APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2006, p. 4489–4491 Vol. 72, No. 6
0099-2240/06/$08.00+0 doi:10.1128/AEM.00346-06

Evaluation of the Duopath *Legionella* Lateral Flow Assay for Identification of *Legionella pneumophila* and *Legionella* Species Culture Isolates

Jürgen Herbert Helbig,[1]* Paul Christian Lück,[1] Britta Kunz,[2] and Andreas Bubert[2]

Institute of Medical Microbiology and Hygiene, Medical Faculty of the Technical University Dresden, Dresden,[1] and Life Science & Analytics Division, Merck KGaA, Darmstadt,[2] Germany

Received 13 February 2006/Accepted 10 April 2006

Duopath *Legionella* (Merck KGaA, Darmstadt, Germany) is a new immunochromatographic assay for the simultaneous identification of cultured *L. pneumophila* and *Legionella* species other than *L. pneumophila*. In tests of 89 *L. pneumophila* strains and 87 *Legionella* strains other than *L. pneumophila* representing 41 different species, Duopath and a widely used latex agglutination assay detected *L. pneumophila* with 100% and 98% accuracy, respectively, whereas the percentages differed significantly for other *Legionella* spp. (93% versus 37% [$P < 0.001$]). Since many countries' regulations require the identification of *Legionella* spp. in water and environmental samples, the use of Duopath *Legionella* to comply with those regulations could contribute to significantly fewer false-negative results.

Legionellae are ubiquitous bacteria in the natural aquatic environment and often colonize man-made aquatic environments. When inhaled in aerosol form, legionellae may cause a severe, atypical pneumonia named Legionnaires' disease. Currently, the genus *Legionella* is known to include 50 species (see J. P. Euzéby's List of Prokaryotic Names with Standing in Nomenclature [www.bacterio.cict.fr/]). Some of the species have been isolated from only environmental sources to date, but it is generally accepted that all species may cause pneumonia, especially in immunocompromised persons. For monitoring of water systems, the "gold standard" method in a routine laboratory for identification of legionellae is enrichment on glycine-vancomycin-polymyxin B-cycloheximide (GVPC) agar plates with subsequent confirmation that is mostly done by serological methods. Unfortunately, the huge number of *Legionella* spp. and *L. pneumophila* serogroups represent a very wide serological heterogeneity which can lead to false-negative results. Molecular methods could circumvent this limitation, but these methods are generally not user-friendly and convenient. A new immunochromatographic identification (lateral flow) assay named Duopath *Legionella* has been recently developed by Merck KGaA and is intended for the simultaneous recognition of *L. pneumophila* and other *Legionella* spp. on the same test device. Separate recognition of *L. pneumophila* within the genus *Legionella* is based on the use of monoclonal antibodies that recognize species-specific and genus-specific epitopes of the Mip proteins (3). Here we describe the evaluation of this new assay in comparison with the widely used latex agglutination assay (*Legionella* latex test) from Oxoid, Basingstoke, United Kingdom, which recognizes the most important *Legionella* spp. causing pneumonia. While the latex assay contains latex suspensions for other *Legionella* spp. separate from those for *L. pneumophila* serogroup 1 and for serogroups 2 to 14, Duopath *Legionella* is composed like a pregnancy test, with separate detection zones for *Legionella* spp. and for *L. pneumophila* (all serogroups) on the same test device.

For evaluation of Duopath *Legionella*, *Legionella* type strains or water samples were cultured on GVPC agar (Merck, Darmstadt, Germany) for 3 to 5 days. The prolongation of growth for up to 2 weeks had no influence on the results (data not shown). Patient isolates were grown on BMPA agar (Oxoid, Wesel, Germany). One suspect *Legionella* colony (approximate colony diameter, 1 to 2 mm) was resuspended in a 0.9% NaCl solution containing 1% Tween 20. After the addition of polymyxin B (*Bacillus cereus* selective supplement; Merck, Darmstadt, Germany), the suspension was incubated for 2 to 5 min at room temperature followed by 5 min at 95°C and cooled to room temperature for pipetting into the sample port of Duopath *Legionella*. Results at test and control zones were read after 30 min without a magnifying glass. The ability of Duopath *Legionella* to identify *L. pneumophila* and other *Legionella* spp. was compared with that of the appropriate latex assay of Oxoid recognizing *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2 to 14, or other *Legionella* spp. Tests were performed according to the manufacturer's description.

Specificity of Duopath *Legionella*. The specificity was calculated by testing a total of 87 bacterial strains isolated from water samples and grown on GVPC agar plates (Merck, Darmstadt, Germany) or from human sources and grown on BMPA agar (Oxoid, Wesel, Germany). All of these strains were able to grow on blood agar (Merck, Darmstadt, Germany), which is the first exclusion criterion for identification of *Legionella* spp. Afterwards they were identified using the API system (bioMerieux, Nürtingen, Germany) as *Acinetobacter baumannii* (2 strains), *Acinetobacter haemolyticus* (2), *Acinetobacter* sp. (3), *Aeromonas hydrophila* (1), *Alcaligenes faecalis* (1), *Brevundimonas diminuta* (1), *Burkholderia cepacia* (4), *Citrobacter freundii* (2), *Escherichia coli* (2), *Pseudomonas aeruginosa* (40),

* Corresponding author. Mailing address: Institute of Medical Microbiology and Hygiene, Medical Faculty of the Technical University Dresden, Fetscherstr. 74, D-01307 Dresden, Germany. Phone: 49-351-4586577. Fax: 49-351-4586310. E-mail: j.helbig@mailbox.tu-dresden.de.

TABLE 1. Identification of strains of *Legionella* other than *L. pneumophila* by Duopath *Legionella* and latex agglutination assay

Legionella sp. (serogroup)	Designation	*Legionella* type strain test result[a]		No. of water isolates positive/total no. tested[b]	
		Duopath *Legionella*	Latex agglutination	Duopath *Legionella*	Latex agglutination
L. adelaidensis	ATCC 49625	o	o	0/0	0/0
L. anisa	ATCC 35292	+	+	2/2	2/2
L. birminghamensis	ATCC 43702	+	o	0/0	0/0
L. bozemanae (1)	ATCC 33217	+	+	0/0	0/0
L. bozemanae (2)	ATCC 35545	+	+	0/0	0/0
L. bozemanae				3/4	4/4
L. brunensis	ATCC 43878	+	o	0/0	0/0
L. cherrii	NCTC 11976	+	+	1/1	1/1
L. cincinnatiensis	NCTC 12438	+	+	0/0	0/0
L. dumoffii	ATCC 33279	+	+	1/1	1/1
L. erythra (1)	ATCC 35303	+	o	1/1	0/1
L. fairfieldensis	ATCC 49588	+	o	0/0	0/0
L. feeleii (1)	ATCC 35072	+	o	0/0	0/0
L. feeleii				4/4	0/4
L. geestiana	ATCC 49504	+	o	0/0	0/0
L. gormanii	ATCC 33297	+	+	1/1	1/1
L. gratiana	ATCC 49413	+	+	0/0	0/0
L. hackeliae (1)	ATCC 35250	+	o	0/0	0/0
L. israelensis	NCTC 12010	+	o	0/0	0/0
L. jamestowniensis	ATCC 35298	+	o	1/1	0/1
L. jordanis	ATCC 33623	+	+	2/2	2/2
L. lansingensis	ATCC 49751	+	o	0/0	0/0
L. londiniensis	ATCC 49505	+	o	3/3	0/3
L. longbeachae (1)	ATCC 33462	+	+	0/0	0/0
L. longbeachae (2)	ATCC 33484	+	+	0/0	0/0
L. maceachernii	ATCC 35300	+	o	5/5	0/5
L. micdadei	ATCC 33218	+	+	3/3	3/3
L. moravica	ATCC 43877	+	o	0/0	0/0
L. nautarum	ATCC 49506	+	o	0/0	0/0
L. oakridgensis	ATCC 33761	+	o	1/1	0/1
L. parisiensis	ATCC 35299	+	+	0/0	0/0
L. quateirensis	NCTC 12376	+	o	0/0	0/0
L. quinlivanii (1)	ATCC 43830	+	o	0/0	0/0
L. rubrilucens	ATCC 35304	+	o	4/4	0/4
L. sainthelensi (1)	ATCC 35248	+	o	1/1	0/1
L. santicrucis	NCTC 11989	o	o	0/0	0/0
L. shakespearei	ATCC 49655	+	o	0/0	0/0
L. spiritensis (1)	NCTC 11990	+	o	0/0	0/0
L. spiritensis (2)	NCTC 12082	+	o	0/0	0/0
L. steigerwaltii	ATCC 35302	o	+	3/3	3/3
L. taurinensis				5/5	0/5
L. tucsonensis	NCTC 12439	+	+	1/1	0/1
L. wadsworthii	ATCC 33877	o	o	0/0	0/0
L. waltersii	NCTC 13017	+	o	0/0	0/0
L. worsleiensis	ATCC 49507	+	o	1/1	0/1
Legionella genomospecies A				1/1	0/1

[a] +, positive; o, negative. Of 42 strains tested, 38 (90.5%) gave positive results by Duopath *Legionella* and 15 (35.7%) gave positive results by the latex agglutination assay.

[b] Of 45 strains tested, 43 (95.6%) gave positive results by Duopath *Legionella* and 17 (37.8%) gave positive results by the latex agglutination assay.

Pseudomonas alcaligenes (2), *Pseudomonas putida* (2), *Pseudomonas stutzeri* (1), *Pseudomonas* sp. (4), *Serratia marcescens* (2), and *Stenotrophomonas maltophilia* (18). None of those bacteria tested positive by Duopath *Legionella*; thus, the specificity of the testing was 100%.

Identification of *L. pneumophila*. A total of 89 *L. pneumophila* strains were tested with both assays. The details of the strains and isolates tested were as follows: (i) type strains of monoclonal subgroups of serogroup 1 ($n = 10$) according to Joly et al. (4), (ii) ATCC serogroup type strains of serogroups 2 to 15 ($n = 14$), and (iii) water or patient isolates ($n = 65$) confirmed as *L. pneumophila* by use of MONOFLUO anti-*Legionella* staining reagent (Bio-Rad, Munich, Germany). By Duopath *Legionella*, all of them were detected as providing bands at the detection zones for both *L. pneumophila* and other *Legionella* spp. When the agglutination assays for serogroup 1 and serogroups 2 to 14 were used, two of the *L. pneumophila* isolates gave reproducibly negative results with the Oxoid test. The possibility of a prozone phenomenon was excluded. Serogroup typing of these strains failed with rabbit sera and serogroup-specific monoclonal antibodies produced in our laboratory (2) as well as with monovalent fluorescein isothiocyanate-conjugated anti-*Legionella* (serogroups 1 to 14) rabbit sera (Prolab Diagnostics, Neston, United Kingdom).

Recognition of *Legionella* spp. For recognition of *Legionella* spp., 42 *Legionella* ATCC or NCTC type strains belonging to 39 different species other than *L. pneumophila* were included in this study (Table 1). For three species (*L. bozemanae*, *L. longbeachae*, and *L. spiritensis*), members of both known serogroups were analyzed. In addition, 45 environmental isolates belonging to 20 *Legionella* spp. (one to five strains per species) were tested. These isolates were classified on the species level by *mip* gene sequencing (5) according to the guidelines and databases of the European Working Group for *Legionella* Infections, which are available in the web site www.ewgli.org (link: "Typing and Identification Schemes").

Duopath *Legionella* recognized 38 (90%) of the 42 *Legionella* type strains other than *L. pneumophila* (Table 1), while the latex assay recognized only 15 of these 42 strains (36%). In tests of the environmental isolates, Duopath *Legionella* was positive for 43 of the 45 strains tested (96%) and the latex assay was positive for 17 (38%). Interestingly, for Duopath *Legionella* there was no absolute agreement to the species level represented by the type strains as seen for *L. steigerwaltii*. The three environmental isolates' test results were positive, whereas the type strain result was negative. With all positive-testing *Legionella* strains other than *L. pneumophila*, Duopath *Legionella* always provided a specific signal only at the *Legionella* sp. test zone but never at the *L. pneumophila* test zone.

Superiority of Duopath *Legionella* over the latex assay for identification of legionellae in water systems. In summary, the latex agglutination assay is aimed at recognizing the *Legionella* species most frequently causing Legionnaires' disease (*L. anisa*, *L. bozemanae*, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. longbeachae*, and *L. micdadei*) but not the wide range of legionellae found in water systems, which are also suspected to be pneumonia pathogens. According the manufacturer's instructions, cross-reactions have been reported to occur occasionally with certain serotypes with at least eight other *Legionella* spp. Among all of the *Legionella* strains other than *L. pneumophila* involved in this study ($n = 87$), the Duopath test correctly detected 93% whereas the agglutination assay identified significantly fewer (37% [$P < 0.001$]).

The guidelines of the Centers for Disease Control and Prevention (1) and of the European Working Group for *Legionella* Infections (www.ewgli.org [link: "EQA Water Scheme"]) for monitoring of water systems recommend testing for other *Legionella* spp. as well as for *L. pneumophila*. Given the low number of *Legionella* spp. recognized in our testing, it can be assumed that a significant number of false-negative results occur when the latex assay is used. Here, Duopath *Legionella* revealed that it possesses an important advantage over the latex assay and that its use would make the phenotypic diagnostic gap significantly smaller. Therefore, Duopath *Legionella* can be considered a user-friendly, simple, and reliable test for the simultaneous identification of *L. pneumophila* and other *Legionella* strains.

We thank Birgit Bubert for excellent consultancy and Sigrid Gäbler, Jutta Paasche, Kerstin Seeliger, and Ines Wolf for excellent technical assistance.

REFERENCES

1. **Centers for Disease Control and Prevention.** 2003. Guidelines for environmental infection control in health-care facilities. Morb. Mortal. Wkly. Rep. **52**(RR-10)**:**1–43.
2. **Helbig, J. H., J. B. Kurtz, M. Castellani Pastoris, C. Pelaz, and P. C. Lück.** 1997. Antigenic lipopolysaccharide components of *Legionella pneumophila* recognized by monoclonal antibodies: possibilities and limitations for division of the species into serogroups. J. Clin. Microbiol. **35:**2841–2845.
3. **Helbig, J. H., B. Ludwig, P. C. Lück, A. Groh, W. Witzleb, and J. Hacker.** 1995. Monoclonal antibodies to *Legionella* Mip proteins recognize genus- and species-specific epitopes. Clin. Diagn. Lab. Immunol. **2:**160–165.
4. **Joly, J. R., R. M. McKinney, J. O. Tobin, W. F. Bibb, I. D. Watkins, and D. Ramsay.** 1986. Development of a standardized subgrouping scheme for *Legionella pneumophila* serogroup 1 using monoclonal antibodies. J. Clin. Microbiol. **23:**768–771.
5. **Ratcliff, R. M., J. A. Lanser, P. A. Manning, and M. W. Heuzenroeder.** 1998. Sequence-based classification scheme for the genus *Legionella* targeting the *mip* gene. J. Clin. Microbiol. **36:**1560–1567.