Evaluation of IgM lateral flow assay as screening tool for *Mycoplasma pneumoniae* infection in childhood pneumonia

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Diagnosis of *Mycoplasma pneumoniae* (*Mp*) infection with community-acquired pneumonia (CAP) is challenging [1]. Current diagnostic tests include *Mp*-specific IgM serology and polymerase chain reaction (PCR) of upper respiratory tract (URT) specimens [2,3], but these tests are also positive in asymptomatic patients carrying *Mp* in the URT [1,4]. IgM may be detected from 1 week after symptom onset [1,5-8]. The “gold standard” is considered a ≥4-fold rise in antibody levels [1], despite the need of paired sera and low sensitivity [4,8]. We demonstrated that the measurement of *Mp*-specific IgM antibody-secreting cells (ASCs) by enzyme-linked immunospot (ELISpot) differentiates between *Mp* infection and carriage [4].

ASC-ELISpot cannot be performed at bedside [5] and no point-of-care (POC) test is available for direct *Mp* detection in Europe and USA [1]. Therefore, we here assessed a new immunochromatographic POC IgM lateral flow assay (LFA) (Biocard *Mycoplasma pneumoniae* IgM, Labsystems Diagnostics, Vantaa, Finland) as on-site screening tool to randomize *Mp* CAP children in a future interventional trial on the efficacy of macrolide antibiotics.

A set of 239 pediatric serum samples from 94 CAP patients (median 6.3 years, interquartile range [IQR] 4.3–10.2) and 145 healthy controls (HC) (median 6.0 years, IQR 4.5–8.3) was used from a previous study [4,5]. Study flow of patients including previous results from PCR, IgM-ELISA, and IgM-ASC-ELISpot is provided in Fig. S1. CAP samples were collected median 7.0 days (IQR 3.0–12.0) after symptom onset. The study was approved by the local ethics committee (no. 2016–00148).

The IgM-LFA was performed according to the manufacturer’s instructions (Fig. 1A). Results were visually read after 10min and graded based on the
appearance/intensity of the blue test line [9]: grade 0 = negative; grade 1 = weakly positive; grade 2 = moderately positive; and grade 3 = strongly positive (Fig. 1C).

Statistical methods are detailed in Supplemental Material.

IgM-LFA grading results after 10 min varied between authors only in 11.7% (n=28/239) (Cohen kappa coefficient=0.70), but most frequently between grade 0 and 1 (50.0%, n=14/28). Results were identical for both fingertip blood and serum samples (Fig. 1B). Compared to IgM-ELISA, IgM-LFA-negative results (grade 0) were true negative in 97.8% (n=178/182), and IgM-LFA-positive results (grade 1–2–3) true positive in 87.7% (n=50/57; grade 1: n=7/14, grade 2: n=8/8, and grade 3: n=35/35) (Cohen kappa coefficient=0.87). Diagnostic performances and receiver operating characteristic analyses of IgM-LFA against different reference standards are shown in Table 1 and Fig. S2, respectively. The IgM-LFA also obtained positive results for all individuals tested positive with IgM-ELISA+PCR (n=41) (Fig. 2A) and IgM-ASC-ELISpot (n=29) (Fig. 2B). The best threshold compared to IgM-ASC-ELISpot was IgM-LFA grade ≥2 (Table 1).

In summary, Mp-specific IgM serology may lead to false positive results due to limited assay performance and age-/host-dependent characteristics [6,10], or false negative results early in disease course and after re-infection [1,7]. Despite these shortcomings and the possibility of false positive IgM-LFA grade 1 results, IgM-LFA results with grade 2–3 are predictive for Mp infection. However, IgM-LFA cannot currently replace diagnostic tests and therefore results need to be confirmed with Mp-specific PCR, IgM-ELISA, and/or IgM-ASC-ELISpot assay.
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AUTHOR CONTRIBUTIONS

P.M.M.S. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: P.M.M.S.;

Acquisition of data: E.P.;

Analysis and interpretation of data: all authors;

Drafting of the manuscript: P.M.M.S.;

Critical revision of the manuscript for important intellectual content: all authors;

Statistical analysis: P.M.M.S., L.M.B., L.A.;

Administrative, technical, or material support: C.B.;

Study supervision: P.M.M.S.
REFERENCES


FIGURE LEGENDS

Fig. 1. Biocard Mycoplasma pneumoniae IgM-LFA test results. (A) Illustrative examples of positive, negative, and invalid test results. 5μl of each serum sample was added into the sample diluent tube (not shown, provided in the kit; sample dilution factor, 1/100). 3 drops of the diluent sample were added with a calibrated disposal micropipette (provided in the kit) to the sample application window ("S"). Test results were visually read after 10 min for the appearance of both the blue test line ("T") and the red control line ("C"). A red control line was a prerequisite for a valid test. (B–C) Representative examples show test results for different specimens (B) and gradings (C), i.e., grade 0=negative, grade 1=weakly positive, grade 2=moderately positive, and grade 3=strongly positive. All test results were read by two of the authors. Samples with a diverging test reading were discussed between the authors to find a consensus about the final test result.

Fig. 2. Correlation between IgM-LFA and IgM-ELISA among the full cohort (A) which was also tested with PCR (n=239) and a subset of individuals (B) who were additionally tested with the IgM-ASC-ELISpot (n=74). Dots represent data for individual children (CAP patients and HC), including those tested positive by PCR (light blue, A) or IgM-ASC-ELISpot (blue, B). The dashed line represents the cutoff for the IgM-ELISA (17U/ml). Spearman rank correlation coefficient (R, rho) was used for analyses of correlation. Abbreviations: ASC, antibody-secreting cell; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot; Ig, immunoglobulin; LFA, lateral flow assay.
### Table 1. Diagnostic performances with 2x2 contingency tables of IgM-LFA compared to IgM-ELISA, PCR, IgM-ELISA+PCR, and IgM-ASC-ELISpot.

<table>
<thead>
<tr>
<th>Reference standard</th>
<th>IgM-LFA result (Grades)</th>
<th>IgM-LFA performance (95% CI)</th>
<th>IgM-LFA threshold* (Grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM-ELISA (n=239)</td>
<td>Positive 50 4</td>
<td>Sensitivity: 92.6% (82.4%–97.1%)</td>
<td>≥1</td>
</tr>
<tr>
<td></td>
<td>Negative 7** 178</td>
<td>Specificity: 96.2% (92.4%–98.2%)</td>
<td></td>
</tr>
<tr>
<td>PCR (n=239)</td>
<td>Positive 42 10</td>
<td>LR+: 24.47 (11.79–50.81)</td>
<td></td>
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<tr>
<td></td>
<td>Negative 15 172</td>
<td>LR-: 0.08 (0.03–0.20)</td>
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<tr>
<td></td>
<td></td>
<td>DOR: 317.86 (89.45–1129.46)</td>
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<tr>
<td></td>
<td></td>
<td>AUC: 0.96 (0.92–1.00)</td>
<td></td>
</tr>
<tr>
<td>IgM-ELISA+PCR (n=239)</td>
<td>Positive 41 0</td>
<td>Sensitivity: 100.0% (91.4%–100.0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative 16*** 182***</td>
<td>Specificity: 99.6% (92.7%–98.4%)</td>
<td></td>
</tr>
<tr>
<td>IgM-ASC-ELISpot (n=74)</td>
<td>Positive 29 0</td>
<td>LR+: 12.38 (7.74–19.90)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative 10 35</td>
<td>LR-: 0.00 (0.00–NA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOR: NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC: 0.91 (0.85–0.98)</td>
<td></td>
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</tbody>
</table>

* The threshold is the optimal cut-off that maximizes the distance to the identity (diagonal) line in the receiver operating characteristic (ROC) curve in Fig. S2 according to Youden’s J statistic using the “coords” function in R software environment (version 4.0.0).

** All with IgM-LFA result grade 1.

*** IgM ELISA-negative + PCR-negative (n=174): 3.4% (n=6) IgM-LFA-positive (all grade 1) and 96.6% (n=168) IgM-LFA-negative.

Abbreviations: ASC, antibody-secreting cell; AUC, Area under the ROC curve (AUC); CI, confidence interval; DOR, diagnostic odds ratio; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot; Ig, immunoglobulin; LFA, lateral flow assay; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NA, not available; PCR, polymerase chain reaction.