JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1986, p. 768–771
0095-1137/86/040768-04$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 23, No. 4

# Development of a Standardized Subgrouping Scheme for *Legionella pneumophila* Serogroup 1 Using Monoclonal Antibodies

JEAN R. JOLY,[1]* ROGER M. McKINNEY,[2] JOHN O. TOBIN,[3] WILLIAM F. BIBB,[2] IAN D. WATKINS,[3]† AND DANIELLE RAMSAY[1]

*Département de Microbiologie, Faculté de Médecine, Université Laval, Quebec, Canada G1K 7P4[1]; Biotechnology Branch, Division of Bacterial Diseases, Center for Infectious Disease, Centers for Disease Control, Atlanta, Georgia 30333[2]; and Sir William Dunn School of Pathology, University of Oxford, Oxford, England[3]*

Received 7 November 1985/Accepted 6 January 1986

**A panel of monoclonal antibodies to *Legionella pneumophila* serogroup 1 and a subclassification scheme were developed in a collaborative project among three laboratories. The seven most useful monoclonal antibodies were selected from three previously developed panels on the basis of indirect fluorescent antibody patterns with 83 strains of *L. pneumophila* serogroup 1 that were obtained from widely distributed geographic locations. The isolates were divided into 10 major subgroups on the basis of reactivity patterns that can be readily reproduced in any laboratory and are not subject to major inconsistencies of interpretation of staining intensity. A standard protocol for the indirect fluorescent antibody procedure was also developed.**

*Legionella pneumophila* were first recognized as the etiological agent of Legionnaires disease in 1977 following an epidemic of acute pneumonia in Philadelphia (12, 16). Since then, at least 10 distinct serogroups (1, 3, 9, 10, 18, 21, 22) and more than 20 related species (4) of this bacterium have been identified. Although many of these bacterial species or serogroups have been related to human disease, *L. pneumophila* serogroup 1 is the most frequent etiological agent of Legionnaires disease (25). This same serogroup of *L. pneumophila* is also the one most frequently identified in environmental samples (11). Indeed, it has been found in lakes, cooling towers, and water distribution systems of hospitals, hotels, and private homes (6–8, 26, 28).

This ubiquity has led numerous research teams to propose different subgrouping schemes that could be used in epidemiological studies of Legionnaires disease. Among them are protein profiles as revealed by polyacrylamide gel electrophoresis (15), plasmid profiles (5, 24), agglutination reactions observed with absorbed antisera (5, 27), and agarose immunodiffusion (30). Another procedure that has been proposed frequently is the indirect immunofluorescent antibody technique with monoclonal antibodies (13, 19, 29). However, the different subgroups of *L. pneumophila* serogroup 1 defined by this latter technique are highly dependent on the monoclonal antibodies that are used and, to a lesser extent, on the method used to prepare the antigen.

A collaborative project conducted in three laboratories led to the selection of a panel of 7 of 29 monoclonal antibodies previously reported by these three laboratories (13, 19, 29). These were selected on the basis of reactivity, reproducibility, and ease of interpretation with 83 strains of *L. pneumophila* serogroup 1 obtained from widely distributed geographic locations. Ten distinct subgroups of *L. pneumophila* serogroup 1 were identified with this panel of seven monoclonal antibodies.

* Corresponding author.
† Present address: Amersham International PLC, Forest Farm, Whitchurch, Cardiff, Wales, United Kingdom.

## MATERIALS AND METHODS

**Bacterial strains.** The bacterial strains used in this study belong to 10 antigenic subgroups. *L. pneumophila* Philadelphia 1 ATCC 33152 is representative of the subgroup also containing Adelaide 1, Albuquerque 1, Birmingham 1, Buffalo 1, Cambridge 1, Chamblee 1, Kingston P129, London JR7E, Lyon 1, Lyon 7, New London 1, Oxford 74/81, Pontiac 1, SK-267, SK-273, and SK-304. Allentown 1 ATCC 43106 and Camperdown 1 ATCC 43113 are the only strains of their respective subgroups that were identified. Benidorm 030E ATCC 43108 is representative of the subgroup also containing Los Angeles 2, Lyon 3, Muenchen 1, Orlando 1, Oxford 6842E, Oxford 7384E, Philadelphia 5, Stockholm, Toma 1181, and West Palm Beach 1. Knoxville 1 ATCC 33153 is representative of the subgroup also containing Burlington 1, Burlington 26, Concord 4, Corby 6E, Davenport 1, Indianapolis 10, Kingston 1, Miami Beach 1, and Toma 955a. France 5811 ATCC 43112 is representative of the subgroup also containing France 4999, France 5006, France 5050, France 5871, and Lyon 6. OLDA ATCC 43109 is representative of the subgroup also containing Ann Arbor 6, Bloomington 1, Burlington 2, Burlington 3, Chicago 5, Dallas 1, Darby 1, Houston 1, Houston 3, Long Beach 3, Oxford 10975, San Francisco 9, Tucson 1, and Wadsworth LA ES402. Oxford 4032E ATCC 43110 is representative of the subgroup also containing London JR6E, London JR9E, Portland 2, and Porton 1093E. Heysham 1 ATCC 43107 is representative of the subgroup also containing France 5041, Oxford JR11E, Oxford 8141, and Oxford 105/81E. Bellingham 1 ATCC 43111 is representative of the subgroup also containing Cairo BE, Denver 5, Indianapolis 3, Indianapolis 9, Nottingham RH, Salt Lake City 2, Togus 2, West Haven 2, West Haven 3, West Haven 4, and Winnipeg 2. All these strains were characterized as being *L. pneumophila* serogroup 1 on the basis of their ability to grow on buffered charcoal-yeast extract agar supplemented with 1% alpha-ketoglutarate, their inability to grow on blood agar plates, and their reactivity with specific anti-*L. pneumophila* serogroup 1 fluorescein-labeled rabbit antisera.

**Growth conditions and preparation of the bacterial antigen.**



Downloaded from http://jcm.asm.org/ on September 18, 2020 by guest

TABLE 1. Immunogen, antibody class, and source of each monoclonal antibody used in this study

| Hybridoma | Immunogen | Antibody class | Source | Availability |
|---|---|---|---|---|
| MAB1 | OLDA | IgG | Atlanta | ATCC |
| MAB2 | Knoxville 1 | IgG | Atlanta | ATCC |
| MAB3 | Knoxville 1 | IgG | Atlanta | ATCC |
| W32 | Bellingham 1[a] | IgG | Oxford | NA[b] |
| 33G2 | Philadelphia 1 | IgG | Québec | ATCC |
| 32A12 | Philadelphia 1 | IgG | Québec | ATCC |
| 144C2 | Bellingham 1 | IgG | Québec | ATCC |

[a] Identified in previous publications as Washington (29).
[b] NA, Not available.

Although the bacterial growth conditions were relatively similar in all three laboratories, the methods of preparing the bacterial antigens were highly different. Details of these procedures have been published previously (13, 19, 29). The procedure described here is that found to be optimal (both in terms of ease of preparation and results) by the three collaborating laboratories.

Bacteria were grown for 48 to 72 h on buffered charcoal-yeast extract medium at 37°C. Organisms were harvested from the surfaces of plates with a glass rod and suspended in 1% (vol/vol) Formalin in 0.85% (wt/vol) saline. This suspension was left overnight at room temperature, and optical density was adjusted to 0.35 ± 0.05 at 600 nm.

**Monoclonal antibodies.** The hybridomas used in this study, their antibody classes, and their origins, as well as the bacterial strains that were used to produce them, are listed in Table 1. All of these hybridomas except 144C2 have previously been reported, and all were produced by standard techniques (13, 19, 29). Hybridoma 144C2 was produced and characterized by procedures previously described (13); the fusion partner for this antibody was the NS-0 cell line. The antibodies secreted by these hybridomas were used in the indirect fluorescence assay either as tissue culture supernatant buffered with 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.3), as antibodies purified by ammonium sulfate precipitation (from ascites fluids or tissue culture supernatants), as undiluted ascites fluids, or as ascites fluids diluted 1:5 in 0.2 M boric acid in 0.85% (wt/vol) saline adjusted to pH 7.5 with a concentrated solution of NaOH.

**Indirect immunofluorescence assay.** As for antigen preparation, the details of this technique differed widely between the collaborating laboratories. The one that follows is the one found to be optimal. The bacterial suspension (10 μl) was allowed to dry at room temperature on each well of Teflon-coated microscope slides. The slides were then immersed in acetone for 10 min and air dried. Monoclonal antibodies were added to the slides, and incubation was performed either at room temperature or at 37°C for 30 or 45 min in a humid chamber. After incubation, slides were washed twice for 5 min in 10 mM phosphate-buffered saline (pH 7.2), rinsed briefly in distilled water, and air dried. Fluorescein-labeled rabbit anti-mouse immunoglobulin G (IgG) and IgM diluted in rhodamine-conjugated rabbit anti-*L. pneumophila* serogroup 1 antiserum was then added. Anti-*L. pneumophila* antiserum was prepared with the Knoxville and OLDA strains as described by McKinney et al. (20) and conjugated with 30 μg of tetramethylrhodamine isothiocyanate dissolved in a minimal amount of dimethylformamide per milligram of purified IgG (17). Incubation and washings were done as described above. After brief rinsing in distilled water, the slides were allowed to dry and mounted.

Slides were examined with a Zeiss Standard 14 epi-illumination fluorescence microscope (Carl Zeiss, Oberkochen, Federal Republic of Germany) with a 40× objective, 10× oculars, and an HBO 50-W lamp. Organisms were located with a BP 450-490 exciter filter, an FT 510 dichromatic beam splitter, and an LP520 barrier filter.

## RESULTS

**Preliminary experiments.** The 83 strains of *L. pneumophila* serogroup 1 used in this study were selected from the bacterial collection of each investigator. These were first exchanged between the three laboratories and subgrouped with each panel of monoclonal antibodies. These experiments led to the recognition of 13 subgroups of *L. pneumophila* with the Atlanta set of monoclonal antibodies, 17 with the Oxford set, and nine with the Québec panel.

Because of the large number of strains and monoclonal antibodies to be tested, investigators from each collaborating laboratory selected four monoclonal antibodies within their own panel. These antibodies were selected on the basis of their utility in discriminating different *L. pneumophila* serogroup 1 subgroups and intensity and reproducibility of staining. The selected antibodies were then tested in each of the collaborating laboratories by the subgrouping procedure that was in current use in that laboratory and, for a number of strains, by the method in use in the other laboratories.

**Development of a standardized subgrouping scheme.** In preliminary experiments, antigens, antibody concentrations, anti-mouse immunoglobulins conjugates, and the scoring system were different in each laboratory. Despite these numerous variables, the results were generally reproducible, although discordant results were occasionally obtained. Factors thought to be important in generating these conflicting results were further evaluated.

The following variables were tested before a standard procedure was adopted: (i) antigen preparation (100°C for 10 min, 65°C for 60 min, or Formalin killing at room temperature overnight), (ii) bacterial concentration in the antigen, (iii) age of bacterial culture (24, 48, or 72 h), (iv) monoclonal antibody concentration, (v) length of incubation with monoclonal antibodies and anti-mouse immunoglobulin conjugates (30 or 45 min), (vi) temperature of incubation (room temperature or 37°C), (vii) type of conjugate used to reveal the presence of monoclonal antibodies, and (viii) magnification (×400 or ×630) used to read results. Of the tested variables, the following did not influence the ultimate results with the selected monoclonal antibodies: age of bacterial culture, monoclonal antibody concentration, temperature and time of incubation of the antibodies or conjugates, and magnification used to read slides. With the selected monoclonal antibodies, the method used to prepare the antigen was generally of little importance in the ultimate results. With certain strains, however, boiling for longer than 5 min led to discrepant results probably due to uncovering of antigens deeper in the cell wall. Because of simplicity and reproducibility, we elected to use Formalin-killed bacteria. The addition of a rhodamine-conjugated anti-*L. pneumophila* antiserum in the conjugate was found to be extremely useful in the subgrouping procedure. The use of such an antiserum enabled us to resolve many of the discrepancies in results that were observed between the different laboratories. Indeed, with this rhodamine-conjugated anti-*L. pneumophila* serogroup 1 antiserum, we could easily identify the presence of bacterial subpopulations in certain strains. The

Downloaded from http://jcm.asm.org/ on September 18, 2020 by guest

use of this antiserum also enabled us to focus more easily on the bacteria, and it acted as a control for the presence of bacteria on slides. Cells that were negative with the monoclonal antibodies were easily observed by the pale, reddish-orange fluorescence of the rhodamine stain. When cells were strongly positive with monoclonal antibodies, the weaker rhodamine fluorescence was completely masked by the yellow-green emission of fluorescein.

**Selection of monoclonal antibodies.** Of the 12 monoclonal antibodies selected by the three laboratories, 7 were found to be of particular value in the subgrouping scheme (Table 1). Of the five monoclonal antibodies originally selected and eventually deleted, two (one from Oxford and one from Québec) were found to recognize the same or a closely linked epitope on the bacteria as MAB2 did. The last three monoclonal antibodies were deleted because of the generally nonreproducible results obtained between the different laboratories or because of the intermediate bacterial staining that was obtained with them. MAB1 was selected because it reacted with all strains of *L. pneumophila* serogroup 1 and could be used as a positive control. All the other monoclonal antibodies were selected for their value in discriminating different subgroups and for the reproducibility of the obtained results. The sole exception to this rule was monoclonal antibody 32A12. Although this antibody gave variable results with bacteria belonging to the Knoxville subgroup, it gave clear-cut results with all the other subgroups. The different subgroups identified with these seven monoclonal antibodies are given in Table 2.

## DISCUSSION

The criteria used in this study for the selection of monoclonal antibodies for subgrouping purposes were extremely stringent and resulted in the selection of less than 25% of the antibodies that were initially used. These criteria were as follows: strong fluorescence of the strain used for the production of this monoclonal antibody (positive control), rare intermediate staining of strains to be subgrouped (this resulted most frequently in an all-or-none staining pattern), little overlap between the different subgroups identified by the different monoclonal antibodies, and absence of antigenic variations within a strain upon serial passage on artificial medium. Because such stringent selection criteria were used, we believe that this subgrouping scheme will be generally reproducible in different laboratories.

The number of subgroups identified up to now with this panel of monoclonal antibodies is relatively limited. In addition to the 10 major subgroups already identified, each of the collaborating laboratories could also identify minor antigenic variations within each of the main subgroups recognized in this study when other monoclonal antibodies were used. Although these subgroups may be of use under certain circumstances, we did not believe that the observed differences warranted the addition of these antibodies to the panel and separation of the strains into additional subgroups. The main reason for excluding these antibodies was that they did not give consistent results with all the strains. The proposed subgroups may thus be viewed as major antigenic variants in serogroup 1 of *L. pneumophila*. In a recent study, using absorbed polyclonal antisera, Thomason and Bibb (27) identified 17 distinct subtypes of *L. pneumophila* serogroup 1 that could be grouped in three large subgroups. Their elegant study suggests that these bacteria are antigenically much more variable than what was found by us. However, the use of monoclonal antibodies offers major advantages over absorbed antisera, both in terms of availability of reagents and reproducibility of results when selected monoclonal antibodies are used. Other major subgroups may be identified in the future with the use of other monoclonal antibodies or when the number of strains examined with the current panel is enlarged.

The exact epidemiological value of this subgrouping scheme is still uncertain, although evidence for its utility is accumulating. Indeed, each of the three collaborating laboratories has already published studies in which a given subgroup of *L. pneumophila* serogroup 1 was epidemiologically linked to an outbreak and, in some studies, with possible environmental sources (2, 14, 29). Other laboratories, using either monoclonal or polyclonal antibodies, have also demonstrated the potential utility of a subgrouping scheme for this bacterium (23, 30). A recent study by Watkins et al. showed that certain subgroups of *L. pneumophila* serogroup 1 may be more frequently associated with human disease than are other subgroups (29a). It is thus probable that subgrouping will be as important in the study of legionellosis as other subtyping procedures were for the understanding of the epidemiology of other bacterial diseases.

TABLE 2. Subgroups of *L. pneumophila* identified with the seven monoclonal antibodies selected from the different collaborating laboratories

| Representative strain | Indirect immunofluorescence assay staining intensity with:[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | MAB1 | MAB2 | MAB3 | W32 | 33G2 | 32A12 | 144C2 |
| Philadelphia 1 | 3 | 3 | 0 | 0 | 3 | 3 | 0 |
| Allentown 1 | 3 | 3 | 0 | 0 | 3 | 0 | 0 |
| Benidorm 030E | 3 | 3 | 0 | 0 | 3 | 0 | 3 |
| Knoxville 1 | 3 | 3 | 3 | 0 | 0 | V[b] | 0 |
| France 5811 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| OLDA | 3 | 0 | 0 | 0 | 0 | 3 | 3 |
| Oxford 4032E | 3 | 0 | 0 | 0 | 0 | 3 | 0 |
| Heysham 1 | 3 | 0 | 3 | 0 | 0 | 3 | 3 |
| Camperdown 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bellingham 1 | 3 | 0 | 0 | 3 | 0 | 0 | 3 |

[a] Staining intensity: 3, bright fluorescence; 2, good fluorescence; 1, barely visible fluorescence; 0, no fluorescence.

[b] Variable results were obtained with this monoclonal antibody and the different *L. pneumophila* serogroup 1 strains belonging to this subgroup.

## ACKNOWLEDGMENTS

This study was supported in part by grant MA-7689 from the Medical Research Council of Canada, a grant from the British Medical Research Council, and Inveresk Research International Ltd.

We thank all the microbiologists who provided us with the different strains of *L. pneumophila* serogroup 1 used in this study.

## LITERATURE CITED

1. **Bibb, W. F., P. M. Arnow, D. L. Dellinger, and S. R. Perryman.** 1983. Isolation and characterization of a seventh serogroup of *Legionella pneumophila*. J. Clin. Microbiol. **17**:346–348.
2. **Bibb, W. F., P. M. Arnow, L. Thacker, and R. M. McKinney.** 1984. Detection of soluble *Legionella pneumophila* antigens in serum and urine specimens by enzyme-linked immunosorbent assay with monoclonal and polyclonal antibodies. J. Clin. Microbiol. **20**:478–482.
3. **Bisset, M. L., J. O. Lee, and D. S. Lindquist.** 1983. A new serogroup of *Legionella pneumophila*, serogroup 8. J. Clin. Microbiol. **17**:887–891.
4. **Brenner, D. J., A. G. Steigerwalt, G. W. Gorman, H. W.**

Downloaded from http://jcm.asm.org/ on September 18, 2020 by guest

**Wilkinson, W. F. Bibb, M. Hackel, R. L. Tyndall, J. Campbell, J. C. Feeley, W. L. Thacker, P. Skaliy, W. T. Martin, B. J. Brake, B. S. Fields, H. V. McEachern, and L. K. Corcoran.** 1985. Ten new species of *Legionella*. Int. J. Syst. Bacteriol. **35**:50–59.

5. **Brown, A., R. M. Vickers, E. M. Elder, M. Lema, and G. M. Garrity.** 1982. Plasmid and surface antigen markers of endemic and epidemic *Legionella pneumophila* strains. J. Clin. Microbiol. **16**:230–235.
6. **Cordes, L. G., D. W. Fraser, P. Skaliy, C. A. Perlino, W. R. Elsea, G. F. Mallison, and P. S. Hayes.** 1980. Legionnaires' disease outbreak at an Atlanta, Georgia, country club: evidence for spread from an evaporative condenser. Am. J. Epidemiol. **111**:425–431.
7. **Dennis, P. J., J. A. Taylor, R. B. Fitzgeorge, C. L. R. Bartlett, and G. I. Barrow.** 1982. *Legionella pneumophila* in water plumbing systems. Lancet **i**:949–951.
8. **Dondero, T. J., R. C. Rendtorff, G. F. Mallison, R. M. Weeks, J. S. Levy, E. W. Wong, and W. Schaffner.** 1980. An outbreak of Legionnaires' disease associated with a contaminated air conditioning cooling tower. N. Engl. J. Med. **302**:365–370.
9. **Edelstein, P. H., W. F. Bibb, and G. W. Gorman.** 1984. *Legionella pneumophila* serogroup 9: a cause of human pneumonia. Ann. Intern. Med. **101**:196–199.
10. **England, A. C. III, R. M. McKinney, P. Skaliy, and G. W. Gorman.** 1980. A fifth serogroup of *Legionella pneumophila*. Ann. Intern. Med. **93**:58–59.
11. **Fliermans, C. B., W. B. Cherry, L. H. Orrison, S. J. Smith, D. L. Tison, and D. H. Pope.** 1981. Ecological distribution of *Legionella pneumophila*. Appl. Environ. Microbiol. **41**:9–16.
12. **Fraser, D. W., T. F. Tsai, W. Orenstein, W. E. Parkin, H. G. Beecham, R. G. Scharrer, J. Harris, G. F. Mallison, S. M. Martin, J. E. McDade, C. C. Shepard, P. S. Brachman, and the Field Investigation Team.** 1977. Legionnaire's disease: description of an epidemic of pneumonia. N. Engl. J. Med. **297**:1189–1197.
13. **Joly, J. R., Y.-Y. Chen, and D. Ramsay.** 1983. Serogrouping and subtyping of *Legionella pneumophila* with monoclonal antibodies. J. Clin. Microbiol. **18**:1040–1046.
14. **Joly, J. R., and W. C. Winn.** 1984. Correlation of subtypes of *Legionella pneumophila* defined by monoclonal antibodies with epidemiological classification of cases and environmental sources. J. Infect. Dis. **150**:667–671.
15. **Lema, M., and A. Brown.** 1983. Electrophoretic characterization of soluble protein extracts of *Legionella pneumophila* and other members of the family *Legionellaceae*. J. Clin. Microbiol. **17**:1132–1140.
16. **McDade, J. E., C. C. Shepard, D. W. Fraser, T. R. Tsai, M. A. Redus, W. R. Dowdle, and the Laboratory Investigation Team.** 1977. Legionnaire's disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N. Engl. J. Med. **297**:1197–1203.
17. **McKinney, R. M., and J. T. Spillane.** 1975. An approach to quantitation in rhodamine isothiocyanate labeling. Ann. N.Y. Acad. Sci. **254**:55–64.
18. **McKinney, R. M., L. Thacker, P. P. Harris, K. R. Lewallen, G. A. Hébert, P. H. Edelstein, and B. M. Thomason.** 1979. Four serogroups of Legionnaires' disease bacteria defined by immunofluorescence. Ann. Intern. Med. **90**:621–624.
19. **McKinney, R. M., L. Thacker, D. E. Wells, M. C. Wong, W. L. Jones, and W. F. Bibb.** 1983. Monoclonal antibodies to *Legionella pneumophila* serogroup 1. Possible applications in diagnostic tests and epidemiologic studies. Zentralbl. Bakteriol. Mikrobiol. Hyg. I Abt. Orig. A **255**:91–95.
20. **McKinney, R. M., B. M. Thomason, P. P. Harris, L. Thacker, K. R. Lewallen, H. W. Wilkinson, G. A. Hebert, and C. W. Moss.**1979. Recognition of a new serogroup of Legionnaires disease bacterium. J. Clin. Microbiol. **9**:103–107.
21. **McKinney, R. M., H. W. Wilkinson, H. M. Sommers, B. J. Fikes, K. R. Sasseville, M. M. Yungbluth, and J. S. Wolf.** 1980. *Legionella pneumophila* serogroup six: isolation from cases of legionellosis, identification by immunofluorescence staining, and immunological response to infection. J. Clin. Microbiol. **12**:395–401.
22. **Meenhorst, P. L., A. L. Reingold, D. G. Groothuis, G. W. Gorman, H. W. Wilkinson, R. M. McKinney, J. C. Feeley, D. J. Brenner, and R. van Furth.** 1985. Water-related nosocomial pneumonia caused by *Legionella pneumophila* serogroups 1 and 10. J. Infect. Dis. **152**:356–364.
23. **Para, M. F., and J. F. Plouffe.** 1983. Production of monoclonal antibodies to *Legionella pneumophila* serogroups 1 and 6. J. Clin. Microbiol. **18**:895–900.
24. **Plouffe, J. F., M. F. Para, W. E. Maher, B. Hackman, and L. Webster.** 1983. Subtypes of *Legionella pneumophila* serogroup 1 associated with different attack rates. Lancet **ii**:649–650.
25. **Reingold, A. L., B. M. Thomason, B. J. Brake, L. Thacker, H. W. Wilkinson, and J. N. Kuritsky.** 1984. *Legionella pneumophila* in the United States: the distribution of serogroups and species causing human illness. J. Infect. Dis. **149**:819.
26. **Stout, J., V. L. Yu, R. M. Vickers, J. Zuravlef, M. Best, A. Brown, R. B. Yee, and R. Wadowsky.** 1982. Ubiquitousness of *Legionella pneumophila* in the water supply of a hospital with endemic Legionnaires' disease. N. Engl. J. Med. **306**:466–468.
27. **Thomason, B. M., and W. F. Bibb.** 1984. Use of absorbed antisera for demonstration of antigenic variation among strains of *Legionella pneumophila* serogroup 1. J. Clin. Microbiol. **19**:794–979.
28. **Tobin, J. O., J. Beare, M. S. Dunnill, M. Franch, P. J. Morris, S. Fisher-Hoch, R. G. Mitchell, and M. F. Muers.** 1980. Legionnaires' disease in a transplant unit: isolation of the causative agent from shower baths. Lancet **ii**:118–121.
29. **Watkins, I. D., and J. O. Tobin.** 1984. Studies with monoclonal antibodies to *Legionella* species, p. 259–262. *In* C. Thornsberry, A. Balows, J. C. Feeley, and W. Jakubowski (ed.), Legionella: proceedings of the 2nd International Symposium. American Society for Microbiology, Washington, D.C.

29a. **Watkins, I. D., J. O. Tobin, P. J. Dennis, W. Brown, R. Newham, and J. B. Kurtz.** 1985. *Legionella pneumophila* serogroup 1 subgrouping by monoclonal antibodies—an epidemiological tool. J. Hyg. **95**:211–216.

30. **Zanem-Lim, O. G., N. J. van den Broek, P. G. J. M. Rietra, and H. C. Zanen.** 1984. Comparison of strains of *Legionella pneumophila* serogroup 1 isolated in four hospitals from patients and hot-water supplies. J. Infect. Dis. **150**:508–512.

Downloaded from http://jcm.asm.org/ on September 18, 2020 by guest