

Rapid and Simple Diagnosis of *Chlamydomphila pneumoniae* Pneumonia by an Immunochromatographic Test for Detection of Immunoglobulin M Antibodies[∇]

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Received 8 March 2008/Returned for modification 2 April 2008/Accepted 6 May 2008

To evaluate a newly developed immunochromatographic test (the MySet test) for the detection of *Chlamydomphila pneumoniae*-specific immunoglobulin M antibodies, the results obtained by the MySet test were compared with those obtained by two serological tests. The sensitivity and specificity of the MySet test were 100% and 92.9%, respectively. The MySet test is rapid and simple to use and is thought to be a useful tool for the selection of appropriate antibiotic therapy.

Community-acquired pneumonia (CAP) is a common, but serious, respiratory disease. Despite substantial progress in therapeutic options, CAP remains a significant cause of morbidity and death worldwide, and there continue to be major controversies concerning the antimicrobial management of this infection. Rapid and simple diagnostic tests, such as sputum Gram staining, the urinary antigen test, and an antigen detection assay with nasopharyngeal swab specimens, are useful tools for the early presumptive diagnosis of CAP caused by some pathogens. These results can be used to initiate appropriate antibiotic treatment (pathogen-oriented treatment) and prevent antimicrobial resistance through the use of narrow-spectrum antibiotics.

Chlamydomphila pneumoniae, an obligate intracellular pathogen, has been proven to cause both epidemic and endemic respiratory tract infections in many areas of the world (1, 3, 4, 7). It is a significant cause of both lower and upper acute respiratory illnesses and accounts for approximately 10% of cases of CAP (1, 3, 4, 7). Until recently, however, no rapid, simple tests have been available for the diagnosis of *C. pneumoniae* infection (2). A rapid and simple immunochromatographic test for the detection of immunoglobulin M (IgM) antibodies against *C. pneumoniae* (the MySet *C. pneumoniae* IgM test; Ani Biotech Ltd., Oy, Vantaa, Finland) has recently been developed. The test requires only 1 drop (10 μ l) of blood from the fingertip or 5 μ l of serum, and it can be performed and the results can be evaluated in 5 to 10 min. In the present study, we evaluated the MySet test for the accuracy of diagnosis of *C. pneumoniae* infection and compared it with the results

obtained with conventional serological tests, the AniLab *C. pneumoniae* enzyme immunoassay (EIA; Ani Labsystems [AniLab] Ltd., Oy, Vantaa, Finland) (10) and a microimmunofluorescence (MIF) test, which is the current “gold standard” for serological testing for *C. pneumoniae* infections worldwide (2, 4).

The MySet test has been developed from the previously available AniLab EIA, and the method has been modified by use of a two-step test with the predilution of specimens. The principle of the MySet test is an immunochromatographic method. Prediluted specimens are collected with a sample stick which contains gold-labeled antibodies, and the stick is placed in the well of the test device. Subsequently, the sample and the labeled antibodies move into the reading window of the device. If the sample contains the appropriate antibodies, they bind to the gold-labeled antibodies and with the stationary antigen in the test line. The test also contains an integrated control system, and a red control line indicates the proper functioning of the test (Fig. 1). The antigen used is same as that used in the AniLab EIA (10) and comprises elementary bodies (EBs) of a European isolate of *C. pneumoniae*. Purified EBs are used in the test line of the device. The AniLab EIA is an indirect solid-phase EIA based on an antigen from *C. pneumoniae* devoid of lipopolysaccharide (LPS). The results for IgA and IgG are expressed as enzyme immunounits (EIU), which are calculated as follows: $[(A_{\text{sample}} - A_{\text{blank}})/(A_{\text{calibrator}} - A_{\text{blank}})] \times n$, where A is the absorbance and n is the number of samples (10). The criterion for a diagnostically significant change in the EIU values for IgG and IgA is a 1.5-fold change of EIU in the zones below 130 EIU for IgG and 50 EIU for IgA. When the first sample shows an EIU value of 130 or more for IgG and 50 or more for IgA, a 1.3-fold change is considered significant. Instead of the EIU values for IgG and IgA, the result for IgM is expressed as a signal/cutoff (S/CO) ratio after subtraction of the value for the blank. Samples with an S/CO

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[∇] Published ahead of print on 14 May 2008.

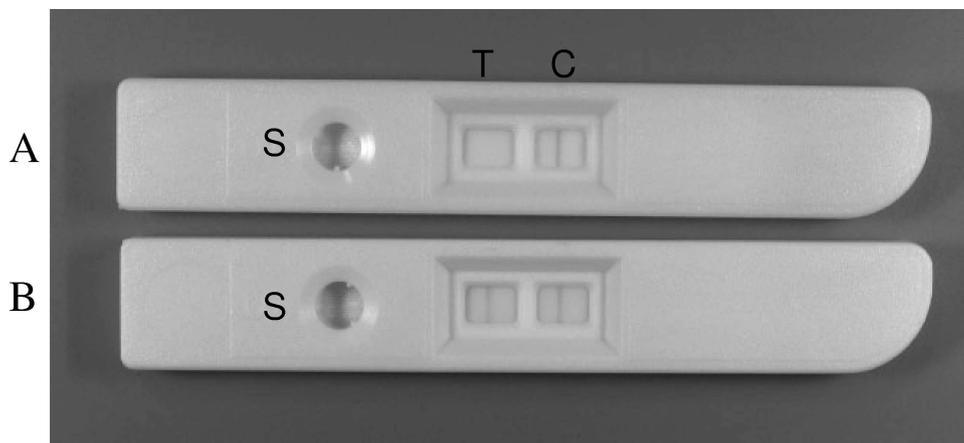


FIG. 1. Results obtained with the test device. (A) Negative result; (B) positive result. After placement of the sample in the sample field (S) on the device, the test result is positive if a red control line appears in the control field (C) and a red line appears in the test field (T). Red indicator lines appear dark gray here.

ratio of more than 1.1 are considered positive (10). For the MySet test, 5 μ l of serum in sample buffer is shaken several times, and then the sampling stick is placed into the sample buffer for 10 to 15 s. Next, the sample stick is removed from the buffer and placed into the test device. The result is evaluated in 5 to 10 min. The test result is positive if a red control line appears in the control field and a light to dark red line forms in the test field.

The MIF test was primarily developed for the detection of *C. trachomatis* by Wang and Grayston (13), but it was later adapted for use for the serodiagnosis of *C. pneumoniae* infections (14). The EBs of strain TW-183 were purified by continuous Urografin (Schering AG, Berlin/Bergkamen, Germany) gradient centrifugation (40 to 52%). The EBs were resuspended in a solution of 2% yolk sac in phosphate-buffered saline (pH 7.2) containing 0.02% formalin. IgM, IgA, and IgG antibodies against *C. pneumoniae* were detected with commercial fluorescein isothiocyanate-conjugated goat anti-human IgM, IgA, and IgG (Medical and Biological Laboratories, Nagoya, Japan). Rheumatoid factor (RF) was absorbed with GullSORB (Meridian Bioscience Inc.) before IgM titrations. An anti-*C. pneumoniae* antibody titer of $\geq 1:32$ for IgM or a fourfold increase in IgA or IgG titer is considered an indication of acute infection.

Culturing for *C. pneumoniae* with nasopharyngeal swab specimens was performed in cycloheximide-treated HEp-2 cells grown in a 24-well cell culture plate, as reported previously (6). Following incubation, a genus-specific fluorescein isothiocyanate-conjugated monoclonal antibody (Chlamydia FA Seiken; Denka Seiken, Tokyo, Japan) and *C. pneumoniae* species-specific monoclonal antibodies were used to stain the inclusions. PCR was also performed as described previously (9). The *C. pneumoniae*-specific sequences of the PCR primers and the probe were selected from the 53-kDa protein of *C. pneumoniae* with a sequence detection system (version 1.6.3; Applied Biosystems) and were synthesized by Applied Biosystems (9). PCR was performed in 96-well MicroAmp optical plates (Applied Biosystems) with reaction mixtures consisting of 12.5 μ l of the TaqMan universal master mix including dUTP and uracil *N*-glycosylase (AmpErase UNG; Applied Biosys-

tems). The primers and the probe were used in a total reaction volume of 25 μ l for the purified *C. pneumoniae* DNA series and clinical specimens. Amplification and detection of the PCR products were performed with an ABI Prism 7700 sequence detection instrument (Applied Biosystems), as suggested by the manufacturer, by using all of the default program settings. A sequence detection system (version 1.6.3) was used for analysis after the real-time PCR. Sera and nasopharyngeal swab specimens were obtained from all subjects after their informed consent was obtained; the study protocol was approved by the Ethics Committee of the Kawasaki Medical School.

The IgM antibody titers of nine serum samples collected periodically from a patient with acute *C. pneumoniae* pneumonia were determined using the MySet test, the AniLab EIA, and MIF test (Table 1). A complement fixation (CF) test, which is useful for the detection of primary infection with *C. pneumoniae* (2), was also tested with samples from this patient (Table 1). The patient was a 39-year-old man who had arrived at the Kawasaki Medical School Hospital complaining of cough, and his chest radiograph revealed consolidation in the right middle and lower lung fields. The *C. pneumoniae* organism and the 53-kDa protein gene were detected in a bronchoalveolar lavage fluid specimen by cell culture and PCR, respec-

TABLE 1. IgM antibody titer determined by different serological tests in a patient with *C. pneumoniae* pneumonia

| Serum sample no. | No. of days after onset | MySet test result | AniLab EIA result (S/CO ratio) | IgM antibody titer | |
|------------------|-------------------------|-------------------|--------------------------------|--------------------|----------------|
| | | | | MIF test result | CF test result |
| 1 | 5 | – | 0.88 | <1:16 | <1:4 |
| 2 | 24 | + | 4.22 | 1:512 | 1:64 |
| 3 | 32 | + | 7.88 | 1:512 | 1:32 |
| 4 | 55 | + | 7.80 | 1:512 | 1:32 |
| 5 | 68 | + | 7.33 | 1:128 | 1:16 |
| 6 | 110 | + | 3.55 | 1:16 | 1:8 |
| 7 | 144 | + | 2.37 | <1:16 | 1:4 |
| 8 | 296 | – | 0.90 | <1:16 | <1:4 |
| 9 | 415 | – | 0.12 | <1:16 | <1:4 |

tively. Seroconversion to positivity for *C. pneumoniae*-specific antibodies was observed thereafter. The variations in the IgM antibody titers detected by these four tests were almost identical.

To evaluate the clinical sensitivity of the MySet test, serum samples collected from patients with CAP were tested. The patients had visited the outpatient clinic or had been admitted to the Kawasaki Medical School Hospital, Kurume University Hospital, or the National Hospital Organization Tokyo Medical Center between April 2003 and December 2006. The microbial etiology was determined as reported previously (6), and those patients with an unknown etiology were excluded from this study. Finally, a total of 140 CAP patients (74 males and 66 females; mean age, 38.7 years; age range, 2 to 56 years) were evaluated. Initial serum samples for the detection of IgM antibodies were collected from 10 to 21 days after the onset of illness. Convalescent-phase serum samples were collected at intervals of at least 4 weeks after initial serum sample collection. Of these, 41 patients were diagnosed with *C. pneumoniae* pneumonia and the remaining 99 patients were diagnosed with bacterial pneumonia. Among the patients with *C. pneumoniae* pneumonia, all patients showed positive IgM reactions by both the MIF test and the AniLab EIA, and paired serum samples from these patients showed significant changes in IgA and/or IgG antibody titers. Positive reactions by *C. pneumoniae* culture and PCR were observed in 7 and 9 of the 41 patients, respectively. Five cases were culture positive and PCR positive, while six cases were positive but with discrepancies in either the culture or the PCR results. No positive results by culture, PCR, or detection of IgM antibodies to *C. pneumoniae* were observed in patients with bacterial pneumonia. The correlations between the MySet test and the other serological tests are presented in Table 2. Forty-one patients diagnosed with *C. pneumoniae* pneumonia showed a positive reaction in all serological tests, and a further 7 of 99 patients with non-*C. pneumoniae* pneumonia (bacterial pneumonia) showed positive reactions by the MySet test. To examine the reactivity of the MySet test, we measured IgM using another established serological test, a recombinant EIA (rEIA) based on the recombinant LPS of *Chlamydia* as an antigen (Medac, Wedel, Germany). Several studies have demonstrated good agreement between the rEIA and the MIF test for the serodiagnosis of acute *C. pneumoniae* infection (10). Since none of the seven samples tested positive by rEIA, these MySet test-positive reactions were considered to be false positive. Thus, the sensitivity, specificity, and positive and negative predictive values of the MySet test were 100%, 92.9%, 85.4%, and 100%, respectively.

Currently, the most reliable serological test for the diagnosis of *C. pneumoniae* infection is the MIF test, and the use of only this test is strongly recommended (2). However, most clinicians and researchers do not use the MIF test for routine diagnosis because this assay is technically demanding and time-consuming and antigen preparation is difficult. In addition, the MIF test involves an element of subjective assessment. Thus, automated commercial kits have widely been used for routine diagnosis, but each test has its own sensitivity and specificity limitations. Therefore, the development of a new means of diagnosis of *C. pneumoniae* infection that is rapid, simple, and reliable is expected to be useful in the clinical field.

TABLE 2. Results of the IgM reaction in sera from 140 patients with CAP by MySet test and other serological tests

| Organism(s) | No. of patients with positive reaction for IgM by: | | |
|--|--|----------|------------|
| | MySet test | MIF test | AniLab EIA |
| <i>C. pneumoniae</i> ($n = 41$) ^a | 41 | 41 | 41 |
| Bacteria ($n = 99$) | 7 ^b | 0 | 0 |

^a Paired serum samples from 41 patients with positive reactions for IgM in all serological tests showed significant changes in IgA and/or IgG antibody titers, and all patients were diagnosed with *C. pneumoniae* pneumonia.

^b Seven patients with negative IgM reactions by both the AniLab EIA and the MIF test were negative by rEIA.

Among the Ig classes, IgM antibodies are very helpful for the rapid diagnosis of acute *C. pneumoniae* infection because of their early serological response to *C. pneumoniae* infection compared to the times of response of IgG and IgA antibodies (2, 4). In a clinical setting, single rather than paired acute- and convalescent-phase serum samples are used to diagnose acute infections. In this study, we evaluated a newly developed diagnostic test, the MySet *C. pneumoniae* IgM test, for the detection of anti-*C. pneumoniae*-specific IgM antibodies and found good agreement with the results of the MIF test using serum samples periodically collected from a patient with acute *C. pneumoniae* pneumonia. Of the 140 samples from patients with CAP, 41 showed significant reactions by all three serological tests for *C. pneumoniae* infection. Seven samples showed false-positive reactions by the MySet test, although the sensitivity of the MySet test was almost equivalent to that of the MIF test.

It is well-known that RF sometimes causes false-positive IgM reactions for *C. pneumoniae* (2, 12). Therefore, an RF-absorbing reagent is used in the MySet test and no cross-reactions have been detected. In addition to RF, we suggested that chronic lung diseases such as chronic obstructive pulmonary disease, collagen disease-associated interstitial lung disease, and cryptogenic organizing pneumonia may cause false-positive reactions for IgM with another enzyme-linked immunosorbent assay kit (8). However, no patients with chronic lung disease or high levels of RF were included in the present study, and we could not determine the cause of the false-positive reaction in the MySet test. To determine the possible causes of the false-positive results by the MySet test, such as other underlying conditions, further studies are required.

In the clinical setting, simple and rapid diagnostic tests that can be performed and whose results can be evaluated within 15 min are thought to be very useful for the selection of appropriate initial antibiotic therapy. Among the currently available rapid and simple diagnostic tests, urinary antigen tests for the detection of *Streptococcus pneumoniae* and *Legionella pneumophila* have been well evaluated and are widely used (5, 11). The MySet test also has the potential advantages of rapid and simple detection, similar to that of urinary antigen tests. However, it has some drawbacks. First, the usefulness of the MySet test may be limited compared to that of the urinary antigen test because the IgM antibody appears only ~2 to 3 weeks after the onset of illness (2). Thus, the MySet test may not be suitable as a point-of-care test with relevance to initial antimicrobial therapy. Second, in some cases of *C. pneumoniae* reinfection, the

IgM antibody may not appear (2). Thus, the MySet test may be useful only in cases of primary infection and in some cases of reinfection. Further studies are obviously necessary to determine the precise timing for the collection of test specimens in relation to the time of disease onset and the reaction to reinfection by the MySet test.

This work was supported by the Mext Kakenhi (19591190) and project research grants from the Kawasaki Medical School (grants 13-401, 14-402, 15-405A, 16-405 M, 17-402 M, 18-401, and 19-402 M).

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