

## Letter to the Editor

### Vircell Assays for Detection of Antibodies against *Legionella pneumophila*

We read with interest the study by Diederer et al. (1), in which the serological assays from Vircell were evaluated. We found that this work underlines the importance of immunoglobulin M (IgM) detection in the diagnosis of legionellosis previously described by our group (3). Yet, we would like to point out some disagreements with some of the information given in the aforementioned study.

The authors used enzyme-linked immunosorbent assay (ELISA) as a reference technique for calculating the sensitivity and specificity of Vircell's assays. We consider that the references cited in the paper do not show enough validation to the two commercial tests for either of them to be considered a "gold standard": in the cited studies, sensitivities of 78% and 74%, respectively, were calculated for the Serion IgG and IgM ELISAs. Besides, in a previous comparison of the immunofluorescence assay (IFA) and ELISA IgG tests of the same commercial brands (2), completely different results were obtained: 67% out of 117 patients from a *Legionella* outbreak were serologically diagnosed by the Vircell IFA against 40% diagnosed by the Serion IgG ELISA. Therefore, only samples from patients with a diagnosis confirmed by other direct methods should have been included in calculations of the sensitivities of the assays for both commercial brands; the remaining samples should have been included only for the purpose of determining the diagnostic concordance between tests. Otherwise, additional serological tests (either commercial or in-house) should have been included to elucidate discrepant results. In any case, the data for the second manufacturer should have been given in Table 1 together with the clinical data (i.e., sensitivity and specificity values) of Vircell's tests. It is obvious that if one single test is considered a reference method, any other assay evaluated against it will render worse results.

The specificities for the different tests (given in Tables 2 and 3) were calculated taking into account the results from 179 samples, 129 of which belonged to patients with legionellosis. That is to say, if the assay of the other manufacturer yielded a negative result, then a positive result in the Vircell assay was considered to be a false positive for the purpose of specificity calculation. In our opinion, this method of calculating false-positive results is not correct. Only samples from populations free of Legionnaires' disease should have been included for the evaluation of the specificity of the tests. The confidence intervals also should have been included in the statistical analysis of the results.

Finally, we found the results shown in Tables 2 and 3 difficult to interpret. It is not clear which of the data in the table correspond to the Vircell assays and which to Serion assays. If the Serion assay results are given in rows, the sensitivity and specificity values for Vircell's tests are not well calculated. As an example, the respective sensitivities for the Vircell IgM, IgG, and IgG-plus-IgM ELISAs should be 97.3%, 55.2%, and 96.0% instead of the 82.0%, 88.9% and 90.0% shown in the footnote of Table 3. On the other hand, if the Serion assay results are given in the columns, as the sensitivities and specificities shown in the tables would indicate, then either Tables 2 and 3 report different numbers of total positive and negative samples scored by the Serion IgM and IgG ELISA assays (for example, 61 positive IgM samples in Table 2 versus 91 in Table

3) or Table 3 reports different numbers depending on the column (compare the total number of samples positive by IgM ELISA with the number positive by IgM-plus-IgG ELISA [91 versus 82] and the total number of samples negative by IgM ELISA with the number negative by IgM-plus-IgG ELISA) [82 versus 89]). In that sense, it would be desirable for the authors to clarify these data.

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Almudena Rojas  
José Rojas\*  
Joaquín Mendoza  
Vircell S.L.  
Pza. Domínguez Ortiz 1  
18320 Santa Fe, Granada, Spain

\*Phone: 34 958441264  
Fax: 34 958510712  
E-mail: immunology@vircell.com

#### Authors' Reply

We thank Rojas et al. for their letter, in which they raise several concerns about our study.

In our study, we included 129 serum samples of 65 patients with proven Legionnaires' disease (LD) and 50 serum samples of 29 patients with respiratory tract infections caused by other microorganisms (4). If a diagnostic test is evaluated with specimens from persons whose infection status is known with certainty, the proportion of positive test results for specimens from infected persons provides an unbiased estimate of sensitivity, and the proportion of negative test results for specimens from uninfected persons provides an unbiased estimate of specificity. The letter authors state that because one of the inclusion criteria was a positive result in the Serion enzyme-linked immunosorbent assay (ELISA), it would be more correct to say that the assays were validated only for seropositive cases of LD in the Serion ELISA. We do not agree with this. Although the Materials and Methods section does not clearly state it, all included patients with LD had evidence for LD in at least two laboratory tests, most often a positive urinary antigen test in combination with serology. None of the patients were diagnosed based on serology alone. Because the Serion ELISA was not under evaluation, and positive reaction in this assay was part of the inclusion criteria, it would not have been correct to assess this assay in terms of clinical sensitivity and specificity. In the study cited by Rojas et al. (9), the Vircell ELISA for immunoglobulin M (IgM), the Vircell ELISA for IgG plus IgM, and the Vircell immunofluorescence assay (IFA) for IgM were able to diagnose 72.3%, 60.5% and 51.4%,

respectively, of outbreak-related patients. Our evaluation included patients from various sources in a nonepidemic setting and therefore differs from studies of patients in an epidemic setting, where only a single *Legionella pneumophila* strain is evaluated. In addition, outbreak-related studies provide no information regarding the specificity of serological assays. In our study (4), the clinical specificity was estimated as follows: 96.6% for the IgM IFA (95% confidence interval [CI], 81 to 100%), 88.0% for the IgG IFA (95% CI, 69 to 97%), 100% for the IgM ELISA (95% CI, 86 to 100%), 96.6% for the IgG ELISA (95% CI, 81 to 100%), and 100% for the IgM-plus-IgG ELISA (95% CI, 85 to 100%). We agree with Rojas et al. that we tested a relatively small number of patients in the non-LD group (29 patients). This means that the calculated specificities for the assays are more uncertain, as indicated by the CIs that are provided here.

Another way to validate an assay is to compare different assays with each other, randomly choosing one validated assay as the "gold standard" (8). We have chosen to compare Vircell assays with the Serion ELISA. The Serion ELISA detects IgM and IgG antibodies to serotypes 1 to 7 in separate assays, and optimal test sensitivity is achieved using both IgG and IgM antibodies (5). The use of the Serion ELISA has been described in several other studies (1, 2, 3). The specificity of the assays has been determined by Boshuizen et al.: While none of the 480 samples from a serum bank in The Netherlands tested positive for *L. pneumophila* IgM antibodies, 4.5% were seropositive for IgG. Sensitivities of 89% for the IgG test, 56% for the IgM test, and 93% for the IgG and IgM results combined were found (2). A recent report of a study in which serum samples from outbreak-related LD patients from The Netherlands were used (10) showed sensitivities of 64%, 61%, and 44%, respectively, for the Serion ELISA, IFA, and a rapid microagglutination test. In an ELISA, 48% of patients showed a seroconversion in IgM and 50% a seroconversion in IgG. The letter authors state that the Serion ELISA displays a suboptimal performance, indicated by the low sensitivity (40%) of IgG antibody detection in another recent study (7). The difference found may be due to differences in the study population and the design of the study but most likely is because the study's authors tested only the ability of Serion ELISA to detect IgG antibodies. Studies have clearly shown that, in many patients with legionellosis, the immune response is primarily IgM and that IgM tests must thus be included for optimal sensitivity (6, 11).

The letter authors have pointed out that the results given in Tables 2 and 3 are difficult to interpret, and after reconsidering these we must agree. The calculated sensitivity and specificity values presented in the tables were not correct. We apologize for this mistake and have requested that a correction be published.

Notwithstanding that some values were calculated incorrectly and that we evaluated a relatively small group of patients with respiratory tract infections other than LD, our results indicate that the predictive value of a positive diagnostic result obtained by the Vircell IgM IFA and the Vircell IgG IFA will be low when used in a population with a low prevalence of LD. The Vircell IgM ELISA and the IgM-plus-IgG ELISA show

high sensitivities and specificities, and therefore a reliable serodiagnosis can be made using these assays. The Vircell IgG ELISA shows moderate sensitivity, but high specificity, and could be included together with IgM or IgM-plus-IgG for optimal clinical decision making.

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Bram M. W. Diederer\*  
 Marcel F. Peeters  
 Laboratory for Medical Microbiology  
 and Immunology  
 St. Elisabeth Hospital  
 P.O. Box 747  
 5000 AS Tilburg, The Netherlands

Jan A. J. W. Kluytmans  
 Laboratory for Microbiology  
 and Infection Control  
 Amphia Hospital  
 Breda, The Netherlands

\*Phone: 31 13 539 2655  
 Fax: 31 13 544 1264  
 E-mail: bramdiederer@gmail.com

Ed. Note: An author's correction for the article discussed here (B. M. W. Diederer, J. A. J. W. Kluytmans, and M. F. Peeters, *Clin. Vaccine Immunol.* 13:361-364, 2006) appears in this issue.

## AUTHOR'S CORRECTION

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

Bram M. W. Diederer, 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

Volume 13, no. 3, p. 361–364, 2006. Page 362: Table 2 should appear as shown below.

TABLE 2. Comparison of the Vircell *Legionella* IFA to the Serion classic ELISA for detection of *L. pneumophila*-specific IgM and IgG antibodies<sup>a</sup>

Serion ELISA result	No. of samples tested by Vircell <i>Legionella</i> IFA <sup>b</sup>					
	Positive		Negative		Equivocal	
	IgM	IgG	IgM	IgG	IgM	IgG
Positive	47	35	24	24	5	2
Negative	11	17	88	88	2	5
Equivocal	2	4	0	3	0	1

<sup>a</sup> Samples were tested for antibodies specific for *L. pneumophila* serogroup 1 in IFAs for IgM and IgG as well as in ELISAs for IgM and IgG and for antibodies specific to *L. pneumophila* serogroups 1 to 6 in an ELISA for both IgM and IgG. Results presented are compiled from the individual and combined ELISAs.

<sup>b</sup> For IFA compared to ELISA, the agreement, sensitivity and specificity were, respectively, 79.4%, 66.2%, and 88.9% for IgM and 75.0%, 59.3%, and 83.8% for IgG.

Page 363, Table 3: Footnote *b* should read as follows. "Agreement, sensitivity, and specificity were 89.5%, 97.3%, and 83.3%, respectively, for the Vircell IgM ELISA; 81.9%, 55.2%, and 96.3%, respectively, for the Vircell IgG ELISA; and 93.5%, 96.0%, and 91.5%, respectively, for the Vircell IgM and IgG combined ELISA."