagent specific for *L. pneumophila* would also cross-react with pseudomonads, a two-laboratory study was undertaken to test both monoclonal and polyclonal reagents against 33 isolates of *Pseudomonas* spp., including 25 *Pseudomonas aeruginosa*, 4 *P. putida*, 2 *P. maltophilia*, 1 *P. fluorescens*, and 1 *P. alcaligenes*. Four antisera were tested; polyclonal anti-legionella antisera pools A and B (Centers for Disease Control [CDC], Atlanta, Ga.), polyclonal 1-6 antisera (BioDx, Inc., Denville, N.J.), and a monoclonal antibody reagent produced by Genetic Systems Corp., Seattle, Wash. All reagents were labeled with fluorescein. Cross-staining reactions were found with the BioDx *L. pneumophila* antisera and 10 isolates of *Pseudomonas*. Four of these isolates demonstrated cross-staining with CDC pool A. When tested with individual serotype-specific reagents (CDC), three of four cross-reacted with *L. pneumophila* serotype 1 antisera; the fourth cross-reacted with serotype 3. No cross-staining reactions were noted with the monoclonal reagent and any of the pseudomonads tested, demonstrating that the Genetic Systems Corp. monoclonal reagent is the most specific of the four reagents tested.**

The diagnosis of legionella pneumonia is most readily established by the direct detection of the causative agent in respiratory secretions, tissues, or fluids by using fluorescein-labeled legionella antisera (2, 6, 10). Several investigators have noted, however, that such antisera may cross-react with nonlegionella organisms, including several species of *Pseudomonas*, occasional *Bacteroides* spp., and members of the *Flavobacterium-Xanthomonas* group (1, 5, 11), resulting in false-positive reactions.

Our previous evaluations of the monoclonal legionella reagent developed by Genetic Systems Corp., Seattle, Wash. (GSC) demonstrated it to produce more uniform staining patterns around legionella bacilli and to produce more definitive staining of *Legionella pneumophila* serogroups 7, 8, and 9 than a polyclonal reagent (12). However, problems with cross-reactions between the polyclonal and monoclonal reagents and *Pseudomonas* spp. were not addressed. To determine whether such cross-reactions are less common with a monoclonal legionella reagent, the Seattle and Los Angeles (Wadsworth) Veterans Administration Medical Centers (SVAMC and LAVAMC) tested 33 isolates of *Pseudomonas* spp. with three different polyclonal legionella antisera and one monoclonal antibody reagent specific for *L. pneumophila* (9).

The 33 study organisms (Table 1) were obtained from the Centers for Disease Control, Atlanta, Ga. (CDC), American Type Culture Collection, Rockville, Md., and Cutter Laboratories, San Francisco, Calif., and their identification was confirmed at the SVAMC microbiology laboratory. Some of these strains had previously been noted to cross-react with antisera from BioDx, Inc., Denville, N.J. or CDC. Isolates were identified as *Pseudomonas aeruginosa* if they grew on Trypticase soy agar (BBL Microbiology Systems) at 42°C, were resistant to kanamycin by disk diffusion testing, produced pigment on pseudomonas A agar (Flo agar; Difco Laboratories, Detroit, Mich.) or pseudomonas B agar (Tec agar; Difco) at 37°C, and grew on cetrimide slants. Organisms which did not fit these criteria were identified by additional procedures described by Gilardi (7, 8) including: indophenol oxidase; growth on MacConkey agar (Difco) at 37°C; production of DNase, lysine decarboxylase, and arginine dihydrolase; liquification of gelatin; reduction of nitrate to nitrite or gas; hydrolysis of urea, *o*-nitrophenyl-β-D-galactopyranoside, or casein; oxidation of glucose, maltose, xylose, or mannitol; and motility. The number and arrangement of flagella for each non-*P. aeruginosa* isolate was determined by electron microscopy.

All study isolates were initially tested with BioDx *L. pneumophila* polyclonal 1-6 reagent and with GSC *L. pneumophila* reagent according to the package inserts. Both reagents are fluorescein labeled. The slides were examined with a 40× glycerol immersion objective and 10× eyepieces (at SVAMC total magnification, 400×) or a 40× dry objective with a cover slip in conjunction with 12.5× eyepieces (at LAVAMC; total magnification, 500×). Organisms demonstrating fluorescence with the BioDx reagent were further tested with CDC pool A (*L. pneumophila* serogroups 1 through 4), CDC pool B (*L. pneumophila* serogroups 5 and 6, *Legionella dumoffii* serogroup 1, and *Legionella longbeachae* serogroup 2), and CDC type-specific conjugates for

* Corresponding author.

TABLE 1. Identification of study organisms

| Organism no. | Identification[a] |
|---|---|
| 17 | *P. aeruginosa* IATS 14 |
| 23 | *P. aeruginosa* |
| 78 | *P. putida* |
| 89 | *P. putida* |
| 91 | *P. aeruginosa* Fisher 2 |
| 128 | *P. aeruginosa* IATS 3 |
| 143 | *P. aeruginosa* IATS 9 |
| 175 | *P. putida* |
| 185 | *P. aeruginosa* IATS 16 |
| 191 | *P. maltophilia* |
| 266 | *P. aeruginosa* Fisher 1 |
| 302 | *P. maltophilia* |
| 402 | *P. aeruginosa* |
| 411 | *P. aeruginosa* Fisher 4 |
| 528 | *P. aeruginosa* Fisher 5 |
| 538 | *P. aeruginosa* IATS 6 |
| 569 | *P. aeruginosa* |
| 604 | *P. fluorescens* |
| 606 | *P. aeruginosa* IATS 12 |
| 673 | *P. aeruginosa* IATS 2 |
| 698 | *P. aeruginosa* IATS 7 |
| 700 | *P. aeruginosa* Fisher 7 |
| 731 | *P. aeruginosa* IATS 1 |
| 732 | *P. aeruginosa* Fisher 6 |
| 735 | *P. aeruginosa* IATS 4 |
| 773 | *P. aeruginosa* IATS 13 |
| 805 | *P. aeruginosa* IATS 5 |
| 842 | *P. alcaligenes* CDC ABB50 |
| 896 | *P. aeruginosa* |
| 902 | *P. aeruginosa* Fisher 3 |
| 934 | *P. putida* |
| 991 | *P. aeruginosa* IATS 17 |
| 992 | *P. aeruginosa* |

[a] The organisms were identified by the SVAMC as described in the text. Serotyping was confirmed by GSC.

*L. pneumophila* serotypes 1 through 6 prepared by the Diagnostics Division of the CDC as outlined in the instruction brochure. The intensity of fluorescence was graded on a scale from 4+ to 1+ at the SVAMC and from 4+ to 0.5+ at the LAVAMC. Intensity of 4+ denoted brilliant, apple-green fluorescence around >90% of all bacilli present; 3+ denoted bright fluorescence around >90% of the bacilli present; 2+ denoted discernable green fluorescence around >90% of the bacilli present; 1+ denoted dim fluorescence around >90% of the bacilli present; and 0.5+ denoted dim fluorescence around <50% of the bacilli present. A positive test as defined by the CDC, BioDx, and GSC package inserts corresponds to 2+ or greater fluorescence around 90% of the bacilli present.

The results obtained at the two medical centers are shown in Table 2. Neither center found cross-staining of pseudomonads with the GSC monoclonal reagent. Both laboratories found cross-staining of five *P. aeruginosa* isolates (organism numbers 185, 673, 805, 902, 991), two *P. putida* isolates (numbers 89 and 175), one *P. fluorescens* isolate (number 604), and one *Pseudomonas alcaligenes* isolate (number 842) when the BioDx reagent was used. The SVAMC noted one additional organism (*P. putida*, organism number 78) that stained at low intensity (Table 2). Three of the ten organisms demonstrating fluorescence with the BioDx reagent also demonstrated fluorescence with CDC pool A reagent at both laboratories (organism numbers 89, 175, and 842). SVAMC noted low-level staining of one additional organism (number 991) with CDC pool A. Three of the four demonstrated cross-staining with *L. pneumophila* serogroup 1 antiserum. The remaining organism (number 842) cross-reacted with serogroup 3. There were no significant cross-reactions between the pseudomonas isolates and any of the antisera contained in the CDC pool B reagent. Neither laboratory noted any cross-staining reactions with either the BioDx or CDC negative control sera.

TABLE 2. Cross-reactions between study organisms and GSC, BioDx, and CDC immunofluorescent typing reagents

| Organism no. | Cross-reaction with the following fluorescent-labelled *L. pneumophila* typing reagents[a]: | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GSC | BioDx | | CDC pool A | | CDC monospecific conjugates of serogroup | | | | | | | |
| | | | | | | 1 | | 2 | | 3 | | 4 | |
| | | S | L | S | L | S | L | S | L | S | L | S | L |
| 78 | 0 | 1+ | 0 | 0 | NT | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 89 | 0 | 4+ | 2+ | 4+ | 2+ | 3+ | 2+ | 0 | 0 | 0 | 0 | 0 | 0 |
| 175 | 0 | 4+ | 2+ | 4+ | 2+ | 4+ | 4+ | 0 | 0 | 0 | 0 | 1+ | 0 |
| 185 | 0 | 3+ | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 604 | 0 | 2+ | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 673 | 0 | 3+ | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 805 | 0 | 1+ | 0.5+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 842 | 0 | 1+ | 2+ | 3+ | 2+ | 0 | 0 | 0 | 0 | 3+ | 3+ | 0 | 0 |
| 902 | 0 | 1+ | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 991 | 0 | 1+ | 1+ | 1+ | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] S, Seattle Veterans Administration Medical Center, Seattle, Wash.; L, Los Angeles (Wadsworth) Veterans Administration Medical Center, Los Angeles, Calif. Fluorescence is indicated as follows: 4+, brilliant apple-green fluorescence around >90% of the bacilli present; 3+, bright fluorescence around >90% of the bacilli; 2+, discernable fluorescence around >90% of the bacilli; 1+, dim fluorescence around >90% of the bacilli; and 0.5+, dim fluorescence around <50% of the bacilli. NT, Not tested.

These results demonstrate that the GSC monoclonal reagent is more specific than are the CDC or BioDx polyclonal reagents. The cross-reactivity of the polyclonal reagents is already known (5, 11). Collins and co-workers (3) have identified shared antigens between several *Legionella* species and a *P. aeruginosa* common antigen by using serum from rabbits hyperimmunized with different *Legionella* species. The GSC monoclonal reagent apparently does not react with this *P. aeruginosa* common antigen and thus holds promise for greater diagnostic accuracy than the polyvalent antisera. The recent study of frozen respiratory tract specimens with the GSC monoclonal antiserum demonstrated the monoclonal reagent to be as sensitive and specific as polyclonal reagents (4). The data reported here suggest that the monoclonal reagent, in fact, is more specific than the polyclonal reagents, making it the preferred reagent for diagnostic use. It is unclear to us whether the weak-intensity cross-reactions noted with some of the pseudomonas isolates would cause confusion in diagnostic testing, although false-positive reactions with at least some *Pseudomonas* spp. undoubtably occur in clinical specimens. Specimens demonstrating low-intensity fluorescence around bacilli morphologically consistant with legionella should initiate a request for additional specimens for further testing.

In summary, the GSC reagent did not exhibit any cross-staining with a variety of *Pseudomonas* spp., including several organisms noted previously to cross-stain with other *L. pneumophila* typing sera.

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