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Confirmation of *Legionella pneumophila* Cultures with a Fluorescein-Labeled Monoclonal Antibody

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We compared a fluorescein-labeled monoclonal antibody directed against an outer membrane protein of *Legionella pneumophila* (Genetic Systems Corp. [GSC], Seattle, Wash.) with a similarly labeled polyclonal reagent (*L. pneumophila* serogroups 1 to 6, poly; BioDx, Inc., Denville, N.J.) for the confirmation of *L. pneumophila* isolates grown in culture. Duplicate suspensions of 52 organisms, including 21 *L. pneumophila* and 8 non-*L. pneumophila* species of legionella, were placed on individual glass slides, fixed, and stained with both reagents, and the results were compared. Both antisera correctly identified all *L. pneumophila* serogroups 1 to 6, but only the GSC reagent produced definitive staining of the *L. pneumophila* isolates of serogroups 7, 8, and 9. Additionally, the GSC reagent produced more uniform staining patterns around the legionella bacilli and displayed little background fluorescence when compared with the BioDx reagent.

Legionella pneumophila is the causative agent of legionellosis, a pneumonic illness often accompanied by systemic manifestations (4, 6, 7). This organism not only causes epidemic and sporadic disease in the community but is a significant cause of nosocomial infections as well (7). Rapid laboratory confirmation of legionellosis often requires the use of fluorescein-conjugated antisera for the direct demonstration of legionella in clinical specimens and the identification of culture isolates (1, 2). Most of the commercially available antisera are polyclonal reagents and are subject to lot-to-lot variation. To circumvent this problem of variable reactions, a monoclonal antibody against an outer membrane protein of *L. pneumophila* was developed (5). In this report, we compare this fluorescein-conjugated monoclonal antibody (Genetic Systems Corp. [GSC], Seattle, Wash.) with a commercially available polyclonal reagent (*L. pneumophila* serogroup 1 to 6, poly; BioDx, Inc., Denville, N.J.) for the confirmation of *L. pneumophila* grown in culture.

A total of 52 organisms, including 21 *L. pneumophila* isolates obtained from the American Type Culture Collection (Rockville, Md.) and the Centers for Disease Control (Atlanta, Ga.) were assigned random numbers, subcultured to both Columbia blood agar plates (Prepared Media Laboratories) and buffered charcoal-yeast extract agar (prepared according to the method of Feeley et al. [3] at the Seattle Veterans Administration Medical Center), and incubated at 35°C in 5% CO_2 at 80% humidity. All organisms grew on buffered charcoal-yeast extract agar, Columbia blood agar, or both media by 48 h. When growth was visible on both media, organisms were selected from the buffered charcoal-yeast extract agar plate for further analysis.

Suspensions of the test organisms were prepared as directed in the package insert of each product. For preparation of the BioDx slides, organisms were suspended in 1% neutral Formalin to the density of a 1.0 McFarland standard. Suspensions for the GSC slides were inoculated to the same density in 1% Formalin containing 0.005% Triton X-100. Each suspension was applied to a 10-well Teflon-coated slide (Carlson Scientific, Peotone, Ill.) with a Pasteur pipette and immediately aspirated back into the pipette. This resulted in approximately 200 to 300 organisms per ×400 field. On the GSC slide, only well 1 was seeded, whereas wells 1 and 3 were seeded on the BioDx slides. All slides were air dried, and the organisms were fixed by passing the slides through an open flame. GSC reagent (15 μl), which contained Evans blue counterstain, was applied to well 1 of each GSC slide. After incubation in a humidified chamber at 35°C in the dark for 30 min, the reagent was aspirated from the slide, and the slides were placed in a Coplin jar containing distilled water for 5 min. After removal from the rinse, the slides were drained on paper towels, aspirated to dryness, and observed under phosphate-buffered glycerol (pH 9.0) with a Zeiss Universal epi-illuminated microscope with a 40× Plan-Neofluar glycerol immersion objective.

For each of the BioDx slides, 15 μl of *L. pneumophila* serogroup 1 to 6 polyvalent antiserum was applied to well 1 and 15 μl of BioDx negative control antiserum was applied to well 3. Both antisera contained Evans blue counterstain. The slides were incubated in humidified chambers at room temperature for 30 min in the dark. After incubation, the negative control serum was aspirated from well 3 of each slide, and then the polyvalent antiserum was aspirated from well 1. The slides were then rinsed with a stream of 0.01 M phosphate-buffered saline (pH 7.2) with well 3 uppermost to prevent contamination with residual antiserum from well 1. The slides were then placed in a Coplin jar containing phosphate-buffered saline for 10 min, drained, aspirated to dryness, and observed as described above. Both sets of slides were examined independently by two readers. The identities of the organisms were not revealed until the completion of the study.

The results of the cultures and stains are presented in Table 1. No false-positive or false-negative results were noted with the GSC reagent, which gave a strong 3+ reaction with each of the *L. pneumophila* serogroups 1 to 9. The BioDx reagent demonstrated slightly stronger reactions

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TABLE 1. Growth and staining characteristics of study organisms

Organism[a]	No. tested	Growth on[b]:		Intensity of staining[c]	
		CBA	BCYE	BioDx	GSC
Legionella pneumophila 1	13	0	+	4	3
Legionella pneumophila 2	1	0	+	4	3
Legionella pneumophila 3	1	0	+	4	3
Legionella pneumophila 4	1	0	+	4	3
Legionella pneumophila 5	1	0	+	4	3
Legionella pneumophila 6	1	0	+	4	3
Legionella pneumophila 7	1	0	+	±	3
Legionella pneumophila 8	1	0	+	±	3
Legionella pneumophila 9	1	0	+	±	3
Legionella micdadei	2	0	+	0	0
Legionella bozemanii	1	0	+	0	0
Legionella dumoffii	1	0	+	0	0
Legionella longbeachae	2	0	+	0	0
Legionella sainthelensi	1	0	+	0	0
Legionella wadsworthii	1	0	+	0	0
Escherichia coli	4	+	+	0	0
Klebsiella pneumoniae	5	+	+	0	0
Lactobacillus sp.	3	+	0	0	0
Pseudomonas fluorescens	1	+	+	0	0
Pseudomonas aeruginosa	5	+	+	0	0
Streptococcus pyogenes	5	+	0	0	0

[a] Suspensions of organisms were prepared and stained as described in the text.
[b] CBA, Columbia blood agar; BCYE, buffered charcoal-yeast extract agar.
[c] 4+, Brilliant yellow-green staining; 3+, bright staining; ±, dim fluorescence around <50% of bacterial cells; 0, no fluorescence noted.

than did the GSC reagent for the serogroups against which it was prepared (1 to 6) but gave indeterminate reactions with *L. pneumophila* serogroups 7, 8, and 9. Neither of the reagents cross-reacted with any of the non-*L. pneumophila* species of legionella or with any of the other test organisms. Several qualitative differences in the two reagents were noted. The GSC reagent consistently produced more uniform staining around the legionella bacilli and produced noticeably less background fluorescence than did the BioDx reagent, making it easier to read despite its slightly lessintense fluorescence. The difference in background fluorescence may be related to both the use of a detergent and the monoclonal nature of the antibody in the GSC reagent. Theuseof the Triton X-100 suspension fluid with the BioDx reagent, however, did not noticeably reduce backgroundfluorescence with that product. Since the concentration of Evans blue counterstain in each reagent is not reported in comparable units, the effect of the counterstain on the degree of background fluorescence cannot be determined. Both reagents were relatively easy to use and are stable for 90 days after reconstitution.

In summary, both the GSC monoclonal antibody reagent and the BioDx reagent were 100% sensitive and specific for the identification of *L. pneumophila* serogroups 1 to 6. The GSC reagent, however, also stained *L. pneumophila* groups7, 8, and 9, produced a more uniform staining pattern, and demonstrated less background fluorescence than did the BioDx product, making it the preferred reagent.

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