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Evaluation of 10 serological assays for diagnosing *Mycoplasma pneumoniae* infection ☆

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ABSTRACT

In this study, the performance of 10 serological assays for the diagnosis of *Mycoplasma pneumoniae* infection was evaluated. A total of 145 sera from 120 patients were tested. They were obtained from patients who were serologically positive for *M. pneumoniae* infection as well as from patients who were infected with micro-organisms that may cause interstitial pneumonia. The following assays were utilized: SeroMP IgM and IgG, SeroMP recombinant IgM, IgA and IgG, Liaison *M. pneumoniae* IgM and IgG and *M. pneumoniae* IgM, IgA and IgG ELISA Medac. The SeroMP Recombinant and Liaison assays both showed low IgM specificity, and crossreactivity was mainly observed in groups of patients with acute cytomegalovirus and Epstein-Barr virus infections. For IgA, the Medac assay was less specific than the SeroMP Recombinant assay. Discrepancies between the four tests were observed in IgG analyses, and due to the lack of a gold standard, 22 results were removed prior to determining the sensitivity and specificity. Therefore, the overall performance of IgG assays may be overstated; nevertheless, the SeroMP assay demonstrated a lack of sensitivity. The seroprevalence of IgG appears to be very low, raising concerns regarding whether the serological techniques can detect IgG levels over time. Serology remains a biological tool of choice for diagnosing *M. pneumoniae* infection, but improvement and standardization of the assays are needed, particularly for the determination of IgG.

1. Introduction

Mycoplasma pneumoniae is a leading cause of bacterial community-acquired pneumonia (Cillóniz et al., 2012; Strålin et al., 2006), accounting for 15–20% of cases, and up to 40% in children. It may also be responsible for upper respiratory tract infections and extra-pulmonary manifestations. This infection is endemic, with epidemic peaks occurring every 4 to 7 years (Bébéar, 2007), as was observed in several European countries from 2010 to 2011 (Jacobs, 2012). Laboratory diagnosis was previously performed with cultures of the organism, but this technique is slow and less sensitive than serological or nucleic acid amplification assays (She et al., 2010). The nucleic acid amplification assays appear to be the most sensitive methods; however, those techniques cannot distinguish between asymptomatic and acute infections (Dorigo-Zetsma et al., 2001; Foy, 1993; Ieven and Goossens, 1997; Loens et al., 2003). Serology still has a place of choice in the diagnosis of *M. pneumoniae* infection, but this must rely on the analysis of two coupled sera taken at 2–3 weeks. Serological diagnosis can confirm a recent infection when there is an apparition of the IgG or when there is a significant increase in IgG levels between the two sera because upon reinfection, IgM may not be present (Waites and Talkington, 2004). Complement fixation has been replaced with a variety of commercially available techniques, specifically with the enzyme-linked immunosorbent assays (ELISA), which allow for a precise quantification of IgM, IgA or IgG (Bébéar, 2007). The aim of this work is to evaluate the performance of 10 serological assays for the diagnosis of *M. pneumoniae* infection.

2. Materials and methods

2.1. Sera

A total of 145 sera from 120 patients (54 women and 66 men) were evaluated. Fifty sera were coupled, and the interval between the two sera collections varied from 5 days to 5 months. The average age of the patients was 23.63 years (median: 15.5 years). Sera were sorted into different groups (Table 1): *M. pneumoniae* infections (n = 32), non-specific *M. pneumoniae* IgM (n = 25), other infections causing an

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Table 1
List of sera.

	Description	Criteria of selection	n
M. pneumoniae infection	Serologically confirmed *M. pneumoniae* infection	Apparition or significant augmentation of IgG between paired serum (with or without IgM and IgA)	26
	Possible *M. pneumoniae* infection	High amount of *M. pneumoniae* IgG, IgA and IgM	6
Non-specific IgM	Non-specific *M. pneumoniae* IgM	Persistent *M. pneumoniae* IgM over time without appearance of IgG	25
Other causes of interstitial pneumonia	*Chlamydia pneumoniae* infection	Seroconversion in anti-MOMP IgG (n = 2) or high levels of anti-LPS IgA and IgG (n = 5)	7
	Legionella pneumophila infection	Positive *L. pneumophila* antigen in urine (n = 2) or positive serology with immunofluorescence (n = 2)	4
	RSV infection	Positive RSV culture (n = 5) or positive RSV serology with complement fixation (n = 5)	10
	Adenovirus infection	Positive adenovirus culture (n = 4) or positive adenovirus serology with complement fixation (n = 5)	9
	Parainfluenza virus infection	Positive parainfluenza culture (n = 5) or positive parainfluenza serology with complement fixation (n = 5)	10
	Influenza A or B infection	Positive influenza A or B culture (n = 5) or positive influenza serology with complement fixation (n = 4)	9
	CMV infection	Positive CMV culture (n = 2) or positive CMV serology with Abbott Architect (n = 3) or both (n = 3)	8
	VZV infection	Positive VZV culture (n = 1) or positive VZV serology (n = 4) or both (n = 2)	7
	Measles infection	Positive measles culture on a respiratory sample (n = 1) or positive serology with evocative clinical examination (n = 8)	9
	Human metapneumovirus infection	Positive human metapneumovirus direct immunofluorescence on a respiratory sample (n = 4)	4
	Coronavirus 229E infection	Positive micro-array on a respiratory sample (n = 1)	1
	Aspergillus sp. infection	Positive galactomannan on a respiratory sample and serum (n = 4)	4
EBV acute infection	EBV acute infection	Positive EBV serology with evocative clinical examination and laboratory findings	6
		TOTAL	145

MOMP = major outer membrane protein; LPS = lipopolysaccharide.

interstitial pneumonia and positive Epstein-Barr virus (EBV) serology, which cross-reacts with *M. pneumoniae* serology (Beersma et al., 2005). Cases of *M. pneumoniae* infections were initially chosen in front of an apparition or a significant augmentation of IgG on paired sera using SeroMP (Savyon Diagnostics, Ashdod, Israel) which was in use for the routine analyses in our laboratory. The serological results of those samples were then compared to those obtained with the other techniques evaluated and finally the group of sera representing the *M. pneumoniae* infections was composed either from sera exhibiting an apparition or significant augmentation of IgG between paired samples with at least two of the evaluated techniques and sera with high amount of *M. pneumoniae* IgG, IgA and IgM with SeroMP Recombinant IgM, IgA and IgG (Savyon Diagnostics, Ashdod, Israel) and *M. pneumoniae* IgM, IgA and IgG ELISA Medac (Medac, Hamburg, Germany). Cases of Q fever would ideally have been included, but these were not available. Sera were selected from the serum bank at the Porte de Hal Laboratory, which performs serological analyses for four public university hospitals that are located in Brussels, Belgium.

2.2. Serological assays

- SeroMP IgM and IgG (Savyon Diagnostics, Ashdod, Israel): an ELISA test for the semi-quantitative detection of IgM and IgG antibodies against *M. pneumoniae* in human serum.
- SeroMP Recombinant IgM, IgA and IgG (Savyon Diagnostics, Ashdod, Israel): an ELISA test for the semi-quantitative detection of IgM, IgA and IgG antibodies against *M. pneumoniae* in human serum.
- LIAISON *M. pneumoniae* IgM and IgG (Biotrin International Ltd., Dublin, Ireland): a chemiluminescence immunoassay (CLIA) used for the qualitative (IgM) or semi-quantitative (IgG) determination of antibodies against *M. pneumoniae* in human serum or plasma, performed with the LIAISON analyzer.
- *M. pneumoniae* IgM, IgA and IgG ELISA Medac (Medac, Hamburg, Germany): an enzyme immunoassay for qualitative (IgM) or quantitative (IgA and IgG) determination of *M. pneumoniae* antibodies in human serum.

The results were classified as negative, equivocal or positive, according to the cut-off values that were specified by the manufacturers.

As there is no gold standard for *M. pneumoniae* serology, the sorting of the results into true or false positives or negatives was performed by matching the clinical data with the serological profile. If the interpretation was unclear, a paired sample was analyzed, if available. Despite our careful interpretation, some discrepant results could not be sorted and were not taken into account when calculating the performance of the assays. Of the 145 samples that were evaluated, this occurred for 3 IgM, 5 IgA and 22 IgG results.

3. Results

Table 2 shows the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) results for each assay. The PPV and the NPV are shown for informative purposes, as they depend on the disease prevalence in the population. For IgM, the specificity was recalculated after removing 25 sera with non-specific IgM results, as the

Table 2
Sensitivity, specificity, PPV and NPV of the different assays for IgM, IgA, and IgG.

	SeroMP	SeroMP Rec	Liaison	Medac	SeroMP Rec	Medac	SeroMP	SeroMP Rec	Liaison	Medac
	IgM	IgM	IgM	IgM	IgA	IgA	IgG	IgG	IgG	IgG
Sensitivity (%)	100	100	100	100	100	100	61.76	97.05	100	100
Specificity (Sp) (%)	75.22	68.93	71.68	80.58	98.33	88.13	96.42	100	97.75	100
Sp (without non-specific) (%)	92.04	81.48	82.95	91.56						
PPV (%)	50.87	47.54	47.54	59.18	90.9	58.82	87.5	100	94.44	100
NPV (%)	100	100	100	100	100	100	86.17	98.88	100	100
	n = 142(n = 117 without non-specific)				n = 140		n = 123			