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ORIGINAL ARTICLE

Comparison of Mycoplasma Pneumoniae IgM Antibody and Nucleic Acid Detection in Pediatric Pneumonia

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SUMMARY

Background: Mycoplasma pneumoniae **(MP) is a major cause of acute respiratory infections in children, underscoring the need for accurate diagnostic approaches to guide clinical management. This study aimed to systematically evaluate the correlation between MP-specific IgM antibodies and PCR results, and to analyze epidemiological trends to inform and optimize diagnostic strategies in pediatric practice.**
Methods: **We conducted a retrospective analysis of 736 pediatric patients with acute respiratory infections admitted to Beijing Tiantan Hospital between November 2023 and July 2025. All patients were tested both for MP-IgM antibodies using the colloidal gold method and MP-DNA via real-time fluorescence quantitative PCR.**
Results: **Among the patients, 149 tested positive for IgM antibodies and 246 tested positive by PCR, with a low concordance rate between the two methods (kappa = 0.144, $p < 0.05$). PCR demonstrated consistently high diagnostic performance year-round, particularly in children over 6 years of age. In contrast, rapid IgM testing served as a useful initial screening tool during epidemic periods for children aged ≤ 3 years, though negative results should be confirmed by PCR.**
Conclusions: **Laboratory findings in MP infections are influenced by both age and seasonal factors. A negative IgM result does not rule out active infection, reinforcing the value of PCR as a primary diagnostic tool. We recommend an integrated diagnostic strategy that accounts for age and seasonal variations to enhance the accuracy and effectiveness of MP infection management.**
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INTRODUCTION

Mycoplasma pneumoniae (MP) is a significant pathogen responsible for community-acquired pneumonia in children, with the resultant condition termed *Mycoplasma pneumoniae* pneumonia (MPP), which is frequently encountered in clinical practice [1]. Since MP infection presents with non-specific symptoms in its early stages, such as fever, cough, and dyspnea, it is often difficult to distinguish from other respiratory infections. Without prompt treatment, the disease may progress to severe complications, imposing a substantial health burden on patients and their families. Recent epidemiological data indicate a rising annual proportion of pediatric pneumonia cases attributable to *Mycoplasma pneumoniae*, particularly during winter and spring [2]. This trend is associated with increased hospitalization rates and elevated healthcare costs. Studies have shown that in some regions, the annual incidence of MP infection accounts for 30% to 40% of pediatric respiratory infections [3]. Consequently, obtaining a rapid and precise pathogen diagnosis is critical to enable specific treatment at an early stage, thereby achieving superior patient outcomes.

Currently, the diagnosis of *Mycoplasma pneumoniae* infection relies on clinical symptom assessment, imaging studies, and laboratory investigations. The detection of serum-specific IgM antibodies and nucleic acid amplification testing (NAAT) are the two principal laboratory methods. IgM antibody testing is the most widely utilized technique in clinical practice due to its operational simplicity and rapid turnaround time [4]. As the first antibody to be generated in response to pathogenic stimulation, IgM provides supportive evidence for diagnosing *Mycoplasma pneumoniae* infection [5]. However, the accuracy of IgM testing is constrained by the "window period" of antibody development, which may result in false-negative results during the early stages of infection. Conversely, the persistence of IgM antibodies from prior infections can lead to false-positive interpretations. Moreover, the sensitivity and specificity of IgM serology vary across different age groups and clinical settings, indicating that this method cannot be solely relied upon to fulfill clinical diagnostic requirements [6]. In recent years, nucleic acid testing based on polymerase chain reaction (PCR) has emerged as a pivotal tool for the diagnosis of respiratory pathogens [7]. By directly detecting pathogen nucleic acids with high sensitivity and specificity, PCR enables accurate and early diagnosis of active infections [8].

Nevertheless, comparative studies on these two detection methods remain limited, particularly in pediatric populations. To systematically evaluate their performance in a clinical setting, we conducted a retrospective analysis of 736 children with suspected *Mycoplasma pneumoniae* infection who presented at Beijing Tiantan Hospital, Capital Medical University, between November 2023 and July 2025. This study aimed to assess the concordance and diagnostic efficacy of MP-specific IgM antibody testing versus nucleic acid testing, with the goal of providing information on test selection strategies to enhance early diagnosis, optimization of treatment in children, and improvement of screening protocols.

MATERIALS AND METHODS

Clinical data

A total of 736 pediatric patients with suspected *Mycoplasma pneumoniae* (MP) infection, who presented at Beijing Tiantan Hospital, Capital Medical University, between November 2023 and July 2025, were included in this retrospective analysis. The cohort comprised 346 males and 390 females, with a mean age of 6.41 years (standard deviation [SD] = 3.41). As a retrospective study using anonymized clinical data, this research qualified for a waiver of informed consent and was approved by the Biomedical Ethics Committee of Beijing Tiantan Hospital, Capital Medical University.

Inclusion and exclusion criteria

Inclusion criteria: Patients meeting the following criteria were enrolled: 1) clinical diagnosis of suspected MP infection; 2) presence of symptoms such as fever, headache, or cough; and 3) simultaneous performance of whole-blood MP-IgM testing and nasopharyngeal swab PCR testing within a 24-hour window.

Exclusion criteria: Patients were excluded for any of the following: 1) confirmed co-infection with other pathogens; 2) pre-existing autoimmune diseases; 3) administration of macrolide antibiotics prior to admission; or 4) MP-IgM and PCR tests performed more than 24 hours apart. For patients with multiple test records, only the first available dataset was retained for analysis.

Laboratory methods

Sample collection and processing: For IgM antibody testing, finger-prick whole blood samples were collected and analyzed immediately using the colloidal gold immunochromatography assay. For nucleic acid testing, nasopharyngeal swab specimens were obtained, placed into dedicated transport tubes, sealed, and promptly delivered for PCR analysis.

Colloidal gold immunochromatography assay: MP-specific IgM antibodies were detected using a double-antigen sandwich immunochromatographic technique. In this procedure, the sample is applied to the test card sample well. If MP-IgM is present above the detection threshold, it forms a labeled complex that migrates chromatographically and binds to the test line in the detection zone. A positive result is defined by the appearance of distinct red bands in both the control (C) and test (T) regions, whereas a negative result shows a band only in the control region.

Real-time fluorescent quantitative PCR: Nasopharyngeal swab samples were vortexed thoroughly, and 200 µL of the solution was used for nucleic acid extraction. Extraction was performed automatically using a magnetic bead-based system. Amplification was conducted on a real-time PCR instrument to detect the presence of MP-specific DNA. A cycle threshold (CT) value of < 40 was interpreted as positive, while a CT value ≥ 40 was considered negative.

Observation metrics
1) Positivity rates of MP-IgM antibody detection and real-time quantitative PCR.
2) Comparative performance evaluation of the two detection methods.

Statistical analysis
Statistical analyses were performed using SPSS software (version 27.0). The agreement between MP-IgM and PCR results was assessed using Cohen's kappa statistic. A kappa value ≥ 0.75 was considered to indicate strong agreement, a value between 0.40 and 0.75 indicated fair to good agreement, while a value < 0.40 indicated poor agreement between the two methods.

RESULTS

Comparison of results from colloidal gold testing and real-time fluorescent quantitative PCR
A total of 736 children suspected of *Mycoplasma pneumoniae* (MP) infection were included in this study. The overall positive rate detected by real-time fluorescent quantitative PCR was 33.42% (246/736), significantly higher than the 20.24% (149/736) identified by the colloidal gold-based IgM antibody test (McNemar test, $p < 0.001$).
The concordance and discordance between the two methods are summarized in Table 1. Among the samples, 483 showed consistent results (both positive or both negative), yielding an overall agreement rate of 65.63% (483/736). Discordant results were observed in 253 samples (34.38%), comprising 78 cases (10.60%) that were IgM-positive but PCR-negative, and 175 cases (23.78%) that were IgM-negative but PCR-positive. Statistical analysis indicated low consistency between the two methods (Kappa value = 0.144), and this difference was statistically significant.
No significant differences in the detection patterns were observed between male and female patients.
To assess the potential influence of gender on MP detection profiles, we analyzed the distribution of four distinct detection patterns across 346 males and 390 females. As summarized in Table 2, the composition ratios of each pattern were comparable between genders. The PCR-positive/IgM-negative pattern was the most frequently observed positive profile in both males (22%) and females (25%). Chi-squared tests indicated no statistically significant difference in the distribution of detection patterns between genders ($p > 0.05$), suggesting that gender is not a determining factor in the laboratory presentation of MP infection.

Characteristics of MP detection results across pediatric age groups
To assess the influence of age on MP detection outcomes, patients were stratified into three age groups: infants (≤ 3 years), preschool children (> 3 - 6 years), and school-age adolescents (> 6 - 15 years). Analysis of detection pattern distribution revealed significant differences among these groups (χ^2 test, $p < 0.001$), demonstrating a marked age-dependent trend. As shown in Figure 1, the proportion of the nucleic acid-positive pattern (PCR+/IgM-) increased markedly with age, rising from 6% in the infant group to 37% in the adolescent group, where it became the predominant positive profile. Conversely, the double-negative pattern (PCR-/IgM-) decreased significantly, from 78% in the youngest group to 41% in the oldest. The double-positive pattern (PCR+/IgM+) was also positively correlated with age, peaking at 14% in adolescents, substantially higher than in younger groups. In contrast, the serological-positive pattern (PCR-/IgM+) remained relatively stable across all age groups (8% - 14%). These findings indicate that age is a critical determinant of laboratory detection profiles following MP infection, with nucleic acid positivity being most common in children over 6 years, while a double-negative pattern is more frequent in younger children.

Interaction between age and season in MP detection
To assess the interactive effects of age and season on optimal detection method selection, a multifactorial logistic regression model was employed (Figure 2). The analysis revealed a statistically significant interaction between age and season in shaping detection outcomes.
In children over 6 years old, PCR maintained a high positive rate throughout the year, demonstrating consistent detection efficacy even during non-epidemic seasons (spring and summer), when the positivity rates of alternative methods generally declined. For infants and young children aged ≤ 3 years, detection strategy exhibited a distinct seasonal pattern. During the peak MP transmission seasons (autumn and winter), the positive rate of IgM testing (including both single and dual IgM-positive results) increased significantly, rendering it a valuable initial screening tool. Conversely, during non-epidemic seasons (spring and summer), the IgM positivity rate in this age group decreased markedly, at which point PCR testing demonstrated its superior diagnostic value. Among preschool-aged children (3 - 6 years), the detection profile transitioned from characteristics typical of younger children toward those more commonly seen in older children. During epidemic periods, a combined testing approach may improve diagnostic accuracy, while in non-epidemic seasons, PCR is strongly recommended as the primary diagnostic method.

DISCUSSION

Mycoplasma pneumoniae (MP) is a leading cause of community-acquired pneumonia in children, presenting with a broad spectrum of clinical manifestations ranging from mild, self-limiting disease to severe and refractory cases. It poses a substantial burden on both individual health and the healthcare system. Against the backdrop of its rising incidence in the pediatric population, the

Table 1. Comparison of detection results for MP between the two methods.

MP-IgM Antibody	MP-DNA		Total
	positive (n)	negative (n)	
Positive (n)	71	78	149
Negative (n)	175	412	587
Total	246	490	736

* Consistency analysis. Kappa value = 0.144. * $p < 0.001$.

Table 2. Comparison of MP Detection Patterns by Gender.

Detection Pattern	Gender		p-value
	male n (%)	female n (%)	
PCR-/IgM-	198 (57.2%)	214 (54.9%)	0.521
PCR+/IgM-	76 (22.0%)	99 (25.4%)	0.277
PCR-/IgM+	37 (10.7%)	41 (10.5%)	0.937
PCR+/IgM+	35 (10.1%)	36 (9.2%)	0.685
Total	346 (100%)	390 (100%)	

The p-value was calculated by the Chi-square test, comparing the distribution of the four detection patterns between males and females.

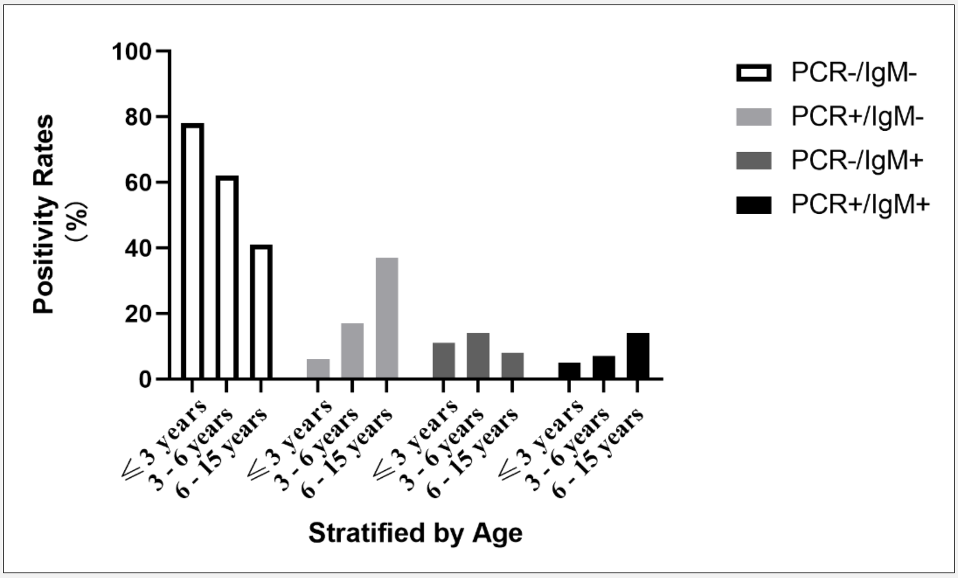

Figure 1. Distribution of MP detection patterns across pediatric age groups.

This bar chart illustrates the composition of four distinct *Mycoplasma pneumoniae* (MP) detection patterns among 736 pediatric patients, stratified into three age groups: ≤ 3 years (infants), > 3 - 6 years (preschool), and > 6 - 15 years (school-age).
The patterns are defined as: PCR+/IgM-, PCR-/IgM+, PCR+/IgM+, and PCR-/IgM-. A significant difference in pattern distribution was observed across age groups (χ^2 test, $p < 0.001$), with the nucleic acid-positive (PCR+/IgM-) pattern becoming predominant in older children.

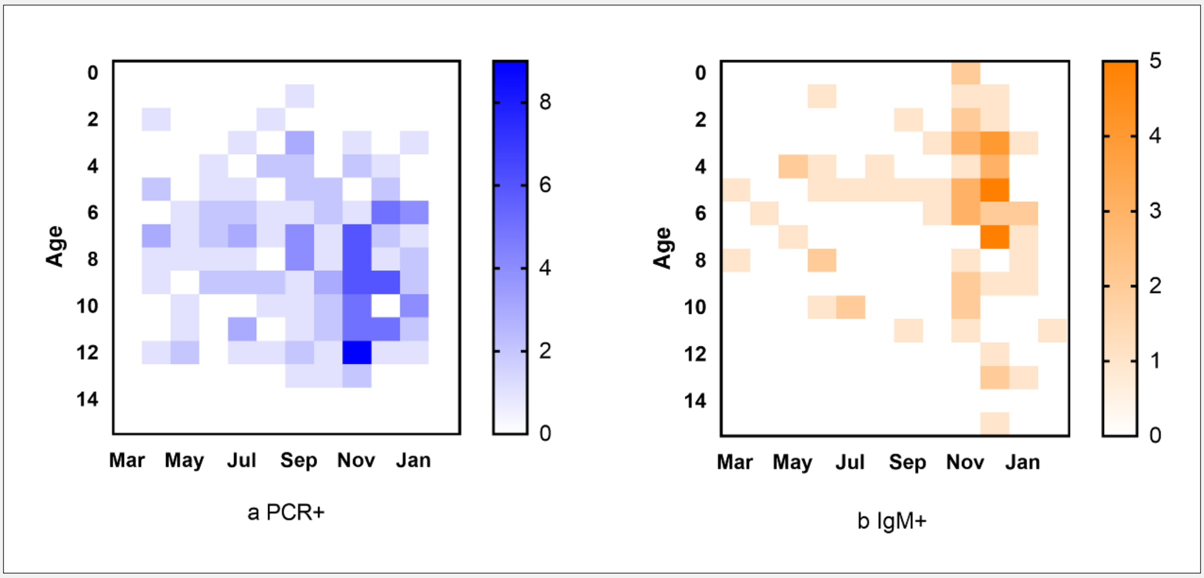

Figure 2. Distribution of positive cases for Mycoplasma pneumoniae by age, season, and detection method.

This heatmap series illustrates the number of positive pediatric cases across different age groups and seasonal periods. The color intensity in each cell corresponds to the absolute number of positive patients, with darker shades indicating a higher case count. Panel a represents patients who tested positive by PCR (blue), while Panel b represents those who tested positive for IgM (orange).

need for early and accurate diagnosis has become increasingly urgent. Conventional diagnostic approaches, such as clinical evaluation and imaging studies, are limited in sensitivity and specificity, highlighting the demand for more reliable laboratory detection methods.

This study aimed to systematically evaluate and compare the diagnostic performance of IgM antibody testing and nucleic acid amplification testing (NAAT) for MP infection, with the goal of providing clinicians with more dependable diagnostic tools. Through a comprehensive analysis of 736 pediatric patients with suspected MP infection, we examined the concordance between these two methods and developed a dynamic, multidimensional laboratory diagnostic profile. A key insight from this profile is that MP infection should not be viewed as a simple binary state of "positive" or "negative", but rather as a continuous spectrum shaped by the patient's age and the season of presentation.

Our data clearly demonstrated significant discrepancies between IgM serology and NAAT in diagnosing pediatric MP infection. This divergence may be attributed to the kinetics of IgM production. IgM typically becomes detectable 7 - 10 days post-infection, reflecting the host's initial immune response to the pathogen [9-11]. In contrast, NAAT directly detects pathogen nucleic acids, providing more accurate information during the early stages of infection, typically within the first week after symptom onset, especially when pathogen replication is active [12,13]. Thus, from a mechanistic standpoint, the high sensitivity of NAAT stems from its direct targeting of MP DNA, whereas the relatively lower sensitivity of IgM testing may relate to the timing of antibody production and clearance. This interpretation is consistent with existing literature, and our study further substantiates the clinical relevance of this mechanism through a large-sample analysis.

In analyzing patient characteristics, we observed that age significantly influences the presentation of MP infection, revealing what we term an "age reversal" phenomenon. While it is generally accepted that immature immune systems in young children may lead to higher infection rates, particularly among preschoolers aged 1 - 5 years [14,15], our findings suggest a more complex evolutionary pattern.

In older children (> 6 years), who have had more opportunities for prior pathogen exposure, the nucleic acid-positive/serology-negative (PCR+/IgM-) pattern predominates. This may be attributed to immune imprinting and the original antigenic effect, whereby re-exposure triggers an IgG-focused memory response rather than a robust IgM production, leading to negative IgM results despite active infection [16-18]. This phenomenon has been well-documented in other respiratory pathogens such as influenza [19,20]. Alternatively, effective early immune control in older children may limit systemic IgM response while still allowing nucleic acid detection in local samples. This suggests that in older children, the PCR+/IgM- profile may reflect "effective immune containment" or "re-exposure" rather than merely representing a "window period". In contrast, younger children (≤ 3 years) predominantly exhibited a double-negative (PCR-/IgM-) pattern, which may be attributed to a confluence of factors. These include practical challenges in obtaining high-quality respiratory specimens from uncooperative young patients, as well as the relative immaturity of the humoral immune system, which can lead to delayed or attenuated antibody responses. Additionally, lower pathogen load during primary infection in this age group could further reduce the sensitivity of nucleic acid amplification tests. Consequently, a negative laboratory result in a young child with high clinical suspicion should be interpreted with caution. Management decisions often necessitate integration of radiographic findings and assessment of therapeutic response. These findings collectively indicate that age is a key determinant of host immune response and corresponding laboratory results following MP infection.

Furthermore, through multivariate logistic regression modeling, this study identified significant interactions between age and season in determining the optimal detection strategy for MP. These results provide important evidence for developing a flexible and accurate diagnostic framework. In school-aged children and adolescents over 6 years old, NAAT demonstrated consistently high diagnostic efficacy throughout the year, particularly during non-epidemic seasons when seropositivity rates generally decline, solidifying its role as the most reliable diagnostic tool. Conversely, for infants and toddlers aged ≤ 3 years, diagnostic strategy exhibited strong seasonal dependence, the humoral immunity in infants and young children remains immature, and the production of specific antibodies depends on a sufficient degree of pathogen exposure. This immunological characteristic is a key factor contributing to the seasonal fluctuations observed in serological test results. Epidemiological studies indicate that while *Mycoplasma pneumoniae* (MP) infections occur year-round in China. They exhibit a distinct seasonal pattern, with a peak incidence typically observed between October and December [21]. Therefore, during autumn and winter epidemic peaks, IgM positivity rates rose significantly, supporting its use as a rapid and cost-effective initial screening option. However, during the spring and summer non-epidemic months, IgM detection rates drop considerably, possibly because humoral immune responses in this age group may be less active. At this point, NAAT demonstrates superior sensitivity and specificity for early and direct pathogen detection. Preschool children (3 - 6 years) displayed a transitional profile, shifting from "younger" to "older" characteristics. In summary, "age-guided and season-aware strategies" form the core principle for optimizing MP diagnosis, meaning that the choice of diagnostic method should be tailored according to the child's age and the timing of illness onset, enabling accurate and timely diagnosis across diverse clinical scenarios.

Our findings clearly indicate that simplifying MP laboratory diagnosis to a traditional "either IgM or PCR" model has significant limitations. These two methods are not mutually exclusive; rather, they reflect different stages of infection and immune response. Therefore, we advocate a shift from a dichotomous diagnostic approach to an integrated, collaborative strategy, establishing an evidence-based and precise diagnostic pathway.

Building on the observed "age reversal" phenomenon and seasonal epidemic patterns, our findings offer insights that may provide a more nuanced diagnostic approach: First, for school-aged children and adolescents over 6 years, as well as for any child presenting during epidemic seasons, NAAT should be employed as the first-line diagnostic method. This approach most effectively identifies the predominant PCR+/IgM- population, avoiding large-scale missed diagnoses resulting from overreliance on IgM testing alone. However, for infants and toddlers (≤ 3 years), the utility of rapid IgM testing as an initial screen is substantially limited by their immature humoral immunity and higher rates of double-negative results. In this vulnerable group, clinical suspicion, supported by imaging when indicated, should guide the decision for confirmatory NAAT. Finally, for cases with negative PCR results but high clinical suspicion for MP infection, IgM antibody testing may be considered as a reference following careful evaluation. The interpretation of such results must be cautious: a "PCR-/IgM+" pattern is more likely to indicate a past infection rather than an active acute infection. Acute MP pneumonia should never be diagnosed based solely on this pattern. Clinical decision-making should primarily rely on comprehensive judgment, integrating findings from serial imaging studies, the evolution of clinical manifestations, and response to empirical treatment.

The core advantage of this integrated strategy lies in its precision and cost-effectiveness. By prioritizing the most sensitive method (NAAT) in key populations (older children) and high-incidence periods (epidemic seasons), it maximizes diagnostic yield. At the same time, it reasonably preserves the clinical utility of IgM in specific scenarios (screening in younger children, supplementary testing in NAAT-negative cases), thereby optimizing the overall diagnostic process, reducing misdiagnosis and underdiagnosis, and laying a solid foundation for timely and accurate clinical intervention.

Although this study offers a new perspective on the laboratory diagnosis of MP, several limitations should be acknowledged, which also indicate directions for future research. First, the retrospective design limited the standardization of sampling procedures and restricted the ability to conduct long-term monitoring of relevant clinical parameters. Second, the use of a qualitative IgM assay precluded quantitative analysis. Third, multicenter validation is necessary to enhance the generalizability of the findings. Additionally, clinical correlations with specific outcomes were not systematically analyzed, rendering some interpretations speculative.

Based on these limitations, we propose the following future research directions: Conduct prospective cohort studies with standardized sampling timepoints, applying both quantitative serology and high-sensitivity NAAT to longitudinally characterize the immune response throughout the disease course. Deepen clinical correlation analyses by linking the four laboratory patterns identified in this study with detailed phenotypic data, aiming to clarify the typical manifestations, outcomes, and treatment responses associated with each pattern, and thereby translating laboratory findings into clinically actionable insights. Explore underlying molecular mechanisms through MP genotyping and immune profiling in samples from older children with PCR+/IgM- results, investigating the immunologic basis of the "age reversal" phenomenon from a pathogen–host interaction perspective.

In summary, this study clearly demonstrates significant differences between IgM antibody detection and nucleic acid testing in diagnosing pediatric MP infection. Looking forward, we recommend multicenter studies to validate these findings and further explore the potential of combined IgM and NAAT strategies to optimize early diagnosis and treatment of MP infection.

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Declaration of Generative AI in Scientific Writing:
In the preparation of this work, the author(s) utilized generative AI tools DeepSeek for the purpose of translation from Chinese to English and subsequent language polishing, including grammar checking, sentence structure refinement, and improving overall readability. It is crucial to note that the AI was used solely as a language tool. All core intellectual content, including ideas, arguments, data analysis, and conclusions, originate from and are the sole responsibility of the human author(s). The final manuscript has been thoroughly reviewed, verified, and approved by the author(s).

Declaration of Interest:
The author(s) declare no competing interests.

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