Performance of Three Microimmunofluorescence Assays for Detection of *Chlamydia pneumoniae* Immunoglobulin M, G, and A Antibodies

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The microimmunofluorescence (MIF) test is considered the “gold standard” for laboratory diagnosis of acute and chronic *Chlamydia pneumoniae* infection. The performance of a MIF test based on *C. pneumoniae* antigen from Washington Research Foundation (WRF) was compared with those of assays from Labsystems (LAB) and MRL Diagnostics (MRL) by investigation of sera from three groups of patients: group I, 83 sera from 28 patients with atypical pneumonia; group II, 37 sera from 16 patients with acute *C. pneumoniae* or *Chlamydia psittaci* respiratory tract infection confirmed by PCR or culture; group III, 100 sera from 100 persons enrolled in the Copenhagen City Heart Study. The accordance among the results of the WRF assay and the two commercial assays was excellent for the immunoglobulin M (IgM) antibody detection rate (98%). The accordance in detection rates for IgG and IgA antibodies in sera from patients with acute infections was acceptable (87 and 88%), and in sera from group III, it was excellent (95 and 97%). The determinations of endpoint titers were reproducible with <1 dilution step difference for all three methods, except that the mean IgM antibody titer found by the LAB assay was almost 2 dilution steps higher than that found by the other two methods. Although the three assays use different *C. pneumoniae* strains as antigens, the detection rates and IgG and IgA endpoint titers were similar. The difference in endpoint titers of IgM antibodies is of no major concern, as the diagnosis of acute *C. pneumoniae* infection rests on the presence of IgM antibodies, not on their level.

In 1986, a new *Chlamydia* species was recognized as a cause of respiratory tract infections, and in 1989 it was named *Chlamydia pneumoniae* (6, 7). *C. pneumoniae* has also been associated with asthma, chronic obstructive lung diseases, chronic coronary heart disease, and acute myocardial infarction (8, 16, 18). Because isolation of the bacterium has proven to be difficult, much of the evidence of its pathogenic role has come from serological studies. The microimmunofluorescence (MIF) test was developed by Wang and Grayston in 1968 for the purpose of serotyping *Chlamydia trachomatis* strains and later for the diagnosis of *C. trachomatis* serovar-specific infections (19, 21). Subsequently, the method was used to detect *C. pneumoniae* antibodies, and among the different serological methods available, the MIF test, though not perfect, is still considered the method of choice (3). Moreover, the MIF test has also been used for measuring *C. pneumoniae* antibodies in patients with assumed chronic *C. pneumoniae* infection (15).

The laboratories performing the MIF test use a variety of in-house assays; assays based on antigens from Washington Research Foundation (WRF) (now Washington University), Seattle, Wash.; or one of the commercially available assays (13). Compared to the test originally developed by Wang and Grayston (21), the different assays each have introduced minor variations in the materials used or in procedures to be followed, e.g., different strains of *C. pneumoniae* as the antigen, different incubation times of sera with the antigen, and the use of fluorescein isothiocyanate-labeled anti-human immunoglobulin (Ig) antibodies from different manufacturers. One study examined the interlaboratory variation in MIF assay results obtained in 13 laboratories analyzing 22 sera from 10 patients (13). The agreement between a reference standard value and the IgM antibody results obtained by the 13 laboratories using 11 different methods was 50 to 95%; four laboratories failed to discern false-positive IgM titers, possibly because of the presence of rheumatoid factor. For IgG antibody analyses, the agreement was 68 to 87%. In another study, one laboratory compared two MIF assays and found a significant difference in IgG and IgA antibody titer levels obtained (5).

The objective of the present study was to assess the performance of two commercially available *C. pneumoniae* MIF assays from MRL Diagnostics (MRL), Cypress, Calif., and Labsystems (LAB), Helsinki, Finland, using a MIF assay based on *C. pneumoniae* antigen from WRF as a reference method. The WRF assay was chosen as the reference method because it had been available for research purposes for decades and because previous Danish studies of the prevalence of *C. pneumoniae*
antibodies had been conducted with the WRF assay (9, 11a). The two commercial assays (LAB and MRL) were under evaluation for use in our routine laboratory. Performance was assessed by testing for C. pneumoniae antibodies in sera from patients with acute respiratory tract infections of known etiology obtained at various intervals after the onset of the disease. Sera from persons enrolled in the Copenhagen City Heart Study (1) were included due to the present interest in the detection of C. pneumoniae antibodies in patients with cardiovascular diseases (9, 16).

MATERIALS AND METHODS

Sera. This study included sera from three groups of patients: two with acute respiratory tract infections and one including patients with possible chronic infections but without known acute infections.

Group I consisted of 83 sera from 28 patients enrolled in the Nordic Atypical Pneumonia (NAP) Study (12). The sera were kindly provided by J. S. Jensen, Mycoplasma Laboratory, Statens Serum Institut, with the permission of P. Saikku, University of Oulu, Finland. The 28 patients were selected because they had serological evidence of infection with C. pneumoniae (13 patients), Mycoplasma pneumoniae (11 patients), or both agents (4 patients). The original MIF analyses for C. pneumoniae antibodies had been performed using antigens from WRF (12).

Group II consisted of 37 sera from 16 patients with acute C. pneumoniae (8 patients) or Chlamydia psittaci (8 patients) respiratory tract infections confirmed by PCR (15 patients) or culture (1 patient) in our routine laboratory. The majority of the 16 patients had developed antibodies detectable in the Chlamydia complement fixation (CF) test; moreover, clinical data were available for all patients with C. pneumoniae infection and for 5 of 8 patients with C. psittaci infection. For the last group of patients, epidemiological data were also available (M. Faber, J. S. Jensen, and I. Lind, Proceedings of the 3rd Nordic-Baltic Congress on Infectious Diseases, abstr. A, p. 12, 1998). Culture was performed in Hep2 and McCoy cells; subsequent identification was obtained with genus- and species-specific staining methods (4). PCR was performed by an in-house method (4, 14).

Group III consisted of 100 sera from 100 persons enrolled in the Copenhagen City Heart Study, which is a prospective, ongoing population study: 20 persons who developed acute myocardial infarction within 3 years after the blood samples were drawn and 80 controls matched according to gender and age at entry without subsequent myocardial infarction (1).

MIF assays. The three MIF assays are described in brief below. Prior to IgM and IgA antibody testing, all sera were tested with GullSORB (Gull Laboratories, Salt Lake City, Utah). All analyses were performed by the same person (L.B.) using a Zeiss Axioskop 20 microscope with a Plan-Neofluar 40×0.75 objective. The procedures followed the recommendations of each manufacturer.

(i) WRF assay. For antigens from WRF, purified C. pneumoniae elementary bodies (strain AR39) were fixed on the slides. The conjugates used were fluorescein-labeled rabbit anti-human IgM, IgG, and IgA (Dako, Glostrup, Denmark). The incubation time was 30 min for both sera and conjugates.

(ii) MRL assay. For MIF assays (IgM, IgG, and IgA) from MRL, the slides were purchased with prefixed antigen dots for C. pneumoniae (strain TW185), C. trachomatis (eight serotypes [D to K]), and C. psittaci (strains 6BC and DD34). For the detection of IgM antibodies, the incubation period with serum was 90 min; for the detection of IgG and IgA antibodies, it was 30 min. The subsequent incubation time with the conjugate (fluorescein-labeled goat anti-human IgG [MRL]) was 30 min.

(iii) LAB assay. The slides for MIF assays (IgM and IgG) from LAB were purchased with prefixed antigen dots for C. pneumoniae (strain Kapaani 6), C. trachomatis (strain LGV/2), and C. psittaci (strains OF 6BC and EAE). For the detection of IgM antibodies, the incubation period with serum was 180 min; for the detection of IgG antibodies, it was 30 min. The subsequent incubation time with the conjugate (fluorescein-labeled goat anti-human IgG [LAB]) was 30 min. All sera were tested in serial twofold dilutions from the following initial dilutions until an endpoint was reached: group I, 1:16 (IgM), 1:64 (IgG), and 1:16 (IgA); group II, 1:16 (IgM) and 1:64 (IgG); group III, 1:16 (IgG) and 1:16 (IgA).

Day-to-day variation. In order to determine the intra- and interday variation, a study of the variation of test results for C. pneumoniae antibodies by the MIF reference method (WRF) was conducted. Two sera were examined for C. pneumoniae IgM, IgG, and IgA antibodies three times a day for 4 days. Thus, for each

| TABLE 1. Overview of experiments performed in assessment of three MIF assays for detection of C. pneumoniae IgM, IgG, and IgA antibodies |
|---------------------------------------------------------------|
| Serum source | Method | Ig antibody class tested* |
|---------------|--------|--------------------------|
|               |        | IgM | IgG | IgA |
| Group I (n = 83) | WRF | + | + | + |
|                | MRL | + | + | + |
|                | LAB | + | + | ND |
| Group II (n = 37) | WRF | + | + | ND |
|                | MRL | + | + | ND |
|                | LAB | + | + | ND |
| Group III (n = 100) | WRF | ND | + | + |
|                | MRL | ND | + | + |
|                | LAB | ND | ND | ND |

*= ND, not done; +, tested.

Statistics. To determine the intraday variation of endpoint titers for each serum for each day, a two-way analysis of variance was performed. The interday variation was estimated with an F test. The variation of reproducibility was estimated for each Ig class and for the three classes together. A comparison of detection rates obtained by the three assays was performed with a likelihood ratio test; for comparison of the antibody titers, Friedman’s nonparametric two-way analysis of variance was used. These analyses were performed with SAS version 8 (SAS Institute Inc.). The 95% confidence intervals (CI) for detection rates were calculated as the proportion (p) ± 2 × \sqrt{1 − p/n} (normal approximation). A P value of < 0.05 was considered significant.

RESULTS

Day-to-day variation. The variation of reproducibility was estimated to 0.41 dilution step for IgM, 0.48 dilution step for IgG, and 0.48 dilution step for IgA antibody titers. Overall, the day-to-day variation of MIF endpoint titers was 0.46 dilution step with 66 degrees of freedom. The 95% CI was 2 × 0.46 = 0.92 = 1 dilution step.

Detection rates of C. pneumoniae antibodies. The presence of C. pneumoniae IgM and IgG antibodies in 120 sera from 65 patients with acute respiratory tract infection (groups I and II) is illustrated in Tables 2, 3, 4, and 5. By the WRF, MRL, and LAB assays, the detection rates of IgM antibody titers of ≥16 were 27, 24, and 28%, respectively (P = 0.16), and the detec-

| TABLE 2. C. pneumoniae IgM endpoint titers in 120 sera from patients with acute respiratory tract infections (groups I and II) |
|---------------------------------------------------------------|
| MRL assay titer | No. of sera for WRF assay titer of: |
|-----------------|-------------------------------------|
|                 | <16 | 16 | 32 | 64 | 128 | 256 | 512 | 1,024 |
| <16 | 85  | 4 | 2 | 2 | 1 | 1 | 1 | 3 |
| 16  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 32  | 3  | 1 | 1 | 1 | 1 | 1 | 1 | 1  |
| 64  | 1  | 1 | 1 | 1 | 1 | 1 | 1 | 1  |
| 128 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  |
| 256 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  |
| 512 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  |
| 1,024 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  |
| Total | 88 | 5 | 4 | 4 | 11 | 7 | 1 | 120 |
tion rates of IgG antibody titers of ≥64 were 45, 53, and 48% (P = 0.43). Concordant results (both tests positive or both tests negative) were demonstrated by the MRL assay compared to the WRF assay in 92% of the sera (111 of 120) for IgM C. pneumoniae antibodies, in 76% of the sera (91 of 120) for IgG antibodies, and in 80% of the sera (66 of 83) (data not shown) for IgA antibodies. The corresponding figures for the results by the LAB assay compared to those by the WRF assay were 97% of the sera (116 of 120) for IgM antibodies and 79% of the sera (95 of 120) for IgG antibodies. When a day-to-day variation of ≤1 dilution step was taken into consideration, the results for the MRL assay versus the WRF assay were concordant in 98, 88, and 88% of the sera for IgM, IgG, and IgA antibodies, and for the LAB assay versus the WRF assay, the results were concordant in 98 and 87% of the sera for IgM and IgG antibodies.

The MRL and the WRF assay results were discrepant (positive versus negative) in 9 of 120 (8%) sera tested for IgM antibodies (Table 2). Two of the three sera with a positive result for C. pneumoniae IgM antibodies by the MRL assay but not by the WRF assay came from a patient with C. psittaci infection verified by PCR analysis. The third serum, from a group I patient, was negative by both the WRF assay and the LAB assay, but subsequent sera from the same patient were found positive for IgM antibodies by all three assays. The six sera with a negative result for C. pneumoniae IgM antibodies by the MRL assay but a positive result by the WRF assay came from two group I patients (three sera from each), and all six sera were also positive for IgM antibodies by the LAB assay.

The LAB and WRF assay results were discrepant in 5 of 120 (4%) sera tested for IgM antibodies (Table 3). The five IgM results were all found in the first or the last of three consecutive sera, the two correspondent sera of which were positive for C. pneumoniae IgM antibodies by both assays. For these five sera, the results by the WRF assay were discordant with those by the MRL assay.

For the majority of sera with discrepant IgG antibody results, the discrepancy was between sera with IgG antibody titers of <64 in one assay and of 64 to 256 in the other assay (Tables 4 and 5). The accordance in detection rates by the MRL assay and the WRF assay for the sera from patients in group III was 92% of the sera (90 of 98) for IgG antibodies and 91% of the sera (91 of 100) for IgA antibodies (Tables 6 and 7). When the day-to-day variation was taken into consideration, the corresponding results were 97 and 95% of the sera, respectively.

**Endpoint titer levels.** Table 8 shows the geometric mean titers found by each of the three MIF assays of all sera with antibody levels considered significant for the diagnosis of C. pneumoniae, i.e., IgM antibody titers of ≥16, IgG antibody titers of ≥64, and IgA antibody titers of ≥32. The geometric mean of IgM antibody titers obtained by the LAB assay was about three times as high as those obtained by the WRF assay and the MRL assay. The overall difference in IgM antibody titers obtained by the three assays was significant (P = 0.018). This difference was due to the diverging results by the LAB assay. Comparing the IgG antibody titers obtained by the three assays for sera from groups I and II, the overall difference demonstrated (P = 0.032) was mainly due to a slightly lower IgG antibody level by the WRF assay than by the other two

### Table 3. C. pneumoniae IgM endpoint titers in 120 sera from patients with acute respiratory tract infections (groups I and II)

| LAB assay titer | No. of sera for WRF assay titer of: | Total |
|-----------------|-------------------------------------|-------|
|                 | <64 | 16 | 32 | 64 | 128 | 256 | 512 | 1,024 |       |
| <64             |     | 85 | 1  | 1  |     |     |     |      | 87    |
| 16              |     | 1  |    |    |     |     |     |      |       |
| 32              |     |    |    |    |     |     |     |      |       |
| 64              |     |    |    |    |     |     |     |      |       |
| 128             |     |    |    |    |     |     |     |      |       |
| 256             |     |    |    |    |     |     |     |      |       |
| 512             |     |    |    |    |     |     |     |      |       |
| 1,024           |     |    |    |    |     |     |     |      |       |
| Total           |     | 88 | 0  | 5  | 4  | 4  | 11 | 7    | 120   |

### Table 4. C. pneumoniae IgG endpoint titers in 120 sera from patients with acute respiratory tract infections (groups I and II)

| MRL assay titer | No. of sera for WRF assay titer of: | Total |
|-----------------|-------------------------------------|-------|
|                 | <64 | 16 | 32 | 64 | 128 | 256 | 512 |       |
| <64             |     | 47 | 3  | 7  |     |     |     | 57    |
| 64              |     | 11 | 6  | 3  |     |     |     | 20    |
| 128             |     | 7  | 9  | 12 | 2   |     |     | 30    |
| 256             |     | 1  | 1  | 1  | 1   |     |     | 4     |
| 512             |     |    |    |    | 2   | 5   | 1   | 8     |
| 1,024           |     |    |    |    |     | 1   | 1   |       |
| Total           |     | 66 | 19 | 25 | 8   | 2   | 1   | 120   |

### Table 5. C. pneumoniae IgG endpoint titers in 120 sera from patients with acute respiratory tract infections (groups I and II)

| LAB assay titer | No. of sera for WRF assay titer of: | Total |
|-----------------|-------------------------------------|-------|
|                 | <64 | 16 | 32 | 64 | 128 | 256 | 512 |       |
| <64             |     | 52 | 4  | 6  | 1   |     |     | 63    |
| 64              |     | 5  | 4  | 6  |     |     |     | 15    |
| 128             |     | 7  | 9  | 7  | 2   |     |     | 25    |
| 256             |     | 1  | 2  | 6  | 1   |     |     | 10    |
| 512             |     | 1  | 4  | 4  |     |     |     | 6     |
| 1,024           |     | 1  | 1  |    |     |     |     |       |
| Total           |     | 66 | 19 | 25 | 8   | 2   | 1   | 120   |
assays. With the WRF assay, 2 of 120 sera (1.7%) had *C. pneumoniae* IgG antibody titers of ≥512 as opposed to 9 of 120 sera (7.5%) with the MRL assay and 7 of 120 (5.8%) with the LAB assay (Tables 4 and 5). However, only five sera were IgG antibody titers of ≥512 demonstrated by both the MRL assay and the LAB assay.

The difference in average endpoint titers of sera positive by two assays can also be expressed in dilution steps. Results by the MRL assay compared to those by the WRF assay were 0.4 dilution step lower for IgM (groups I and II), 0.4 dilution step higher for IgG (groups I, II, and III), and 0.3 dilution step higher for IgA (groups I and II). For the LAB assay compared to the WRF assay, the mean titers for groups I and II were 1.7 dilution steps higher for IgM and 0.4 dilution step higher for IgG antibodies.

In conclusion, the determinations of endpoint titers were reproducible with <1 dilution step difference for all three methods, except that the mean IgM antibody titer found by the LAB assay was almost 2 dilution steps higher than those found by the other two methods.

**Patients with *C. pneumoniae* infection.** A total of 66 sera from 25 patients with either serological evidence of *C. pneumoniae* infection (group I; n = 17) or microbiological evidence of *C. pneumoniae* infection (group II; n = 8) were included.

A total of 51 sera originated from the 17 patients in group I. For IgM antibodies, the accuracy in detection rates were 98% of the sera (50 of 51) for the LAB assay versus the WRF assay and 88% of the sera (45 of 51) for the MRL assay versus the WRF assay, while accuracy in endpoint titer levels, defined as the WRF assay titer ± 1 dilution step, were 78 (40 of 51) and 73% (37 of 51), respectively. For the IgG antibody detection rate, the accuracy was 90% (46 of 51) for both the LAB assay versus the WRF assay and the MRL assay versus the WRF assay, while accuracy in endpoint titer levels was found in 84% (43 of 51) of sera by both assays. For IgA antibodies, the accuracy in the detection rates for the MRL assay versus the WRF assay was 86% (44 of 51), and the accuracy in titer levels was 75% (38 of 51).

By the MRL assay, *C. psittaci* IgM antibodies were detected in 3 of 17 group I patients (7 of 51 sera) with *C. pneumoniae* IgM antibodies; one of these patients also had *C. trachomatis* IgM antibodies. In four of the seven sera, there were <4-fold differences between *C. pneumoniae* and *C. psittaci* IgM antibody titer levels.

By the LAB assay, *C. psittaci* IgM antibodies were detected in 5 of 17 group I patients (13 of 51 sera) with *C. pneumoniae* IgM antibodies; 2 of the 5 patients also had *C. trachomatis* IgM antibodies. In 9 of the 13 sera, there were <4-fold differences between *C. pneumoniae* and *C. psittaci* IgM antibody titer levels.

For group II, Table 9 shows the serological results by all three methods for each patient with confirmed *C. pneumoniae* infection. The accuracy in IgM antibody detection rates for the 15 sera from eight patients was 87% (13 of 15) for the LAB assay versus the WRF assay and 100% (15 of 15) for the MRL assay versus the WRF assay, while the accuracy in endpoint titer levels, defined as the WRF assay titer ± 1 dilution step, was 47 (7 of 15) and 67% (10 of 15). For IgG antibodies, the accuracy in detection rates was 53% (8 of 15) for the LAB assay versus the WRF assay and 47% (7 of 15) for the MRL assay versus the WRF assay, while accuracy in endpoint titer

### Table 7. *C. pneumoniae* IgA endpoint titers in 100 sera from patients without acute respiratory tract infection (group III)

| MRL | assay titer | No. of sera for WRF assay titer of | Total |
|-----|-------------|-----------------------------------|-------|
|     | <16         | 16                                |       |
|     | 16          | 1,12                             | 2     |
|     | 32          | 1,12                             | 4     |
|     | 64          | 1,12                             | 14    |
|     | 128         | 1,12                             | 21    |
|     | 256         | 1,12                             | 12    |
|     | 512         | 1,12                             | 3     |
|     | 1,024       | 1,12                             | 0     |
|     | Total       | 48                                | 11    |

**Table 8.** Geometric mean endpoint titers obtained by three *C. pneumoniae* MIF assays for sera from patients with (groups I and II) and without (group III) acute respiratory tract infections

| MIF test | Groups I and II | Group III |
|----------|-----------------|-----------|
|          | IgM IgG IgA     | IgG IgA   |
| WRF      | 173 116 75      | 113 59    |
| MRL      | 159 131 102     | 114 70    |
| LAB      | 491 145 ND      | ND ND     |

**Table 9.** *C. pneumoniae* IgM and IgG antibody titers by MIF assays for eight patients in whom microbiological findings supported a diagnosis of *C. pneumoniae* infection

| Patient | Day | Titera     |
|---------|-----|------------|
|         |     | WRF IgM IgG | MRL IgM IgG | LAB IgM IgG |
| 1       | 0   | 64 128 256  | N 1,024 256 |
| 2       | 0   | 256 128 256 | N 512 N     |
| 3       | 0   | 512 N 128   | 1,024 N 2,048 N |
| 4       | 0   | 512 N 128   | 1,024 N 2,048 N |
| 5       | 0   | N N N       | N N N       |
| 6       | 0   | 64 64 128   | 4,096 N 4,096 N |
| 7       | 0   | 256 N        | 4,096 N 4,096 N |
| 8       | 0   | 1,024 N 128 | 32 N N      |

ND, not determined.

a Numbers of *C. pneumoniae* antibody-positive sera were as follows: groups I and II, IgM ≥16, 32 (WRF), 29 (MRL), and 32 (LAB); IgG ≥4, 54 (WRF), 63 (MRL), and 57 (LAB); IgA ≥32, 47 (WRF) and 39 (MRL); group III, IgG ≥4, 43 (WRF), and 43 (MRL); IgA ≥32, 45 (WRF) and 51 (MRL). ND, not done.
levels was found in 40% (6 of 15) of sera by the LAB assay and 47% (7 of 15) by the MRL assay.

By all three assays, C. pneumoniae IgM antibodies were demonstrated in 12 of 15 sera and in seven of eight patients. In four patients, cross-reacting antibodies were demonstrated both by the LAB assay and by the MRL assay. In each of these four cases, the antibody titer of C. pneumoniae was at least fourfold higher than the antibody titers toward the two other species.

In conclusion, the accordance in detection rates of IgM antibodies was between 87 and 100% by the two assays compared to the WRF assay, and the accordance in detection rates of IgG antibodies was 90% in sera from group I and about 50% in sera from group II. The accordance in endpoint titer levels between both assays and the WRF assay was somewhat lower than the accordance in detection rates. Cross-reacting IgM antibodies were found in 9 of 25 patients by the LAB assay and in 7 of 25 patients by the MRL assay.

Patients with M. pneumoniae infection. The accordance in C. pneumoniae IgG antibody test results was 91% by the MRL assay versus the WRF assay and 87% by the LAB assay versus the WRF assay in the 32 sera from 11 patients included in group I. By the MRL assay and the WRF assay, the test results for C. pneumoniae IgA antibodies were in accordance in 29 of 32 sera. In none of the sera were C. pneumoniae IgM or IgG antibody titers of $\geq 512$ detected by any of the assays. IgG antibody titers of 64 to 256 were detected in 46 to 57% of sera by the three assays.

Patients with C. psittaci infection. A total of 22 sera from eight patients with C. psittaci infection were analyzed (data not shown). Overall, four of eight patients had C. psittaci IgM antibodies and/or a fourfold rise of C. psittaci IgG titers by the LAB assay. By the MRL assay, one of the eight patients had IgM antibodies toward all three species, and further, one had a fourfold rise of C. psittaci IgG antibody titers. C. pneumoniae IgM antibodies were detected in four sera from two patients by the MRL assay but in none of the sera by the LAB assay or the WRF assay. C. pneumoniae IgG antibody titers of 64 to 256 were detected in sera from three, four, and six patients by the WRF assay, the LAB assay, and the MRL assay, respectively.

Comparison of results for group I with those from the NAP Study. The results for group I tested with antigens from WRF in the present study were compared to the results originally obtained in the NAP Study (12), with the same cutoff applied to both sets of results. The detection rate of C. pneumoniae IgM antibody titers of $\geq 16$ was 24% (CI, 19 to 29%) versus 31% (CI, 26 to 36%) in the NAP Study, and that of IgG antibody titers of $\geq 64$ was 45% (CI, 40 to 50%) versus 69% (CI, 64 to 74%), but the detection rate of IgA antibody titers of $\geq 16$ was 64% (CI, 59 to 69%) versus 40% (CI, 45 to 55%). The endpoint titers obtained in the present study were 2.0 and 1.5 dilution steps lower for IgM and IgG antibodies and 0.3 dilution step higher for IgA antibodies than in the NAP Study.

DISCUSSION

In the present study, it was demonstrated that the accordance between results by the WRF assay and by the commercial assays was excellent for the IgM antibody detection rate (98%). The accordance in detection rates for IgG and IgA antibodies in sera from patients with acute infections was acceptable (87 to 88%), and in sera from group III, it was excellent (95 to 97%). No significant difference among the detection rates of the three assays was seen for any of the Ig classes.

The day-to-day variation in MIF test results implied that a difference of one dilution step is insignificant. This result is in accordance with results from other laboratories experienced in C. pneumoniae MIF analyses (11).

The reproducibility of C. pneumoniae IgG antibody endpoint titers was higher, though IgG antibody titers by the WRF assay were on average 0.4 dilution step lower than those by the other two assays. However, this difference is within the day-to-day variation of one dilution step. Similarly, another recent study comparing IgG antibody titers obtained by the WRF assay and the LAB assay found an insignificant difference of one-dilution-step-higher titer levels by the latter assay (11). The IgM antibody endpoint titers found by the LAB assay were almost two dilution steps higher than those found by the WRF assay and the MRL assay. Labsystem recommends that when testing for IgM antibodies, the incubation time for sera with the antigen should be 180 min, that is, six times as long as originally recommended by Wang (19, 20). The effect of changing the incubation period from 30 to 90 min was assessed by analyzing 22 IgM antibody-positive sera with the WRF assay and the MRL assay (unpublished data). As expected, the results showed that a prolonged incubation time can increase the IgM antibody titer levels; therefore, differences in IgM titer levels obtained by different assays could be due in part to the differences in incubation times.

For the MRL assay, the average endpoint titers were 0.4 dilution step lower than those by the WRF assay for IgM antibodies and 0.5 and 0.3 dilution step higher for IgG and IgA antibodies. Freidank et al. found the average titers for IgG antibodies and IgA antibodies by the MRL assay to be 2.5 and 3.0 dilution steps higher than those obtained by the WRF assay (5). Freidank et al. used anti-human Igs from Medac in the WRF assay, whereas we used anti-human Igs from Dako. Though only minor differences among IgG antibody levels detected by the three assays were found, a difference among the proportions of sera with antibody titers of $\geq 512$ was seen. By the WRF assay in 1.6% and by the MRL assay in 7.5% of sera, IgG antibody titers of $\geq 512$ were demonstrated. Among sera studied by Freidank, 2% had titers of $\geq 512$ with the WRF assay compared to 30% with the MRL test (5).

It has been demonstrated that the use of a C. pneumoniae antigen produced from a local isolate can result in higher detection rates of antibodies in sera and higher titers in the MIF assay than when using a standard antigen (2). The three assays studied each use a different C. pneumoniae strain as the antigen. However, the detection rates and endpoint titers by these three assays were similar when the same panel of sera were tested, with IgM antibody endpoint titers found by the LAB assay as the only exception.

Comparing the results by the MRL assay versus the WRF assay for sera from patients in groups I and II with those from group III, there were no major differences. If anything, the results obtained by the two assays were more concordant for group III sera than for sera from patients with acute respiratory tract infection (groups I and II) (Tables 2 to 7).

In the interlaboratory study by Peeling et al. (13), the agree-
ment among the participating laboratories for IgM titers was 50 to 95%. In the present study, the agreement among the three assays in detecting IgM antibodies in sera from the eight persons with microbiologically verified C. pneumoniae infection was 87 to 100%. Considering the technical complexity of the MIF test and the subjective nature of reading the titers, it is hardly surprising that the interlaboratory variation of the study by Peeling et al. is higher than the interassay variation of the present study.

The agreement between the serological and the microbiological diagnoses for the patients with C. pneumoniae infection was high, in contrast to that for the patients with C. psittaci infection. For most of the patients with psittacosis, sera were obtained early in the course of the disease. Furthermore, there is a greater diversity between C. psittaci strains than between C. pneumoniae strains, and it is possible that the C. psittaci strains used in the LAB assay and the MRL assay did not react with C. psittaci antibodies from Danish patients with infections caused by local strains.

Labsystems has reduced the lipopolysaccharide activity in the C. pneumoniae and C. trachomatis antigens but not in the C. psittaci antigen; therefore, it might be expected that the LAB assay would detect C. psittaci antibodies in sera with positive test results by the Chlamydia CF test. However, in group II, 15 of 16 patients had a positive CF test result, but C. psittaci IgM antibodies were detected in only 4 patients.

The detection rates of C. pneumoniae IgM and IgG antibodies, as well as the antibody endpoint titer levels, were low among sera from group I of the present study compared to the results in the NAP Study; the detection rate of C. pneumoniae IgA antibody was higher in the present study. Although the same antigen for the MIF was used in the two studies, other differences in the test procedures occurred, e.g., the use of different conjugates and interpersonal differences in the reading of slides might explain some of the variations in the results obtained. Furthermore, in the present study the sera had been absorbed before the IgA antibody analyses; removal of IgG has been shown to raise IgA antibody titers in sera with high levels of IgG antibodies (9). Finally, the patients in the NAP Study were recruited between 1990 and 1993 and the sera were analyzed shortly thereafter, whereas in our study the sera were tested after storage at −20°C for up to 8 years.

The prevalence of circulating rheumatoid factor is strongly correlated with age, and the presence of rheumatoid factor in sera containing C. pneumoniae IgG antibodies might give false-positive C. pneumoniae IgM antibody test results (13, 17). In one study, 41 of 286 patients had C. pneumoniae IgM antibody titers of ≥16. Although only 78% of the patients had detectable circulating rheumatoid factor, none of the 41 sera were shown to have C. pneumoniae IgM antibodies after absorption of IgG antibodies (17). Since circulating rheumatoid factor at a level below the threshold of detection by routine screening can cause false-positive IgM results, it is recommended that IgG be removed prior to IgM antibody testing (3). Since the presence of IgM antibodies is considered a sign of acute infection, it is possible that some studies have reported prevalences of acute C. pneumoniae infection that have been inflated due to the inclusion of patients with false-positive IgM antibody results.

In conclusion, the three MIF assays investigated showed about the same ability to detect C. pneumoniae IgM, IgG, and IgA antibodies, but some variation was found in the antibody levels demonstrated. In the light of the established criteria for the serological diagnosis of acute C. pneumoniae infection (6), the difference in average endpoint titer levels of IgM antibodies is of no major concern, as the emphasis of the criteria rests on the presence of IgM antibodies, not on the level. In contrast, the difference in IgG antibody endpoint titers by different assays presents a problem as long as a high level of IgG titers (≥512) is used as an isolated criterion for the serological diagnosis of acute C. pneumoniae infection.

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