

Comparison of PCR for Sputum Samples Obtained by Induced Cough and Serological Tests for Diagnosis of *Mycoplasma pneumoniae* Infection in Children

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Passive agglutination (PA) and immunoglobulin M (IgM), IgA, and IgG enzyme-linked immunosorbent assays (ELISAs) for the diagnosis of *Mycoplasma pneumoniae* were compared with PCR testing of sputum samples obtained from children with lower respiratory tract infections. The sensitivity and specificity of PA were 80.3% and 92.3% at a titer of 1:80. ELISA was found to be less sensitive than PA.

Mycoplasma pneumoniae is an important respiratory pathogen in children as well as adults (6, 9, 13, 14). Laboratory diagnosis of *M. pneumoniae* infection is usually performed by serological methods, such as passive agglutination (PA), complement fixation, and enzyme-linked immunosorbent assay (ELISA). The diagnostic value of these methods has been discussed previously (1–5, 8, 12, 18, 19, 21). Although appropriate respiratory specimens are more difficult to obtain from children than from adults, sputum has been successfully obtained from infants and young children with respiratory infections by inducing coughing (11, 20). The aim of this study was to clarify the diagnostic value of serological methods for the diagnosis of *M. pneumoniae* infection in comparison with PCR using sputum from children.

Enrolled in the study were 339 children (181 males, 158 females; mean age, 2.9 ± 2.6 years; median age, 2 years) who were seen consecutively at Saitama Medical School between January 2000 and August 2004. All patients had respiratory symptoms, such as productive cough, and were clinically diagnosed as having lower respiratory tract infection (LRTI); 263

cases had X-ray-confirmed pneumonia, and 76 had bronchitis. The duration of fever ($\geq 38^\circ\text{C}$) was 3.6 ± 2.6 days. Sputum was obtained by induced coughing from all patients on their initial visit, as described previously (11, 20). After aerobic culture of sputum was performed, the remainder of the sputum was frozen at -80°C . Sputum was thawed and centrifuged at 2,000 rpm for 15 min, and DNA was then extracted using a QIAamp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. *M. pneumoniae* DNA was detected by nested PCR with primer sets for the P1 gene, as described previously (17). The first primer set was ADH2F (5'-GGC AGT GGC AGT CAA CAA ACC ACG TAT-3') and ADH2R (5'-GAA CTT AGC GCC AGC AAC TGC CA T-3'). The second primer set was ADH3F (5'-GAA CCG AAG CGG CTT TGA CCG CAT-3') and ADH3R (5'-GTT GAC CAT GCC TGA GAA CAG TAA-3').

Serum was also obtained from all children. Serum anti-*M. pneumoniae* antibody was assayed using Serodia-MYCO II (Fuji Rebio Ltd., Tokyo, Japan), which is a PA assay (2). PA titers were determined according to the manufacturer's in-

TABLE 1. Comparison of PCR assay results and titers of passive agglutinin antibody in single serum samples

Cutoff PA titer	No. of serum samples ($n = 339$) ^a				% Sensitivity of PA assay (95% CI)	% Specificity of PA assay (95% CI)
	PCR positive ($n = 66$)		PCR negative ($n = 273$)			
	PA positive	PA negative	PA positive	PA negative		
1:40	59	7	47	226	89.4 (78.8–95.3)	82.8 (77.7–87.0)
1:80	53	13	21	252	80.3 (68.3–88.7)	92.3 (88.3–95.1)
1:160	47	19	11	262	71.2 (58.6–81.4)	96.0 (92.7–97.9)
1:320	37	29	7	266	56.1 (43.3–68.1)	97.4 (94.6–98.9)
$\geq 1:640$	33	33	2	271	50.0 (37.6–62.4)	99.3 (97.1–99.9)

^a PCR positivity, 19.5% (66 PCR-positive samples/339); PA positivity ($\geq 1:40$), 31.3% (106 PA antibody-positive samples/339).

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TABLE 2. Passive agglutinin titers in paired serum samples from PCR-positive patients

Interval (days)	No. of serum samples with an increase in PA antibody titer of ^a :						GMT ^b
	1×	2×	4×	8×	16×	≥32×	
2	1	0	2	2	1	0	9.5
3	0	2	2	2	2	1	10.2
4	0	0	0	1	1	1	18.6
5-8	0	1	1	1	0	4	20.2
9-12	0	1	2	0	0	3	17.6
13-16	0	1	0	0	0	4	26.0

^a ≥4-fold increase in PA titers was seen in 30 of 36 samples (83.3%).

^b GMT, geometric mean titer.

structions, and the diagnostic criteria of *M. pneumoniae* infection were evaluated for each antibody titer. When paired sera were available, titer increases of at least fourfold were evaluated as indicating *M. pneumoniae* infection. Immunoglobulin G (IgG)-, IgA-, and IgM-specific anti-*M. pneumoniae* antibodies in serum samples from patients with positive PCR results were assayed using *Mycoplasma pneumoniae* IgG, IgA, and IgM ELISA kits (Medac GmbH, Wedel, Germany). IgG, IgA, and IgM ELISAs were performed according to the manufacturer's instructions. Briefly, serum diluted 1:100 was incubated on an ELISA plate coated with *M. pneumoniae* antigens. The optical density was converted to an antibody value using a standard curve. The cutoff values for IgG and IgA were 10 arbitrary units/ml. The cutoff value of the optical density for IgM was the value for the negative control plus 0.380.

Informed consent was obtained from the parents of all children.

Statistical analysis was performed using Epi Info 6, version 6.04d (Centers for Disease Control and Prevention, Atlanta, Ga.) for exact 95% confidence intervals (CI).

PCR gave positive results in 66 (19.5%) of 339 sputum

specimens. PA titers of ≥1:40 were seen in 106 of 339 serum samples (positivity, 31.3%). A comparison of PCR results and PA titer assay results is shown in Table 1. When cutoff values for PA titers were 1:40, 1:80, 1:160, 1:320, and 1:640 or higher, the sensitivities and specificities of PA serology relative to PCR results were as follows: 89.4 and 82.8%; 80.3 and 92.3%; 71.2 and 96.0%; 56.1 and 97.4%; and 50.0 and 99.3%, respectively.

Among patients positive by PCR, ≥4-fold increases in PA titers of paired sera were seen in 30 of 36 samples (83.3%) (Table 2). The interval of serum sampling was related to the geometric mean of the increased antibody titers.

A comparison of PA titers and cumulative percentages of positivity of the ELISAs is shown in Fig. 1. Percentages of positivity for IgG, IgA, and IgM as determined by ELISA of serum samples from patients with positive PCR results were 44.4% (36/81), 19.8% (16/81), and 66.7% (54/81), respectively, even with PA titers of 1:640 or higher.

The clinical significance of the PCR assay for the diagnosis of *M. pneumoniae* respiratory infections has been discussed both for adults (7, 15, 16, 21) and for children (4, 8, 10, 18, 19). Michelow et al. (10) compared the results of PCR assays for *M. pneumoniae* between nasopharyngeal and oropharyngeal samples. They found discrepancies in three of nine positive samples and combined test results from more than one site in order to improve diagnostic accuracy. In addition, using throat swabs, Dorigo-Zetsma et al. (4) compared PCR and culture with serological tests for *M. pneumoniae* diagnosis among children with respiratory infections. Although they found the same results for culture and for PCR, the sensitivity of PCR using throat swabs was low. They recommended combining PCR and serology for reliable diagnosis of *M. pneumoniae*. On the other hand, Rätty et al. (15) compared PCR results for sputum, nasopharyngeal aspirates, and throat swabs obtained from young adults with pneumonia. Of these, sputum was found to be superior for a diagnosis of *M. pneumoniae*. Because sputum

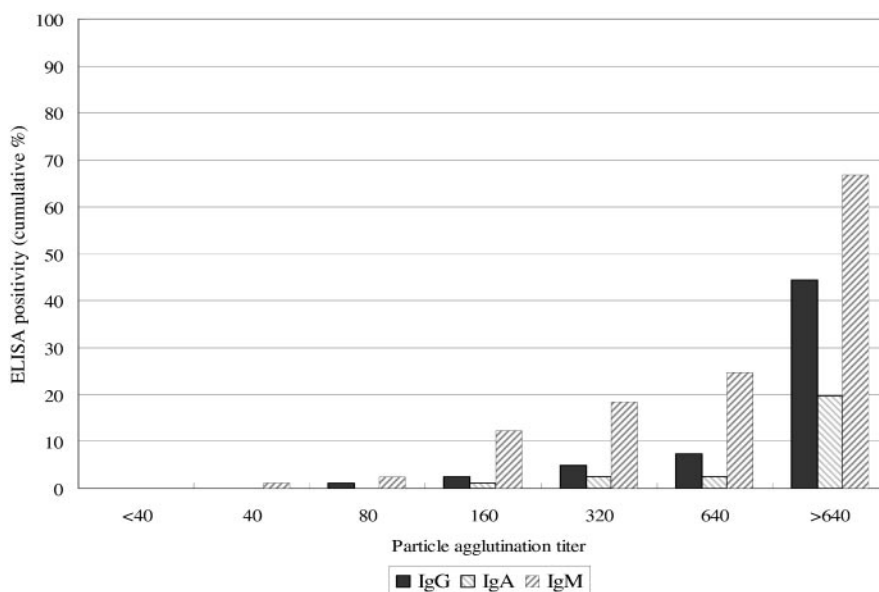


FIG. 1. Comparison of passive agglutinin titer and *Mycoplasma pneumoniae* IgG, IgA, and IgM ELISA results among PCR-positive samples from children.

can be obtained less invasively, we believe that PCR assay using sputum is both rapid and useful for the diagnosis of *M. pneumoniae* LRTI in children.

Our results indicate that the sensitivity and the specificity of PA with single serum samples varied with the titer cutoff values investigated. However, we believe that a titer of 1:80 or 1:160 is useful for the diagnosis of *M. pneumoniae* infection in children. PA titer increases of at least fourfold were seen in 30 of 36 paired serum samples from children with positive PCR results. In addition, none of the paired serum samples from patients with negative PCR results showed PA antibody titer increases of fourfold or more. This suggests that PA using paired sera is clinically useful for the diagnosis of *M. pneumoniae* infection when sputum specimens are not available.

The present results show that the sensitivity of the *Mycoplasma pneumoniae* IgM ELISA is high and that it can be applied to serological diagnosis using unpaired serum. However, the specificity of IgM ELISA should be examined further using sera from patients with negative PCR results. Discrepancies between IgA and IgG ELISA results and PA titers indicate that the PA titer reflects IgM class antibodies. This discrepancy is thus indicative of the patterns of production of immunoglobulin subclasses in the present group of children.

In conclusion, our results demonstrate that PA serology using paired sera shows good agreement with PCR results. In unpaired sera, a PA antibody titer of 1:80 or 1:160 is useful for the serological diagnosis of *M. pneumoniae* infection among children with LRTI.

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