JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1992, p. 1016–1018
0095-1137/92/041016-03$02.00/0

Vol. 30, No. 4

Rapid Identification of Legionellae by a Colony Blot Assay Based on a Genus-Specific Monoclonal Antibody

IVO STEINMETZ, CLAUDIA RHEINHEIMER, AND D. BITTER-SUERMANN*

Institute of Medical Microbiology, Hannover Medical School, 3000 Hannover 61, Germany

Received 9 September 1991/Accepted 31 December 1991

We recently developed a monoclonal antibody immunoglobulin G2a (2125) recognizing a genus-specific epitope on the 60-kDa heat shock protein of all *Legionella* species. In the current study, this antibody was used in a colony blot enzyme-linked immunosorbent assay for the rapid identification of *Legionella* cultures on agar plates. The whole protocol was completed in less than 2 h. All 59 *Legionella* species and serogroups that were tested gave a positive signal. No unspecific reactions with nonlegionellae were observed. This test is a rapid procedure for the identification of legionellae growing on agar medium to the genus level.

The different selective media currently used for the cultivation of legionellae are not sufficiently selective and allow many non-*Legionella* organisms to grow. Procedures such as the pretreatment of specimens by heat (4) or acid (3) cannot abolish contaminating bacterial flora completely. The different *Legionella* species show a heterogeneous morphology and are not always clearly distinguishable from nonlegionellae by morphological criteria. The identification of legionellae grown on buffered charcoal yeast extract (BCYE) agar is a long and complex procedure. Colonies are streaked on different agar media to confirm *Legionella*-specific growth requirements; they are then subjected to biochemical characterization, fatty acid analysis, and immunofluorescent analysis (1). Since primary cultures of legionellae from clinical or environmental specimens are often difficult to obtain and might take several days, time-saving methods must be developed to identify legionellae to the genus level as soon as colonies are visible on the agar medium.

In a prior study, we used a genus-specific monoclonal antibody (MAb) to establish a sandwich enzyme-linked immunosorbent assay which might be useful for a direct examination of environmental or clinical specimens for

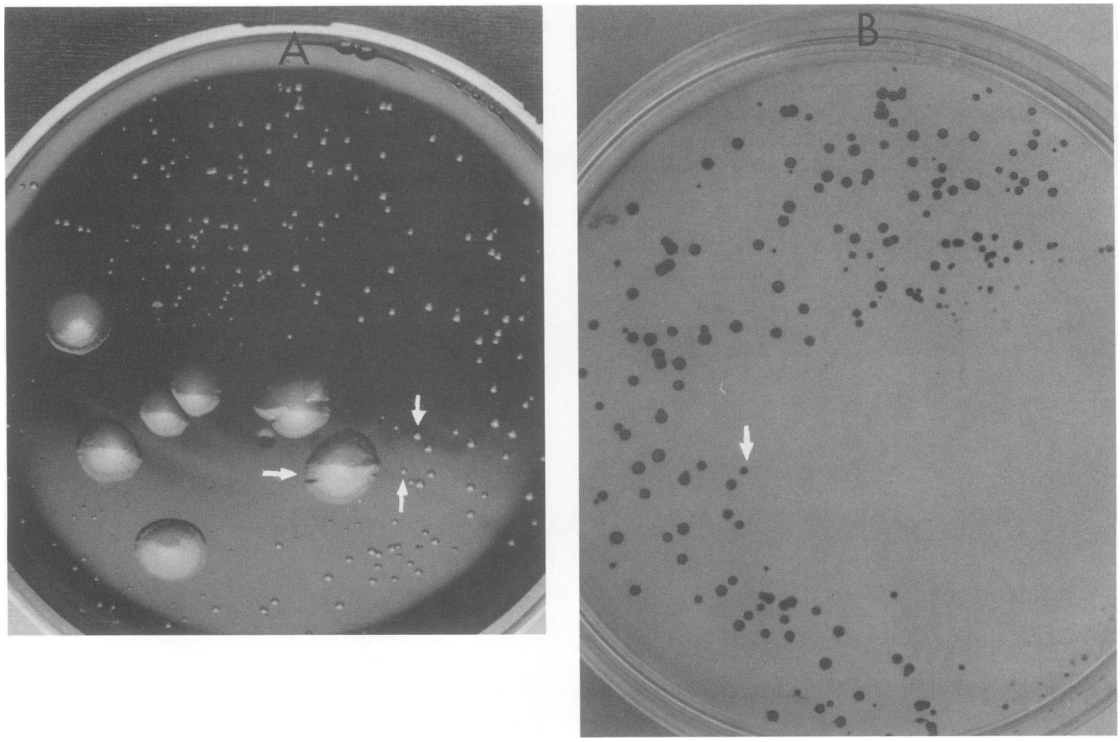

FIG. 1. BCYE agar plate (medium I) containing colonies of *P. aeruginosa* (→), *S. aureus* (↑), and *Legionella oakridgensis* (↓) (A) and the mirror image as the corresponding *Legionella*-specific colony blot (B). A suspension of the three different strains was prepared, seeded on the agar plate, and incubated for 48 h. The colony blot was performed as described in the text.

* Corresponding author.

TABLE 1. Strains used

Organism	Source
L. pneumophila[a]	
Legionella steigerwaltii	ATCC 35302
L. anisa	ATCC 35291
L. cherrii	ATCC 35252
L. erythra	ATCC 35303
L. feeleii	ATCC 35072
L. hackeliae	ATCC 35250
L. jamestowniensis	ATCC 35298
L. maceachernii	ATCC 35300
L. parisiensis	ATCC 35299
L. israelensis	ATCC 43119
L. spiritensis	ATCC 35249
L. santicrusis	ATCC 35301
L. birminghamensis	ATCC 43702
L. bocemanii	ATCC 33217
L. dumoffii	ATCC 33279
L. gormanii	ATCC 33297
L. jordanis	ATCC 33623
L. longbeachae	ATCC 33462
L. micdadei	ATCC 33204
L. oakridgensis	ATCC 33761
L. sainthelensi	ATCC 49322
L. sainthelensi	ATCC 35248
L. wadsworthii	ATCC 33877
L. brunensis	ATCC 43878
L. morawica	ATCC 43877
L. quinlivanii	ATCC 43830
L. tucsonensis	ATCC 49180
L. cincinnatiensis	ATCC 43753
L. rubrilucens	ATCC 35304
L. pneumophila strains 040741 and 038695[b]	IMM[c]
L. pneumophila strain UM2[b]	WFM[d]
P. fluorescens (2 strains)	ATCC 49270, ATCC 49271
P. aeruginosa (2 strains)	ATCC 49266, ATCC 49267
Alcaligenes faecalis	ATCC 15173
Streptococcus agalactiae	IMM
S. faecalis	ATCC 29212
S. pyogenes	IMM
Salmonella typhimurium	IMM
S. enteritidis	IMM
Shigella flexneri	IMM
Enterobacter cloacae	IMM
Serratia marcescens	IMM
Proteus vulgaris	IMM
Morganella morganii	IMM
Yersinia enterocolitica	IMM
Y. pseudotuberculosis	IMM
Vibrio parahaemolyticus	IMM
Plesiomonas shigelloides	IMM
Aeromonas hydrophila	IMM
Pseudomonas cepacia	IMM
Flavobacterium sp.	IMM
Haemophilus influenzae type a[e]	NCTC 8466
H. influenzae type b[e]	NCTC 7279
H. influenzae type c[e]	NCTC 8469
Pseudomonas diminuta[e]	ATCC 19146
Campylobacter fetus (2 strains)[e]	IMM
Staphylococcus epidermidis (2 strains)[b]	IMM
Neisseria sp. (2 strains)[b]	IMM
Escherichia coli[b]	IMM
Citrobacter freundii[b]	IMM
Klebsiella oxytoca[b]	IMM
Proteus mirabilis (2 strains)[b]	IMM
P. aeruginosa (2 strains)[b]	IMM
Xanthomonas maltophilia (2 strains)[b]	IMM
Lactobacillus sp.[b]	IMM
C. albicans (3 strains)[b]	IMM
S. aureus (4 strains)[b]	IMM
Acinetobacter junii (2 strains)[b]	IMM

[a] Twenty-eight *L. pneumophila* strains belonging to serogroups 1 to 14 were obtained from the American Type Culture Collection and the National Collection of Type Cultures.
[b] Clinical isolate from a respiratory tract specimen.
[c] IMM, Institute of Medical Microbiology, Hannover, Germany.
[d] WFM, Wasserforschung Mainz GmbH, Mainz, Germany.
[e] Strains tested in the immunoblot.

legionellae without cultivation (6). In this study, we developed a straightforward and rapid colony blot assay based on this genus-specific MAb which makes it possible to screen hundreds of colonies in one test and to identify legionellae growing on agar medium in less than 2 h.

Table 1 lists the strains used in this study. *Legionella* strains and other species were grown on BCYE agar plates (Biomérieux, Marcy L'Etoile, France) containing 100 μg of vancomycin per 100 ml and 4,500 U of colistin per 100 ml (medium I). All non-*Legionella* strains used in the colony blot were isolated from respiratory tract specimens and were primarily grown on medium I. For identification, these strains were grown on blood agar. In addition, these strains were seeded on BCYE agar plates (Oxoid) containing either BMPA (Oxoid) selective supplement (medium II) or MWY (Oxoid) selective supplement (medium III) and on medium I containing 0.3% (wt/vol) glycine (medium IV). Nearly all non-*Legionella* strains could be cultured on the different selective media (mediums I to IV; *Candida albicans* did not grow on mediums II and III, and one *Staphylococcus aureus* strain did not grow on medium II) and confirm the inability of these selective media to inhibit the growth of nonlegionellae completely.

The production and characterization of the genus-specific MAb 2125 has been described previously (6). MAb 2125 recognizes a genus-specific epitope on the 60-kDa heat shock protein of all *Legionella* species. MAb 2125 was purified by affinity chromatography on protein A Sepharose (Pharmacia, Uppsala, Sweden) and was biotinylated as described previously (6). To perform the colony blot, bacteria were grown for 48 to 72 h at 37°C. The nitrocellulose (NC) filters (0.45-μm pore size, 82 mm [diameter]; BAS 85; Schleicher & Schuell, Germany) were laid on the surface of the agar plate and allowed to adhere for 1 min. NC filters were then carefully removed, placed on a metal grid, and exposed to hot steam for 5 min. For lysis of the adherent bacteria, each NC filter was then immersed in a petri dish containing 10 ml of 1% (wt/vol) Triton X-100 and agitated by gentle rotation for 20 min. The filter disks were washed twice with buffer A (0.01 M potassium phosphate buffer made isotonic with saline, pH 7.5) and then incubated with buffer A–IDM-BGG (buffer A containing 3% [wt/vol] instant dried milk and 0.2% [wt/vol] bovine gamma globulin [BGG; Sigma Chemical Co., St. Louis, Mo.]) for 20 min. The filter disks were washed twice with buffer A and then incubated with biotinylated MAb 2125 diluted 1:1,000 in buffer A–IDM-BGG for 30 min. After washing twice with buffer A, peroxidase-conjugated Streptavidin (Jackson Immunoresearch Laboratories, Inc.) diluted 1:5,000 in 0.05 M Tris buffer made isotonic with saline, pH 7.4, was added for 30 min. The NC filters were developed with 4-chloro-1-naphtol as the substrate. The reaction was stopped by rinsing the filters in tap water.

An effective lysis of the cells seemed to be essential since the main part of the 60-kDa heat shock protein is found in the cytoplasm (6). Colony blots performed with detergents only did not result in a positive signal with all *Legionella* spp. However, treatment with detergents and hot steam resulted in the uniform detection of all *Legionella* spp. *Legionella* cultures could be visualized as distinct dark blue dots on the NC filter (Fig. 1). A positive blotting reaction was obtained with all *Legionella* strains tested (Table 1). In order to evaluate the performance of this test with primary cultures from clinical or environmental samples, we tested primary cultures of *Legionella pneumophila* grown on medium I from bronchial lavages from two patients (strains 040741 and 038695) in the colony blot. Both cultures gave clear positive results (not shown because the colony blots obtained show identical characteristics as the one in Fig. 1). In addition, we tested primary cultures of *L. pneumophila* obtained from tap water (strain UM2) which also gave a positive signal (not shown for the same reason as explained above). Bacterial colonies of non-*Legionella* strains isolated from respiratory tract specimens (Table 1) did not show any reaction when tested either as a monoculture or in combination. A slight reaction of the biotinylated MAb 2125 with colonies of *S. aureus*, probably due to the reactivity of the immunoglobulin with protein A, could be easily eliminated by the addition of bovine gamma globulin in excess to the buffer used to saturate the NC filters and to dilute biotinylated MAb 2125.

With the colony blot assay described herein, it is possible to obtain an immunological confirmation of agar grown legionellae in less than 2 h. A previous study described a membrane immunoassay in which only colonies of *L. pneumophila* serogroups 1 to 8 were detected and diffuse cross-reactions with *Pseudomonas fluorescens* were observed (2).

A recent study described three additional MAbs against the 58-kDa heat shock protein of *L. pneumophila* which do not react with all *Legionella* species and serogroups and cross-react with different gram-negative bacteria (5). In addition to our already described extensive testing for cross-reactivities of the genus-specific MAb 2125, we included those cross-reactive species (Table 1) which were not examined previously and tested them in the immunoblot with MAb 2125 as described previously (6). No cross-reactivities could be observed. We conclude that our *Legionella*-specific colony blot assay based on the genus-specific MAb 2125 provides a highly specific and rapid method for the identification of legionellae.

REFERENCES

1. **American Public Health Association.** 1985. Standard methods for the examination of water and wastewater, 16th ed., p. 934–935. American Public Health Association, Washington, D.C.
2. **Bérubé, A., M. Trudel, and P. Payment.** 1989. Rapid detection and identification of *Legionella pneumophila* by a membrane immunoassay. Appl. Environ. Microbiol. **55:**1640–1641.
3. **Bopp, C. A., J. W. Sumner, G. K. Morris, and J. G. Wells.** 1981. Isolation of *Legionella* spp. from environmental water samples by low-pH treatment and use of a selective medium. J. Clin. Microbiol. **13:**714–719.
4. **Edelstein, P. H., J. B. Snitzer, and J. A. Bridge.** 1982. Enhancement of recovery of *Legionella pneumophila* from contaminated respiratory tract specimens by heat. J. Clin. Microbiol. **16:**1061–1065.
5. **Sampson, J. S., B. B. Plikaytis, C. H. Aloisio, G. M. Carlone, C.-P. Pau, and A. R. Stinson.** 1991. Immunologic characterization and specificity of three monoclonal antibodies against the 58-kilodalton protein of *Legionella pneumophila*. J. Clin. Microbiol. **29:**836–841.
6. **Steinmetz, I., C. Rheinheimer, I. Hübner, and D. Bitter-Suermann.** 1991. Genus-specific epitope on the 60-kilodalton *Legionella* heat shock protein recognized by a monoclonal antibody. J. Clin. Microbiol. **29:**346–354.