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BRIEF REPORT

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Rapid detection of *Mycoplasma pneumoniae* IgM antibodies in pediatric patients using ImmunoCard Mycoplasma compared to conventional enzyme immunoassays

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Mycoplasma pneumoniae is a common cause of community-acquired respiratory infection in children. An estimated 30% of *Mycoplasma pneumoniae* infections in those 5–15 years of age result in pneumonia and, overall, *Mycoplasma pneumoniae* is responsible for 10–40% of all cases of community-acquired pneumonia in children [1, 2]. A specific diagnosis of *Mycoplasma pneumoniae* infection is important since treatment with routinely used β-lactam antibiotics is ineffective against these organisms which lack a cell wall. Proper treatment of infected patients with macrolide antibiotics shortens the duration of overt infection and decreases the severity of respiratory symptoms [2, 3]. Unfortunately, the prompt and accurate diagnosis of *Mycoplasma pneumoniae* infection in children is hampered by the lack of standardized, rapid, and specific testing methods.

Current methods for the specific diagnosis of *Mycoplasma pneumoniae* infection include culture, serology, and polymerase chain reaction (PCR). Serology has been the mainstay for the diagnosis of *Mycoplasma pneumoniae* infections, particularly since culture methods are insensitive, require specialized techniques, and at least 2–3 weeks for results [4]. PCR is a promising diagnostic tool for the identification of *Mycoplasma pneumoniae* in clinical specimens, but it is available only in specialized laboratories. No PCR kits approved by the Food and Drug Administration of the USA are yet available, and in-house performance characteristics vary substantially among laboratories [3–5].

Results of several studies evaluating various serologic methods have been published, but an assessment of the actual performance of the tests is hampered by the lack of standardized, comparative methods [6–9]. Older methods have tested for complement fixing antibodies that measure predominantly immunoglobulin (Ig)G and usually peak 1–3 weeks after the onset of infection. Because complement fixing antibodies can persist for several years after infection, it is necessary to obtain paired acute-phase and convalescent-phase sera to demonstrate seroconversion or a fourfold increase in titer for reliable diagnosis. The need for both specimens typically affords only a retrospective diagnosis of acute infection. Enzyme immunoassay (EIA) and indirect fluorescent antibody test methods may provide a more sensitive and specific diagnosis compared to the complement fixation test [8–10].

The Meridian ImmunoCard Mycoplasma IgM assay (Meridian Bioscience, Cincinnati, OH, USA) is a membrane-based, qualitative EIA in which patient sera is allowed to react with *Mycoplasma pneumoniae* antigen extracts impregnated on filter paper. It requires minimal hands-on time, no additional equipment, and includes an internal control. In the study reported here, the performance of the Meridian ImmunoCard Mycoplasma test was compared to two conventional microtiter plate EIAs for the detection of IgM antibodies to *Mycoplasma pneumoniae* using acute-phase serum samples from 145 pediatric patients. The average age of the patients was 7.1 years (range, 8 months–17 years) and 46% were female.

Initially, all samples were assayed using the ImmunoCard Mycoplasma IgM assay (Meridian Bioscience) according to the manufacturer's instructions. Samples were stored at −30°C, thawed once, and retrospectively analyzed using the ImmunoWELL *Mycoplasma pneumoniae* IgM EIA (GenBio, San Diego, CA, USA) as recommended by the manufacturer. A subset of samples with discrepant results between ImmunoCard and ImmunoWELL were tested by a third EIA method, the Zeus

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Mycoplasma IgM EIA (Zeus Scientific, Raritan, NJ, USA). The ImmunoWELL and Zeus EIAs are quantitative, colorimetric microtiter plate methods for the detection of IgM antibodies to *Mycoplasma pneumoniae* in human sera. Both assays utilize an IgG absorbent to prevent interference by IgG in the serum sample. Serum samples were deemed positive or negative when two or more test methods were in agreement. Samples for which no two methods agreed were considered unresolved and were not included in the statistical calculations.

Testing of 145 acute-phase sera for the presence of IgM antibodies to *Mycoplasma pneumoniae* revealed the essential agreement between the ImmunoCard and ImmunoWELL assays to be 87% (Table 1). Compared to the ImmunoWELL EIA, the ImmunoCard method displayed a sensitivity of 77% (95%CI, 66–85%), specificity of 92% (95%CI, 87–96%), positive predictive value of 83% (95% CI, 71–91%), and negative predictive value of 89% (95% CI, 84–93%). Because the ImmunoWELL EIA results in a quantitative, colorimetric end-point that is read photometrically, an equivocal zone was established by the manufacturer in which neither a positive nor a negative result could be determined. Ten samples considered equivocal by the ImmunoWELL IgM EIA were positive by ImmunoCard in four instances and negative in six.

Samples with discrepant and equivocal results were assessed further using the microtiter-based Zeus Mycoplasma IgM EIA method. Compared to resolved results for these samples, the ImmunoCard displayed a sensitivity of 85% (95%CI, 76–90%), specificity of 97% (95%CI, 92–99%), positive predictive value of 93% (95%CI, 83–98%), and negative predictive value of 93% (95%CI, 89–95%) (Table 1). Overall agreement between the ImmunoCard and resolved EIA results was 93%. Results from three samples remained unresolved due to equivocal readings by the Zeus Mycoplasma IgM EIA.

Previous reports of ImmunoCard Mycoplasma test performance described sensitivities of 80–98% compared to various serologic and clinical methods [6, 8–10]. In fact, two reports suggested that the ImmunoCard is probably more sensitive than other serologic methods for the detection of low levels of IgM in acute-phase sera [6, 8]. In 40 pediatric patients with serologically confirmed *Mycoplasma pneumoniae* pneumonia, acute-phase sera was positive by ImmunoCard in all instances, while complement fixation and particle agglutination tests were positive in 30 and 77.5% of cases, respectively [6]. In adult patients with *Mycoplasma pneumoniae* infection, where the IgM response is typically diminished or absent compared to children, the ImmunoCard was more sensitive than complement fixation, Remel EIA, and ImmunoWELL IgM EIA when acute-phase sera from 64 patients were tested. ImmunoCard detected IgM in 30 of 64 acute-phase sera while Remel EIA, complement fixation, and ImmunoWELL IgM EIA detected IgM in 41, 38, and 23% of samples, respectively [8]. Likewise, in the current study, when initial ImmunoCard-positive and ImmunoWELL-negative or equivocal results were resolved by an additional method, 7 of 11 samples were considered positive for IgM antibodies (Table 1).

Table 1 Performance of ImmunoCard *Mycoplasma* IgM assay compared to two commercial enzyme immunoassays for detecting IgM antibodies to *Mycoplasma pneumoniae* in 145 acute-phase samples

ImmunoCard result	ImmunoWELL result			Resolved by Zeus EIA		
	Pos	Neg	Equiv	Pos	Neg	NR
Pos	34	7	4	41	3	1
Neg	10	84	6	7	91	2

Pos, positive; Neg, negative; Equiv, equivocal zone of interpretation by EIA colorimetric method; NR, not resolved.

The inability of the two different microtiter-based EIAs to resolve equivocal results underscores the lack of a reliable serologic method for the diagnosis of acute *Mycoplasma pneumoniae* infection. In a comparison of four commercially available microtiter-based EIAs, the sensitivities for *Mycoplasma pneumoniae* IgM detection were 66–82% in patients with positive PCR results for *Mycoplasma pneumoniae* [7]. The specificity of currently available methods is variable among assays and among laboratories using the same test, likely due to the lack of a standardized method. It is known that patients with acute viral infections and those that are positive for rheumatoid factor or anti-nuclear antibodies may incur false-positive results with many of the serologic methods for detecting antibodies to *Mycoplasma pneumoniae* [7]. Testing of sera from healthy individuals or from those with other confirmed diagnoses of respiratory infection using commercially available EIAs revealed the presence of IgM antibodies to *Mycoplasma pneumoniae* in 1.6–74% of samples [7]. Since the Zeus EIA was used only to resolve certain discrepant results in this study, we cannot rule out the possibility that false-positive results may have occurred concurrently with both the ImmunoCard and the ImmunoWELL methods.

Clearly there is a need for standardized diagnostic methods that can provide rapid, accurate, and reproducible results for the diagnosis of acute *Mycoplasma pneumoniae* infection so that appropriate treatment can be initiated promptly. PCR alone was not as sensitive as paired sample serology for the detection of *Mycoplasma pneumoniae*, but the combination of PCR and acute-phase IgM serology increased the sensitivity of rapid laboratory diagnosis to 95% [5]. For serologic diagnosis, establishing the cut-off for quantitative, colorimetric EIAs is particularly difficult since the sensitivity and specificity are bargained against one another as the end-point shifts in either direction along the spectrum of colorimetric intensities. In addition, the detection of IgM antibodies may often be insensitive since the production of IgM during the acute phase of *Mycoplasma pneumoniae* infection is inconsistent among individuals as well as among different age groups [4, 6].

The results of our study indicate that a positive ImmunoCard Mycoplasma IgM result allows the diagnosis of a recent *Mycoplasma pneumoniae* infection with high reliability in children based on a single serum sample. The

test affords rapid turn-around time and its sensitivity and specificity compare favorably with microtiter-based EIA methods despite the lack of a standardized reference method.

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