

Labsystems Enzyme Immunoassay for *Chlamydia pneumoniae* Also Detects *Chlamydia psittaci* Infections

In a recent article, Bas and collaborators compared different serological methods to detect chlamydial antibodies in patients with *Chlamydia trachomatis* infections and healthy blood donors (1). Using the Labsystems enzyme immunoassay (EIA) for *C. pneumoniae*, no cross-reaction between *C. trachomatis* and *C. pneumoniae* was found. However, Gnarpe and collaborators recently showed cross-reactions between *C. trachomatis* and *C. pneumoniae*, when the Labsystems EIA for *C. pneumoniae* was used on sera containing high titers of *C. trachomatis* antibodies documented by the microimmunofluorescence (MIF) test (2). A broader cross-reactivity between the different chlamydial species was suspected. The Labsystems EIA test has previously been shown to have high sensitivity and specificity in the diagnosis of acute infections caused by *C. pneumoniae* during an epidemic of *C. pneumoniae* (3).

To examine the Labsystems EIA test for *C. pneumoniae* more extensively, we tested it on paired sera, taken from 43 patients for etiological diagnosis of pneumonia. The immunoglobulin G (IgG) and IgM antibody results for *C. pneumoniae*, *C. psittaci*, and *C. trachomatis* had previously been documented by the MIF tests, and IgG and IgM antibodies were now determined by the Labsystems EIA for *C. pneumoniae*. Of seven patients who were positive by the MIF test for *C. pneumoniae*, only three were positive by the EIA for *C. pneumoniae*, while five out of five patients who were positive by the MIF test for *C. psittaci* were positive by the EIA for *C. pneumoniae*. One patient with positive MIF test results for both *C. pneumoniae* and *C. psittaci* also tested positive for *C. pneumoniae* by the EIA. Sera from the remaining 30 patients, who showed no significant IgG or IgM antibody changes with the MIF test, also produced negative results with the EIA.

Because infections caused by *C. pneumoniae* and *C. psittaci* have important epidemiological differences, it is important to make a correct etiological diagnosis. The study of Gnarpe and collaborators and the present study indicate that cross-reactions between the chlamydial species occur when the Labsystems EIA for *C. pneumoniae* is used. The EIA could be used for the purpose of screening for chlamydial infections, but in nonepidemic situations we suggest that a method that differentiates between the chlamydial species, such as the MIF test, should be used directly (or, alternatively, as a second step after a screen with the EIA).

REFERENCES

1. Bas, S., P. Muzzin, B. Ninet, J. E. Bornand, C. Scieux, and T. L. Vischer. 2001. Chlamydial serology: comparative diagnostic value of immunoblotting, microimmunofluorescence test, and immunoassays using different recombinant proteins as antigens. *J. Clin. Microbiol.* **39**:1368–1377.
2. Gnarpe, J., J. Nääs, and A. Lundbäck. 2000. Comparison of a new commercial EIA kit and the microimmunofluorescence technique for the determination of IgG and IgA antibodies to *Chlamydia pneumoniae*. *APMIS* **108**:819–824.
3. Persson, K., and J. Boman. 2000. Comparison of five serological tests for diagnosis of acute infections by *Chlamydia pneumoniae*. *Clin. Diagn. Lab. Immunol.* **7**:739–744.

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Authors' Reply

In our study (1), we compared two different methods of serodiagnosis for *Chlamydia pneumoniae* infections (an enzyme immunoassay from Labsystems and the microimmunofluorescence [MIF] test) but no statistical analysis concerning possible cross-reactions between anti-*C. trachomatis* and anti-*C. pneumoniae* antibodies was presented. The analysis shows that the percentage of positive samples for immunoglobulin G (IgG) anti-*C. pneumoniae* antibodies, determined by either enzyme immunoassay or MIF, is always higher for positive IgG anti-*C. trachomatis* antibody samples than for negative ones. However, this difference was only significant when the presence of IgG anti-*C. trachomatis* and -*C. pneumoniae* antibodies was determined with the MIF test. Indeed, 92% of IgG anti-*C. trachomatis* antibody-positive samples also had IgG anti-*C. pneumoniae* antibodies while at the same time only 45% of IgG anti-*C. trachomatis* antibody-negative samples were found to be positive for IgG anti-*C. pneumoniae* antibodies ($P = 0.0037$, chi-square test). When the presence of IgG anti-*C. trachomatis* antibodies was documented by the MIF test and that of IgG anti-*C. pneumoniae* was identified by the Labsystems enzyme immunoassay, 77% of IgG anti-*C. trachomatis* antibody-positive but only 48% of the antibody-negative samples also had IgG anti-*C. pneumoniae* antibodies. The difference was not significant.

When the presence of IgG anti-*C. trachomatis* antibodies was determined with other methods, such as enzyme immunoassays using either synthetic peptides derived from species-specific epitopes in the variable domain IV of the major outer membrane protein or *pgp3* as the antigen(s), the presence of IgG anti-*C. pneumoniae* antibodies was found in 62 to 72% of IgG anti-*C. trachomatis* antibody-positive samples and in 50 to 62% of antibody-negative samples. Therefore, though the differences were not significant in most cases, samples positive for anti-*C. trachomatis* antibody had a tendency to be more often anti-*C. pneumoniae* antibody-positive than do samples negative for anti-*C. trachomatis* antibody. However, if *C. trachomatis* infection was proven in these patients, the presence or absence of *C. pneumoniae* or *C. psittaci* was not demonstrated by culture, direct immunofluorescence, or nucleic acid amplification. It is therefore only possible to speculate about the presence or absence of anti-*C. pneumoniae* or anti-*C. psittaci* antibodies and about their probable cross-reactivities.

In conclusion, in our study, the detection of cross-reacting antibodies could be expected to occur more often with the MIF test than with enzyme immunoassays. Several studies have already reported that MIF specificity is lower than that generally thought (2–4, 6–9). Because MIF tests are detecting anti-

bodies against surface protein antigens and because the protein composition of the *C. pneumoniae* outer membrane complex is similar to those described for *C. trachomatis* and *C. psittaci* (5), the detection of cross-reacting antibodies is not surprising. Moreover, recognition of the major outer membrane protein (4, 6, 7) and the 60-kDa proteins of the three species was shown to be cross-reactive (5).

REFERENCES

1. **Bas, S., P. Muzzin, B. Ninet, J. E. Bornand, C. Scieux, and T. L. Vischer.** 2001. Chlamydial serology: comparative diagnostic value of immunoblotting, microimmunofluorescence test, and immunoassays using different recombinant proteins as antigens. *J. Clin. Microbiol.* **39**:1368–1377.
2. **Biendo, M., F. Eb, J. F. Lefebvre, and J. Orfila.** 1996. Limits of the microimmunofluorescence test and advantages of immunoblotting in the diagnosis of chlamydiosis. *Clin. Diagn. Lab. Immunol.* **3**:706–709.
3. **Bourke, S. J., D. Carrington, C. E. Frew, R. D. Stevenson, and S. W. Banham.** 1989. Serological cross-reactivity among chlamydial strains in a family outbreak of psittacosis. *J. Infect.* **19**:41–45.
4. **Kern, D. G., M. A. Neill, and J. Schachter.** 1993. A seroepidemiologic study of Chlamydia pneumoniae in Rhode Island. Evidence of serologic cross-reactivity. *Chest* **104**:208–213.
5. **Melgosa, M. P., C. C. Kuo, and L. A. Campbell.** 1993. Outer membrane complex proteins of Chlamydia pneumoniae. *FEMS Microbiol. Lett.* **112**:199–204.
6. **Moss, T. R., S. Darougar, R. M. Woodland, M. Nathan, R. J. Dines, and V. Cathrine.** 1993. Antibodies to Chlamydia species in patients attending a genitourinary clinic and the impact of antibodies to *C. pneumoniae* and *C. psittaci* on the sensitivity and the specificity of *C. trachomatis* serology tests. *Sex. Transm. Dis.* **20**:61–65.
7. **Ozanne, G., and J. Lefebvre.** 1992. Specificity of the microimmunofluorescence assay for the serodiagnosis of Chlamydia pneumoniae infections. *Can. J. Microbiol.* **38**:1185–1189.
8. **Wagenvoort, J. H., D. Koumans, and M. van de Crujjs.** 1999. How useful is the Chlamydia microimmunofluorescence (MIF) test for the gynaecologist? *Eur. J. Obstet. Gynecol. Reprod. Biol.* **84**:13–15.
9. **Wong, Y. K., J. M. Sueur, C. H. Fall, J. Orfila, and M. E. Ward.** 1999. The species specificity of the microimmunofluorescence antibody test and comparisons with a time resolved fluoroscopic immunoassay for measuring IgG antibodies against Chlamydia pneumoniae. *J. Clin. Pathol.* **52**:99–102.

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