Evaluation of Serodia Myco II particle agglutination test for detecting Mycoplasma pneumoniae antibody: comparison with μ-capture ELISA and indirect immunofluorescence

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Abstract

The Serodia Myco II particle agglutination test, which the manufacturers claim exclusively detects IgM antibody, was compared with two IgM-specific tests, a μ-capture ELISA, and indirect immunofluorescence for their ability to detect recent *Mycoplasma pneumoniae* infection. In general there was good agreement among the three tests, all three having similar sensitivity. One hundred and nine (78%) of serum samples gave concordant results in all three assays. Several sera gave positive particle agglutination titres, however, while being negative by the two other assays, and the Serodia Myco II test may not be as specific for detecting *M pneumoniae* IgM as the other two tests.

While the Serodia Myco II test may be a good screening assay, it is unlikely to be a definitive test for *M pneumoniae* IgM, but may be better than the complement fixation test, particularly in younger patients in whom *M pneumoniae* IgM is found more frequently.

Mycoplasma pneumoniae infection is frequently associated with severe pulmonary and non-pulmonary disease in man.[1] Early diagnosis is important because the infection often responds well to appropriate antibiotics. The culture of *Mycoplasma pneumoniae*, although a sensitive indicator of infection, is difficult, and due to its fastidious nature is too slow to be of diagnostic value. Most laboratories still rely on complement fixation test for diagnosis of *M pneumoniae* infection—a test shown to be both non-specific and insensitive.[2,3] Tests for detection of *M pneumoniae* IgM,[4–7] while providing an accurate indication of recent infection when positive, are only available in a few laboratories.

It was felt that a commercial kit, marketed for detection of recent *M pneumoniae* infection, warranted evaluation against well established μ-capture ELISA and immunofluorescence tests to investigate its diagnostic potential in this context.

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Methods

A total of 140 samples of serum from 96 patients with clinical features compatible with *M pneumoniae* infection were selected.

The complement fixation test was performed as described by Bradstreet and Taylor[8] using a microtitre system, overnight fixation, and antigen provided by the Public Health Laboratory Service Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, Colindale, London.

The μ-capture ELISA was performed as previously described.[5] Negative and positive control sera were selected as previously described[7] and allocated 0 arbitrary units and 100 u, respectively. The positive control serum was diluted in negative control serum to give dilutions containing 33, 10, 3·3, 1, 0·33 and 0·1 u. Sera with $\geqslant$0·33 u of *M pneumoniae*-specific IgM were regarded as positive.

The indirect immunofluorescence antibody (IFAT) test was performed as previously described.[4] IgM, IgA, and IgG were detected by means of anti-human Ig fluorescein isothiocyanate (FITC) conjugate (IgM and IgG, Wellcome Diagnostics, IgA, Miles). Sera with titres of $\geqslant$ 4 (IgM), $\geqslant$16 (IgA), or $\geqslant$64 (IgG) were regarded as indicating recent *M pneumoniae* infection.

The Serodia Myco II gelatin particle agglutination test (Fujirebio, Japan) is marketed in the United Kingdom by Mast Diagnostics, Bootle, and was performed according to the manufacturer's instructions. It is based on the principle that gelatin particles sensitised with *M pneumoniae* cell membrane components are agglutinated in the presence of *M pneumoniae* antibody. Serum samples were inactivated at 56°C for 30 minutes. Rigid U-well microtitre plates (supplied with the kits) were soaked in detergent solution overnight and then rinsed thoroughly under running tap water. They were then washed with distilled water and dried. Using the serum diluent supplied, 25 μl serum samples were double diluted to give dilutions of 1 in 10 to 1 in 10240. Sensitised and unsensitised lyophilised gelatin particles were suspended in diluent. Twenty five microlitre drops of the unsensitised particle suspension were added to the 1 in 10 serum dilutions to give a final dilution of 1 in 20, and 25 μl drops of the sensitised particle suspension were added to the remaining wells giving final dilutions of 1 in 40 to 1 in 20480. The plates were shaken for 30 seconds and then covered and left undisturbed on a level surface at room temperature for three hours (or overnight).

The test was initially calibrated using the control sera dilution series. Each batch of tests included control wells containing 25 μl of

diluent and 25 µl of the particle suspensions and dilutions of a reactive control serum of known titre, supplied with the kit.

Buttons or compact, smooth rings of particles in the bottom of the wells were read as negative agglutination patterns and a more extensive ring as positive. Titres of ⩾40 were regarded as positive.

The control series used for the initial calibration of the particle agglutination test gave titres of ⩾40 only with serum containing ⩾0·33 µ-capture units. These tests are therefore of similar sensitivity.

Results

When the 140 serum samples were tested with the Serodia Myco II particle agglutination, µ-capture ELISA, and indirect immunofluorescence tests, similar results were obtained (figs 1 and 2). Of the 140 sera, only one produced a positive agglutination pattern with the unsensitised particle suspension. Although the manufacturer gives details of a method to absorb out factors causing non-specific agglutination, there was insufficient serum for this to be performed. One hundred and nine of 139 (78%) serum samples gave

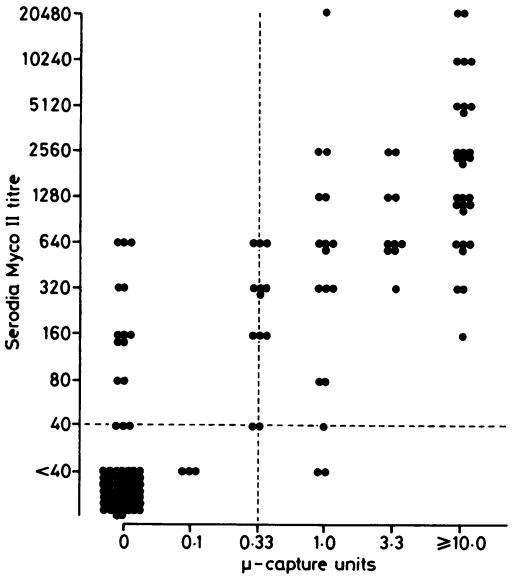

Figure 1 Correlation between results in the Serodia Myco II particle agglutination test and µ-capture ELISA.

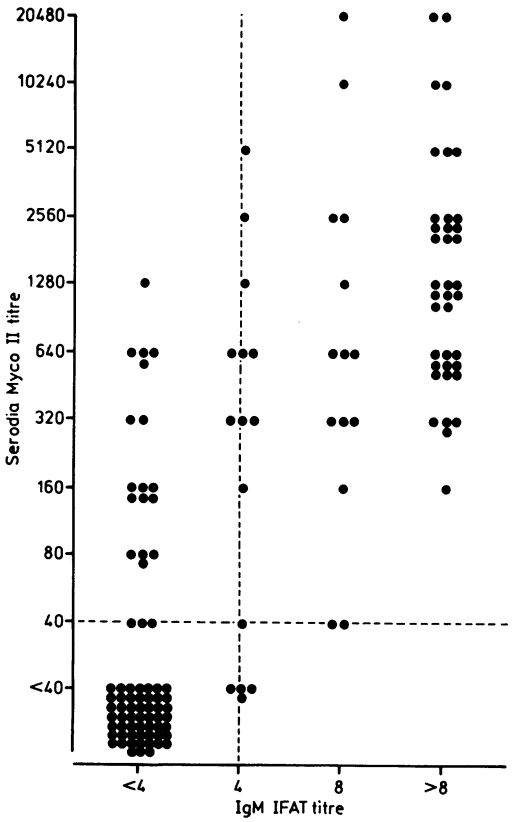

Figure 2 Correlation between results in Serodia Myco II particle agglutination test and IgM IFAT.

Results of serological tests for M pneumoniae antibodies for those sera showing discrepancies between the Serodia Myco II particle agglutination test and both IgM tests

Serum number	Particle agglutination titre	µ-capture ELISA units	IFAT titre			CFT
			IgM	IgG	IgA	
30	80	0	<4	8	<4	64
31	160	0	<4	16	<4	128
32	40	0	<4	16	<4	64
35	640	0	<4	>64	<4	128
73	160	0	<4	16	8	128
74	160	0	<4	16	8	128
79	640	0	<4	32	4	128
106	160	0	<4	32	8	64
116	40	0	<4	32	4	128
124	320	0	<4	64	4	>256
299	80	0	<4	>64	16	>256
420	160	0	<4	>64	4	not tested
34	320	0	8	>64	8	128
135	40	0	8	16	8	64
265	640	0	8	32	16	64
17	40	0·3	<4	32	<4	128
53	640	0·3	<4	>64	8	>256
58	160	0·3	<4	64	8	64
63	80	1·0	<4	16	<4	128
64	80	1·0	<4	16	8	>256
80	160	0·3	<4	16	<4	128
96	640	>10	<4	64	64	>256
188	320	0·3	<4	64	16	>256
410	1280	1·0	<4	<8	4	>256
24	<40	1·0	<4	<8	<4	
407	<40	1·0	<4	64	not tested	256
21	<40	0	4	8	<4	not tested
22	<40	0	4	16	<4	128
26	<40	0	4	8	<4	64
395	<40	0	4	8	16	256

concordant results with all three tests. While generally there was good agreement among the results of μ-capture, particle agglutination, and IgM IFAT, 30 (22%) sera gave discrepant results (table). Of these, 12 gave positive particle agglutination titres of between 40 and 640 in the absence of positive results from the other two tests. All had detectable IgG and eight were shown to have IgA by immunofluorescence, but only three had titres suggestive of recent *M pneumoniae* infection. Another 12 sera were also positive in the particle agglutination test but in only one of the other two IgM tests (table). Another six sera were negative in the particle agglutination test and in one of the IgM tests, but were positive in the remaining IgM test (table).

Discussion

Most methods for the detection of *M pneumoniae* IgM antibody, though sensitive and specific, are not widely available. In view of the response of *M pneumoniae* infection to appropriate antibiotic treatment, there is a need for a more rapid and reliable technique than the complement fixation test. For use in this context, we investigated the Serodia Myco II particle agglutination test, which, the manufacturers claim, "exclusively detects IgM antibody" and compared it with μ-capture ELISA and IgM indirect immunofluorescence tests, which have been found previously to have similar sensitivity and specificity.[5]

We found generally good agreement among the three tests, but 12 (8·6%) sera gave positive particle agglutination titres while being negative by the two reference assays. As all three tests were found to have similar sensitivity for detecting *M pneumoniae* IgM by testing them with a standard dilution series, it is unlikely that the sera giving discrepant results did so because of a difference in sensitivity. The Serodia test may therefore not be as specific for detecting *M pneumoniae* IgM as the other two tests. The μ-capture ELISA and indirect immunofluorescence tests use specific antibodies directed against human IgM; the particle agglutination test does not.

A proportion (66%) of the sera which were positive in the particle agglutination test, but negative in the two other tests, contained *M pneumoniae* specific IgA (table). It is conceivable that these patients may have experienced recent *M pneumoniae* infection, but as there was no correlation between *M pneumoniae*-specific IgA indirect immunofluorescence and particle agglutination antibody titres this seems unlikely. There was also no relation between particle agglutination positivity, IgM negativity and age of patient.

In conclusion, we feel that while the Serodia MycoII test is a useful screening assay (with very few false negative titres), for evidence of recent *M pneumoniae* infection, we, unlike the manufacturers, would be unhappy to consider it as a definitive test for the presence of *M pneumoniae*-specific IgM. It may be better than the complement fixation test, however, as an indicator of recent *M pneumoniae* infection, particularly in younger patients in whom *M pneumoniae* IgM is found more frequently.[7]

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