Evaluation of Vircell Enzyme-Linked Immunosorbent Assay and Indirect Immunofluorescence Assay for Detection of Antibodies against *Legionella pneumophila*

Bram M. W. Diederen, Jan A. J. W. Kluytmans, and Marcel F. Peeters

Laboratory for Medical Microbiology and Immunology, St. Elisabeth Hospital, P.O. Box 747, 5000 AS Tilburg, The Netherlands, and Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda, The Netherlands

Received 3 August 2005/Returned for modification 13 September 2005/Accepted 13 January 2006

We evaluated the abilities of the Vircell immunoglobulin G (IgG) and IgM indirect immunofluorescence assay (IFA) for *Legionella pneumophila* serogroup 1, the IgM and IgG enzyme-linked immunosorbent assay (ELISA) for *Legionella pneumophila* serogroup 1, and the IgM-plus-IgG ELISA for *Legionella pneumophila* serogroups 1 to 6 to diagnose Legionnaires’ disease (LD) in a well-described sample of patients with and without LD. Also, we determined the agreements, sensitivities, and specificities of the different Vircell assays in comparison to a validated ELISA (Serion classic ELISA). Clinical sensitivity and specificity were 74.6% and 96.6%, respectively, for the IgM IFA, 65.1% and 88.0% for the IgG IFA, 92.3% and 100% for the IgM ELISA, 43.3% and 96.6% for the IgG ELISA, and 90.8% and 100% for the IgM-plus-IgG ELISA. Compared to Serion classic ELISA, agreement, sensitivity, and specificity were 80.0%, 83.1%, and 78.4%, respectively, for the IgM IFA, 75.2%, 66.0%, and 79.5% for the IgG IFA, 89.5%, 82.0%, and 97.6% for the IgM ELISA, 81.9%, 88.9%, and 78.0% for the IgG ELISA, and 93.5%, 90.0%, and 96.6% for the IgM-plus-IgG ELISA. The value of a positive diagnostic result obtained by the Vircell IgM IFA, the Vircell IgG IFA, and the Vircell IgG ELISA might not be acceptable for a diagnostic assay. Both the high specificities and sensitivities of the Vircell IgM ELISA and the IgM-plus-IgG ELISA and the high correlation with the Serion classic ELISA indicate that they are useful in the diagnosis of LD.

The genus *Legionella* of the family * Legionellaceae* includes more than 45 species of fastidious gram-negative bacilli, 20 of which have been reported to infect humans. Bacteria of the family *Legionellaceae* are ubiquitous in both natural and man-made aqueous environments, and inhalation or aspiration of contaminated water can cause Legionnaires’ disease (LD), a severe pneumonia. *Legionella* can also cause subclinical infection and extrapulmonary inflammation (5). *Legionella pneumophila* causes 91% of all reported cases of LD, with serogroup 1 being the most predominant serogroup, causing approximately 80% of all culture-confirmed cases (10).

Of the various antibody detection methods that are available to detect *Legionella* infection, indirect immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays (ELISA) are the most commonly used methodologies (1, 3, 8). A fourfold or greater increase in the titer of antibody is considered diagnostic. An ELISA is generally preferred over IFA testing because it is less subjective, is thought to be more sensitive than IFA testing, and has the potential for automated performance (1, 7, 14). The availability of commercial ELISA and IFA kits has resulted in the increasing use of these products, despite the fact that few studies determining their sensitivity and specificity are available.

The aim of our study was to evaluate commercial ELISA and IFA (Vircell, S.L., Santa Fé, Granada, Spain) for the detection of antibodies against *L. pneumophila*.

**MATERIALS AND METHODS**

**Clinical samples.** Serum samples were collected between 1999 and 2004 and stored at −20°C until processing was performed. We included 129 serum samples of 65 patients with proven LD (cases). A proven case of LD was defined as a patient who suffered from symptoms of pneumonia, had radiological signs of infiltration, and showed laboratory evidence of infection with *L. pneumophila*. Laboratory evidence included one or more of the following criteria: isolation of *L. pneumophila* from a respiratory sample; a positive urinary antigen test (Binax Now *Legionella* urinary antigen test; Binax Inc.); a positive PCR result on respiratory tract samples using a 16S rRNA assay (12); a single high titer in immunoglobulin M (IgM) and/or IgG; and/or seroconversion to positive IgM and/or IgG antibodies to *L. pneumophila*. Laboratory evidence included one or more of the following criteria: isolation of *L. pneumophila* from a respiratory sample; a positive urinary antigen test (Binax Now *Legionella* urinary antigen test; Binax Inc.); a positive PCR result on respiratory tract samples using a 16S rRNA assay (12); a single high titer in immunoglobulin M (IgM) and/or IgG; and/or seroconversion to positive IgM and/or IgG antibodies to *L. pneumophila*. Laboratory evidence included one or more of the following criteria: isolation of *L. pneumophila* from a respiratory sample; a positive urinary antigen test (Binax Now *Legionella* urinary antigen test; Binax Inc.); a positive PCR result on respiratory tract samples using a 16S rRNA assay (12); a single high titer in immunoglobulin M (IgM) and/or IgG; and/or seroconversion to positive IgM and/or IgG antibodies to *L. pneumophila*.

In addition, serum samples of patients with respiratory tract infections other than Legionella were tested in a similar manner to serve as controls and to test specificity. These samples were obtained from 29 patients with respiratory tract infections who had a fourfold or greater increase in (complement-fixating) antibodies against influenza A virus, adenovirus, *Chlamydia psittaci*, or *Mycoplasma pneumoniae* (50 samples). Age and sex distributions of controls were as follows: 15 were male, and 14 were female, with ages between 2 and 84 years (mean age, 43.5).

**Legionella pneumophila** IgM and IgG IFA (Vircell, S.L., Santa Fé, Granada, Spain). Samples were tested for *L. pneumophila* serogroup 1 IgM and IgG antibodies by an IFA according to the manufacturer’s instructions (11). IgG
Clinical sensitivity and specificity were 74.6% and 96.6%, respectively, for the IgM IFA and 65.1% and 88.0% for the IgG IFA (Table 1). If the results of both the IgM IFA and IgG IFA of each separate assay were combined, clinical sensitivity and specificity would be 87.5% and 84.0%, respectively. Clinical sensitivity and specificity were 92.3% and 100%, respectively, for the IgM ELISA, 43.3% and 96.6% for the IgG ELISA, and 90.8% and 100% for the IgM-plus-IgG ELISA (Table 1). If the results of both the IgM ELISA and IgG ELISA of each separate assay were combined, clinical sensitivity and specificity would be 91.1% and 96.6%, respectively. A calculated agreement, sensitivity, and specificity of 80.0%, 83.1%, and 78.4%, respectively, were found for the IgM IFA compared to the Serion IgM ELISA and 75.2%, 66.0%, and 79.5%, respectively, for the IgG IFA compared to the Serion IgG ELISA (Table 2). Of the 179 samples tested, 23 (9 in the IgM IFA and 14 in the IgG IFA) were equivocal results that were not included in the calculations.

A calculated agreement, sensitivity, and specificity of 89.5%, 82.0%, and 97.6%, respectively, were found for the IgM IFA compared to the Serion IgM ELISA and 81.9%, 88.9%, and 78.0%, respectively, for the IgG IFA compared to the Serion IgG ELISA (Table 3). A calculated agreement, sensitivity, and specificity of 93.5%, 90.0%, and 96.6%, respectively, were found for the IgM ELISA compared to the Serion IgM ELISA. Of the 179 samples tested, 31 (8 in the IgM ELISA, 13 in the IgG ELISA, and 10 in the IgM-plus-IgG ELISA) were equivocal results that were not included in the calculations.

Several methods of antibody detection against *L. pneumophila* have been developed, such as IFA, microagglutination test, indirect hemagglutination test, and ELISA (1, 6, 7, 9, 13, 15). Historically, the IFA was the first assay used to detect antibodies against *Legionella pneumophila*. Nowadays, commercially available ELISA kits are extensively used for the diagnosis of LD because they are at least as sensitive as IFA, can have an objectively determined end point, and allow automation. The reported sensitivities of serological assays vary substantially, from 41% to 94% (1, 3, 4, 6). This variation may be due to differences in the study population, the design of the study, differences in the antigen preparation or in the valence (mono- or polyvalent) of the antigen used, cross-reacting antibodies, and differences in the ability to detect IgM or IgG. In this study, we evaluated the ability of commercial IFA and ELISA to diagnose LD in a well-described population of patients with and without LD. Also, we determined the agreements, sensitivities, and specificities of the different Vircell assays in comparison to a validated ELISA (1, 2).

The clinical sensitivity of the Vircell ELISA to diagnose LD was significantly higher for the detection of IgM and IgM-plus-IgG antibodies in comparison to that of the IFA for IgM detection (92.3%, 90.8%, and 74.6% for the Vircell IgM ELISA, the IgM-plus-IgG ELISA, and the IgM IFA, respectively; \( P < 0.01 \)). The clinical sensitivity of the Vircell ELISA for the detection of IgG-specific antibodies was significantly lower than that of the IFA for IgG detection (43.3% and 65.1% for the Vircell IgG ELISA and the IgG IFA, respectively; \( P = 0.02 \)).
Compared to that of the Serion classic ELISA, the sensitivity of the Vircell ELISA was higher for the detection of IgM antibodies and IgM-plus-IgG antibodies but not statistically significant (92.3%, 90.8%, and 87.5% for the Vircell IgM ELISA, the IgM-plus-IgG ELISA, and the Serion classic ELISA, respectively). The sensitivity to detect IgG was significantly lower for Vircell in comparison to that of Serion (44.0% versus 76.5% for Serion; \( P < 0.001 \)).

The correlation between Serion and Vircell varied between the assays evaluated. The correlation was highest between the Serion classic ELISA and the Vircell IgM ELISA for the detection of IgM and IgG combined and lowest for the IgG IFA (agreement, sensitivity, and specificity of 93.5%, 90.0%, and 96.6% for the Vircell ELISA and 75.2%, 66.0%, and 79.5% for the IgG IFA). Although equivocal results were not included in the calculations, a considerable percentage of samples gave equivocal results in both the Vircell IFA and ELISA. For example, in the IgM IFA, 6.4% (5/78) of samples positive in the Serion IgM; 81.9%, 88.9%, and 78.0%, respectively, for the Vircell IgM and IgG compared to the Serion IgM.

**TABLE 3. Agreement, sensitivity, and specificity of Vircell Legionella ELISA compared to Serion classic ELISA for detection of__L. pneumophila__ IgM- and IgG-specific antibodies**

| Serion ELISA result | No. of samples<sup>a</sup> | IgM ELISA | IgM IFA | IgM + IgG ELISA | IgM ELISA | IgM IFA | IgM + IgG ELISA |
|---------------------|--------------------------|----------|---------|-----------------|----------|---------|----------------|
| Positive            | 73                       | 32       | 72      | 2               | 26       | 3       | 1              |
| Negative            | 16                       | 4        | 8       | 80              | 104      | 86      | 5              |
| Equivocal           | 2                        | 4        | 2       | 0               | 4        | 0       | 0              |

<sup>a</sup> Samples were tested for _L. pneumophila_ serogroup 1 antibodies in an IgM IFA, IgG IFA, IgM ELISA, and IgG ELISA and _L. pneumophila_ serogroups 1 to 6 in an IgM-plus-IgG ELISA.

<sup>b</sup> Agreement, sensitivity, and specificity were 89.5%, 82.0%, and 97.6%, respectively, for the Vircell IgM compared to the Serion IgM; 81.9%, 88.9%, and 78.0%, respectively, for the Vircell IgG compared to the Serion IgG; 93.5%, 90.0%, and 96.6%, respectively, for the Vircell IgM and IgG compared to the Serion IgM.

ACKNOWLEDGMENT

We thank Vircell, S.L. (Santa Fé, Granada, Spain), for supplying the assays evaluated in this study.

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