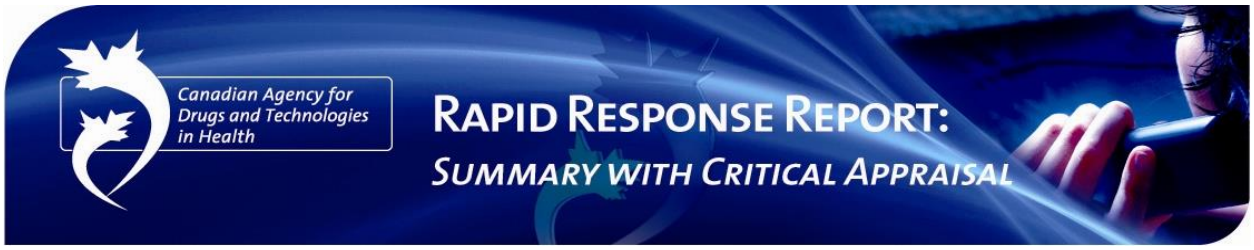

TITLE: Serum IgM and Molecular Tests for *Mycoplasma pneumoniae* Detection: A Review of Diagnostic Test Accuracy, Clinical Effectiveness, Cost-Effectiveness, and Guidelines

DATE: 9 November 2015

CONTEXT AND POLICY ISSUES

Mycoplasma pneumoniae (*M. pneumoniae*) is a bacterium that is responsible for 6% to 40% of lower respiratory tract infections, including community-acquired pneumonia (CAP).[1-3] Since there are multiple pathogens that can cause CAP and other respiratory tract infections, accurate and rapid diagnosis of *M. pneumoniae* is critical to inform appropriate and timely treatment with antibiotics. Serum IgM testing and molecular tests such as polymerase chain reaction (PCR) are two of the available laboratory methods for the diagnosis of acute *M. pneumoniae* infection, but they each have advantages and disadvantages. Serum IgM tests are widely commercially available, commonly used, and allow quantitation of antibody titers; however, these tests may not always be ideal, as IgM antibodies peak at three to six weeks (potentially missing early-stage cases), may persist beyond the stage of acute infection, or may not be present in adults with a history of *M. pneumoniae* infection.[1] Molecular tests are fast, but diagnostic heterogeneity has been reported between studies, and the equipment and reagents required for these tests are expensive.[2,3] It is unclear which type of test is optimal for the diagnosis and subsequent treatment of patients with suspected *M. pneumoniae* infection.

The purpose of this report is to identify the diagnostic test accuracy (DTA), clinical effectiveness, and cost-effectiveness of serum IgM tests and molecular tests, as well as evidence-based guidelines regarding the use of both tests for the detection of *M. pneumoniae* in patients with respiratory infections.

RESEARCH QUESTIONS

1. What is the diagnostic test accuracy of the serum IgM test compared with molecular tests for the detection of *Mycoplasma pneumoniae* in patients with respiratory infections?

2. What is the clinical effectiveness of the serum IgM test compared with molecular tests for the detection of *Mycoplasma pneumoniae* in patients with respiratory infections?

3. What is the cost-effectiveness of the serum IgM test compared with molecular tests for the detection of *Mycoplasma pneumoniae* in patients with respiratory infections?

4. What are the evidence-based guidelines regarding the use of the serum IgM test and molecular tests for the detection of *Mycoplasma pneumoniae* in patients with respiratory infections?

KEY FINDINGS

Six diagnostic test accuracy studies and one evidence-based guideline regarding the use of serum IgM tests and molecular tests for the detection of *Mycoplasma pneumoniae* in patients with respiratory infections were identified. The diagnostic performances of IgM and molecular tests were variable among the six studies and may have been affected by choice of reference standard and specific index test, patient age, and time of specimen collection after disease onset. Four of the six studies concluded that IgM and PCR tests should be performed in combination. One evidence-based guideline recommends acute and convalescent serology for the diagnosis of *Mycoplasma pneumoniae* in children with severe pneumonia. No literature regarding the clinical utility or cost-effectiveness of serum IgM tests or molecular tests was identified.

METHODS

Literature Search Methods

A limited literature search was conducted on key resources including PubMed, The Cochrane Library, University of York Centre for Reviews and Dissemination (CRD) databases, ECRI Institute, Canadian and major international health technology agencies, as well as a focused Internet search. No filters were applied to limit the retrieval by study type. The search was limited to English language documents published between Jan 1, 2005 and Oct 9, 2015.

Rapid Response reports are organized so that the evidence for each research question is presented separately.

Selection Criteria and Methods

One reviewer screened citations and selected studies. In the first level of screening, titles and abstracts were reviewed and potentially relevant articles were retrieved and assessed for inclusion. The final selection of full-text articles was based on the inclusion criteria presented in Table 1.

Table 1: Selection Criteria

Population	Patients (pediatric through geriatric) suspected or having *Mycoplasma pneumoniae*-related respiratory infections
Intervention	Serum IgM test
Comparator	Q1, Q2: Molecular tests (e.g., PCR) as comparator index tests or reference standards Q3: Molecular tests (e.g., PCR); no comparator Q4: No comparator

Table 1: Selection Criteria	
Outcomes	Q1: Diagnostic test accuracy (e.g., sensitivity, specificity, PPV, NPV) Q2: Clinical effectiveness (comparative effectiveness, patient safety) Q3: Cost-effectiveness Q4: Guidelines
Study Designs	Health technology assessments, systematic reviews and meta-analyses, randomized controlled trials, non-randomized studies, diagnostic test accuracy studies, economic evaluations, evidence-based guidelines

IgM = immunoglobulin M; NPV = negative predictive value; PCR = polymerase chain reaction; PPV = positive predictive value.

Exclusion Criteria

Articles were excluded if they did not meet the selection criteria outlined in Table 1, did not include a clear reference standard, they were duplicate publications, were guidelines with unclear methodology, or were published prior to 2005.

Critical Appraisal of Individual Studies

The included DTA studies were critically appraised using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool[4] and guidelines were assessed with the Appraisal of Guidelines for Research & Evaluation (AGREE) II instrument.[5] Summary scores were not calculated for the included studies; rather, a review of the strengths and limitations of each included study were described.

SUMMARY OF EVIDENCE

Quantity of Research Available

A total of 476 citations were identified in the literature search. Following screening of titles and abstracts, 454 citations were excluded and 22 potentially relevant reports from the electronic search were retrieved for full-text review. One potentially relevant publication was retrieved from the grey literature search. Of these potentially relevant articles, 16 publications were excluded for various reasons, while seven publications met the inclusion criteria and were included in this report. Appendix 1 describes the PRISMA flowchart of the study selection.

Additional references of potential interest are provided in Appendix 5.

Summary of Study Characteristics

Study Design

Six DTA studies were identified for inclusion in this report.[6-11] Chang et al.[6] had a retrospective analysis, and all others were prospective studies. Two studies used a case-control design,[10,11] while the others tested a population with signs and symptoms of CAP or other respiratory infections and unconfirmed *M. pneumoniae*.[6-9]

One evidence-based guideline from the British Thoracic Society (BTS) on the management of CAP in children was identified.[12] A systematic literature search and evidence review were performed, and evidence quality was evaluated and categorized according to a provided rating

scheme. The recommendations were developed by expert consensus based on the evidence from the systematic review, and graded according to a provided rating scheme. The guideline was validated by internal peer review.

Country of Origin

The DTA studies were conducted in Taiwan,[6] Japan,[7] Serbia,[8] China,[9] the United States of America,[10] and the Netherlands.[11] The BTS guideline[12] was developed in the United Kingdom.

Patient Population

The DTA studies evaluated a pediatric population[6-8] or a mixed population of children and adults.[9-11] In four studies, patients were included if they had clinical findings of CAP (e.g., cough, fever, radiographic lung abnormalities) and were suspected to have *M. pneumoniae* infection.[6-9] In the two studies with a case-control design, definitive *M. pneumoniae* infection status was determined prior to DTA evaluation to establish patient categorization as either a case or a control subject.[10,11] Thurman et al.[10] defined cases as patients with evidence of CAP that developed during a community outbreak of *M. pneumoniae* (and therefore the cause of the CAP was assumed to be *M. pneumoniae* infection), while controls were asymptomatic household contacts and age-matched controls. In Beersma et al.,[11] patients with PCR-confirmed *M. pneumoniae* infection from two larger prospective studies on lower respiratory tract infections (LRTIs) were selected as cases. Controls were patients with LRTIs that were either PCR-negative for *M. pneumoniae* infection or were documented to be caused by other pathogens.

The target population of the BTS guideline was infants and children with CAP. Excluded from this population were neonates, infants with respiratory syncytial virus bronchiolitis, or children with upper respiratory tract infections, mild fever and wheeze. In addition, specific management of pneumonia in the context of immunosuppressed children or those with pre-existing respiratory conditions was not addressed by the guideline. The intended users of the guideline are medical professionals who treat children with CAP (e.g., physicians, nurses, and respiratory therapists).

Interventions and Comparators

All included studies evaluated the DTA of a serum IgM index test. The type of assays used to detect IgM levels in these studies included enzyme immunoassay (EIA),[7,10,11] enzyme-linked immunosorbent assay (ELISA),[6,8,9] microparticle agglutination (MAG) test,[11] and complement fixation test (CFT).[11] All assays, except one of the EIAs and the CFT in Beersma et al.[11] specifically detected IgM antibodies. The molecular tests evaluated in the DTA studies identified for this report included quantitative PCR (qPCR; also known as real-time PCR)[6,8-11] and loop-mediated isothermal amplification (LAMP).[7] Two studies evaluated the DTA of IgM and qPCR index tests relative to an IgG reference standard;[8,9] two studies evaluated IgM index tests relative to a qPCR reference standard;[6,11] one study evaluated IgM and LAMP assay index tests relative to a reference standard of positive culture and/or seroconversion, or four-fold increase in antibody titers in paired sera;[7] one study evaluated IgM and qPCR index tests relative to a reference standard of a clinically defined case.

The BTS guideline considered interventions and practices related to the assessment, diagnosis, and treatment of CAP in children. The interventions addressed by the guideline of particular

relevance to this report are microbiological investigations for the diagnosis of CAP in children, including acute and convalescent serology for *M. pneumoniae* and the use of PCR testing.[12]

Outcomes

All included DTA studies reported sensitivity, specificity, and positive predictive value (PPV).[6-11] Some studies also reported negative predictive value (NPV),[6-9,11] positive likelihood ratio (PLR),[7-9] and negative likelihood ratio (NLR).[7]

As a whole, the BTS guideline considered CAP incidence and prevalence, etiology and clinical features, investigations and diagnosis, management, morbidity and mortality. DTA outcomes of microbiological investigations are of particular relevance to this report.

Timing

Most studies specified that acute-phase serum was used for IgM testing,[7-9,11] and was typically collected at enrollment or the patients' first visit.[7-9] Throat swabs for qPCR testing were also usually obtained during the patients' first visit.[7-9,11] For studies that evaluated IgG in paired sera as a reference standard, the second serum sample was collected two to four weeks after the initial sample.[8,9]

Beersma et al.[11] collected single and paired samples of acute- and convalescent-phase sera (46 serum samples total). Of the eight single serum samples, seven were acute-phase samples, and three of those seven were collected within seven days of disease onset. The time between the collection of acute- and convalescent-phase sera ranged from seven to 48 days (mean 15.8 days). No further details regarding timing of sample collection were provided.

Thurman et al.[10] did not restrict the time of serum or swab sampling, reporting results for samples collected up to 21 days, 22 to 50 days, and more than 60 days after symptom onset.

Chang et al.[6] did not specify sample collection time in the methods, stating that IgM was tested "when feasible" in a subset of identified CAP patients who were hospitalized, and respiratory samples were assessed by qPCR "on a clinical service basis".

Summary of Critical Appraisal

DTA Studies

Most studies had appropriate inclusion and exclusion criteria,[6-9] and specified objective thresholds for serological test positivity in the study methods.[6,8,9,11] Two studies assessed all patients with the same index tests and reference standards,[7,8] and four studies included all enrolled patients in the analysis of test sensitivity and specificity.[7-10] However, each trial had some notable limitations in study design that may have biased the DTA results.

None of the studies reported whether outcome assessors were blinded to each patient's status (case versus control or *M. pneumonia*-positive versus negative on another test evaluated during the study) when they were running the index tests and reference standards. Knowing this status introduces risk of bias by potentially affecting the way in which the test is conducted or interpreted, especially in the case of subjective outcomes like a colour change on an immunoassay.

Thurman et al.[10] and Beersma et al.[11] used a case-control design, which is not ideal for a DTA study as this may overestimate the diagnostic accuracy of the test; both of these studies report PPV, which may be inflated in studies with a case-control design as PPV is affected by the prevalence of the target condition. Misclassification of cases and controls may also have been an issue in these two studies. The use of clinically defined cases of pneumonia during a community outbreak of *M. pneumoniae* reported by Thurman et al.[10] as a reference standard may have overestimated the prevalence of *M. pneumoniae* infection in the community, as there are other viruses and bacteria that could theoretically cause pneumonia even in the event of an outbreak of one pathogen. Beersma et al.[11] assumed that some controls were *M. pneumoniae*-negative without testing because they had a well-documented history of pneumonia caused by another pathogen; however, it has been reported that coinfection by multiple viruses is common in CAP.[12]

Three of the four studies that did not use a case-control design did not report whether eligible patients were enrolled consecutively or from a random sample of eligible patients, which may have introduced selection bias.[6-8]

Chang et al.[6] and Beersma et al.[11] used qPCR as a reference standard for *M. pneumoniae* detection, but this may not have been an appropriate choice for a DTA study. Beersma et al. selected qPCR as a reference standard due to its reported high sensitivity;[11] however, the BTS guidelines report that serology in paired sera is considered the standard method for diagnosing *M. pneumoniae* infections, which was also discussed by Chang et al.[6,12]

BTS Guideline

The BTS guideline[12] on the diagnosis and management of CAP in children was fairly well done. The objectives, research questions, relevant population and guideline users were clearly defined. Members from appropriate professional groups were involved in guideline development; however, patient values and preferences were not sought during this process. A systematic review was conducted to provide the evidence informing the recommendations, which included a search of multiple databases. A database search strategy for the review was provided but a search of the grey literature was not reported in the methods. Studies were selected in duplicate, risk of bias of each study was assessed during data extraction, and the grading scheme for evaluating the quality of the body of evidence was provided. There was a clear link between the evidence summaries and corresponding recommendations, which were also clearly identifiable. However, the recommendations regarding microbiological diagnostic testing are somewhat ambiguous regarding specific tests, indications, and timing. Methods for formulation of the recommendations was based on expert consensus and not further described, and the guideline was not externally reviewed before publication. There was no discussion of guideline implementation but the BTS provides an audit tool.

Summary of Findings

Six relevant DTA studies and one evidence-based guideline were identified for inclusion in this report. No evidence regarding the clinical effectiveness or cost-effectiveness of the serum IgM test compared with molecular tests for the detection of *M. pneumoniae* in patients with respiratory infections was identified.

What is the diagnostic test accuracy of the serum IgM test compared with molecular tests for the detection of Mycoplasma pneumoniae in patients with respiratory infections?

Six studies that compared the DTA of serum IgM tests and molecular tests were identified for inclusion in this report; five studies compared IgM with qPCR[6,8-11] and one study compared IgM with the LAMP assay.[7] No studies that reported on downstream clinical outcomes of either testing method were identified.

The two studies that used IgG as a reference standard had inconsistent results. Medjo et al.[8] reported that both IgM and qPCR had very similar, high sensitivities (around 81% for both tests) and specificities (around 100% for both tests). However, Qu et al.[9] found low sensitivities for IgM and qPCR (7.4% and 40.7%, respectively). Authors of both studies acknowledged that these results conflicted with previously reported sensitivities and specificities for IgM and qPCR, which were more intermediate. They suggested that the sensitivity of the IgM tests may have been affected by timing of sample collection; Medjo et al. collected sera during the second week after disease onset when IgM titers were likely higher,[8] while Qu et al. evaluated these tests for early diagnosis of *M. pneumoniae* and therefore did not include patients whose disease onset time was longer than seven days.[9] Inconsistencies in qPCR sensitivities and specificities were attributed to the type of qPCR test evaluated, different sample type and collection time, and different gene targets.[8,9]

Thurman et al. assessed the DTA of IgM compared with qPCR in clinically defined pneumoniae cases and controls of unspecified ages during community outbreaks of *M. pneumoniae.*[10] They reported that IgM had a high sensitivity and low specificity, while qPCR had a low sensitivity and high specificity; these test characteristics varied with patient age and time of specimen collection. Sensitivities were highest for the IgM test in children up to nine years old or in samples collected at least 60 days after symptom onset, and were highest for qPCR in patients 19 years of age or older or in samples collected within 21 days of symptom onset.

Kayuka et al. compared the DTA of the serum IgM test with the LAMP assay, a single point-of-care molecular test for *M. pneumoniae*, using a reference standard of positive culture, seroconversion, or four-fold increase in antibody titer on paired sera.[7] They reported that LAMP had high sensitivity (96.8%) and specificity (100%), and the IgM test had a relatively low sensitivity (38.7%) and specificity (76.9%). They also re-tested some LAMP-positive patients and found that approximately 35% of these patients became negative for *M. pneumoniae* at a mean of 12.3 days after fever onset. IgM test results over time were not reported.

Two studies evaluated the DTA of serum IgM tests using qPCR as a reference standard, and found relatively low sensitivities for IgM when testing a pediatric[6] or predominantly adult[11] population. In the study by Chang et al.,[6] the majority of *M. pneumoniae*-positive patients who tested positive for IgM were tested at least seven days after onset of fever, again suggesting that sensitivity of the IgM test depends on specimen collection time. Timing of sample collection was not clearly defined in the study by Beersma et al.,[11] who suggested instead that the observed low sensitivities of the IgM tests may have been related to the use of an adult population (who do not have as strong an antibody response as children) and a highly sensitive reference standard. Furthermore, qPCR does not consistently demonstrate high sensitivity when compared with other reference standards, such as IgM or IgG serology.[2]

Four studies suggested to combine IgM and PCR testing for *M. pneumoniae* to provide the most reliable results.[6,8-10] Two studies concluded that molecular tests may be appropriate or preferred methods for the diagnosis of *M. pneumoniae* infection.[7,11]

What are the evidence-based guidelines regarding the use of the serum IgM test and molecular tests for the detection of Mycoplasma pneumoniae in patients with respiratory infections?

One evidence-based guideline produced by the BTS on the management of CAP in children[12] recommends the use of microbiological diagnostic methods to diagnose children with severe pneumoniae. The guidelines recommend acute and convalescent serology for the diagnosis of *M. pneumoniae* based on some clinical trial evidence; however, IgM tests are not discussed in more detail. Based on a formal combination of expert views, molecular tests such as PCR are recommended for diagnosis of viral pneumonia, which would not include *M. pneumoniae.*

Limitations

No evidence regarding the clinical utility or cost-effectiveness of the serum IgM test or any molecular test was identified for inclusion in this report. Therefore, it is unclear whether the preferential use of any given IgM or molecular test for the detection of *M. pneumoniae* in particular clinical situations would directly lead to improved patient or cost outcomes. Reference standards varied across studies, which introduces inconsistency in DTA characteristics of the same or similar assays. Furthermore, both groups ("serum IgM tests" and "molecular tests") represent a collection of several different assays, which also adds to inter-study inconsistency. Finally, the identified guidelines do not offer specific direction regarding serology tests for the diagnosis of *M. pneumoniae* infection, and no guidelines regarding diagnosis in an adult population were identified.

CONCLUSIONS AND IMPLICATIONS FOR DECISION OR POLICY MAKING

Six DTA studies[6-11] and one evidence-based guideline[12] regarding serum IgM tests and molecular tests for the diagnosis of *M. pneumoniae* were identified for inclusion in this report.

Variable results for the diagnostic accuracy of serum IgM tests and molecular tests were reported. IgM tests had higher sensitivities than molecular tests in three studies[6,8,10] and higher specificities than molecular tests in two.[8,9] Molecular tests had higher sensitivities than IgM tests in two studies[7,9] and higher specificities than IgM tests in three.[6,7,10] One study that evaluated the DTA of several different serum IgM assays against a qPCR reference standard reported low sensitivities and high specificities for most IgM tests.[11] The variation in DTA results may have been due to several factors, including time of specimen collection after disease onset, age of the patients, and specific test selected within the class of assays. In particular for the serum IgM tests, sensitivity improved when samples were collected at least seven days after disease onset (when antibody titers were likely to be higher),[6,8,9] and when tested in children (who typically have a stronger antibody response than adults).[10,11] These findings are consistent with a systematic review of PCR versus serology (not specific to IgM) for the diagnosis of *M. pneumoniae* infection that also found significant heterogeneity between studies and inconsistent diagnostic accuracy results.[2] Though two studies concluded that molecular tests are good options for the diagnosis of *M. pneumoniae* infection,[7,11] most studies concluded that serum IgM and PCR should be tested in combination for most reliable diagnostic results.[6,8-10]

One evidence-based guideline from the BTS[12] recommends performing acute and convalescent serology tests for the diagnosis of *M. pneumoniae* in patients with severe pneumonia, but does not discuss IgM tests in particular. PCR is not currently recommended for the diagnosis of *M. pneumoniae.*

No evidence was identified regarding the clinical utility or cost-effectiveness of serum IgM tests compared with molecular tests for the diagnosis of *M. pneumoniae.* In addition, no guidelines regarding the performance of serum IgM tests and molecular tests for the diagnosis of *M. pneumoniae* in adults with pneumonia were identified. Therefore, it is unclear whether the preferential use of an IgM or molecular test for the detection of *M. pneumoniae* in particular clinical situations (including in an adult population) would directly lead to improved patient or cost outcomes. However, given the DTA results of the included studies, a conservative approach may be to use both serology and molecular tests to maximize diagnostic accuracy.

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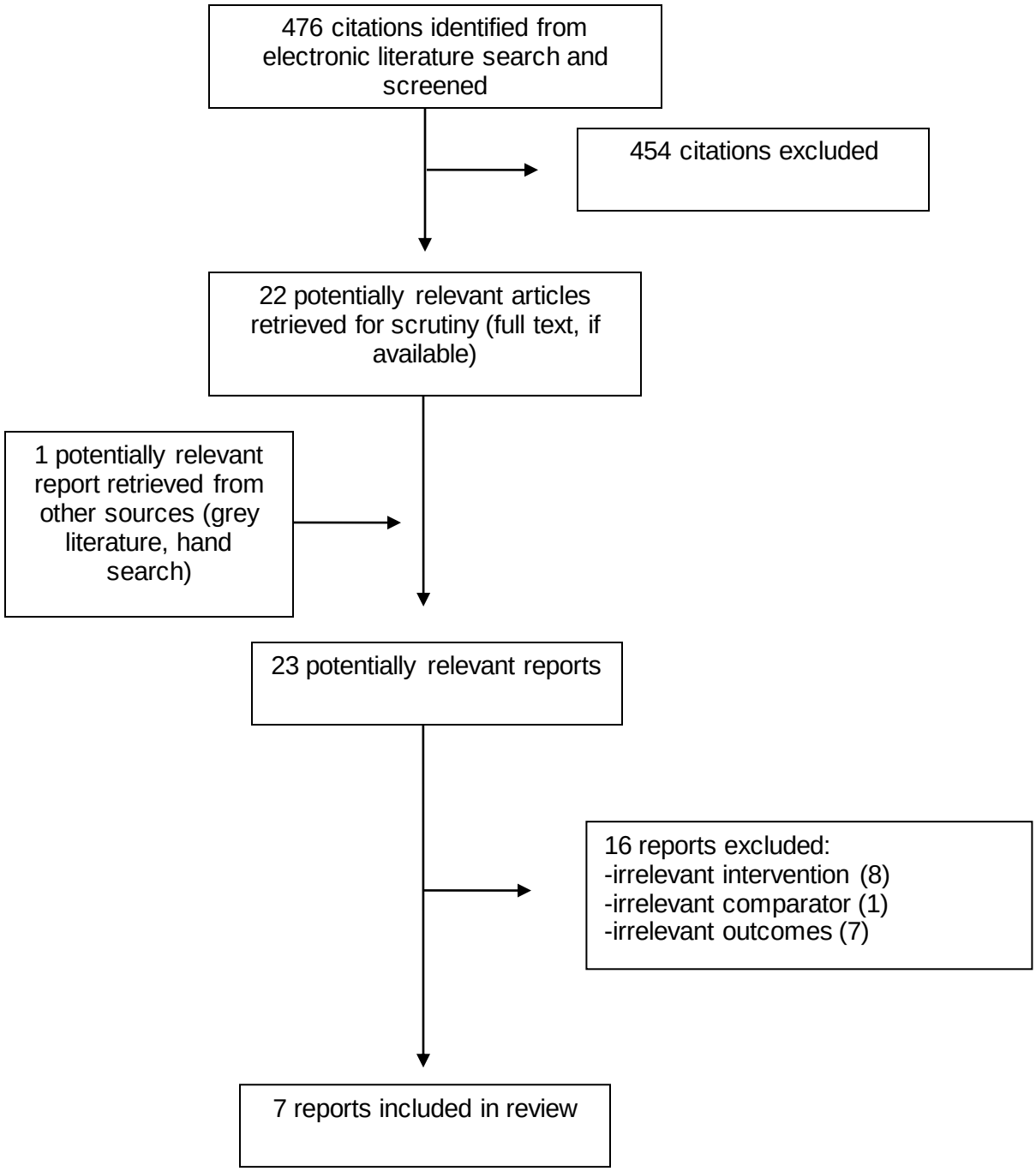
APPENDIX 1: Selection of Included Studies



APPENDIX 2: Characteristics of Included Publications

Table A1: Characteristics of Included Clinical Studies

First Author, Publication Year, Country	Study Design	Patient Characteristics	Intervention(s)	Comparator(s)	Reference Standard	Outcomes
Chang 2014,[6] Taiwan	Retrospective DTA	Pediatric patients (aged ≤ 18 years) with clinical symptoms, physical findings, and radiological evidence of pneumonia; n = 290; hospitalized: n = 244	MP detection in hospitalized patients by IgM ELISA on serum; positive: ≥ 20 BU/ml, borderline: 10 to 20 BU/ml, negative: < 10 BU/ml; n = 182	NA	MP detection by qPCR on throat swab (n = 278), sputum (n = 9), or pleural effusion (n = 3) samples	Sensitivity, specificity, PPV, NPV
Kayuka 2014,[7] Japan	Prospective DTA	Children aged 0 to 18 years with community-acquired LRTI (at least two symptoms among cough, fever, chest pain, dyspnea; plus abnormal breath sounds and/or radiographic lung abnormalities); n = 191	MP detection by IgM EIA on acute-phase sera collected at first visit; positive: definite blue colour in test well	MP detection by LAMP assay on throat swabs collected at first visit	Positive culture and/or seroconversion, or four-fold increase in antibody titers in paired sera	Sensitivity, specificity, PPV, NPV, PLR, NLR
Medjo 2014,[8] Serbia	Prospective DTA	Children aged 1 to 15 years with signs, symptoms, and chest radiographs consistent with CAP; n = 166 Acute MP infection: MP-positive on at least one test method (of culture, PCR, or serology); n = 24	MP detection by IgM ELISA on acute-phase sera collected at enrollment (in the second week after disease onset); positive: > 1.1	MP detection by qPCR on throat swabs collected at enrollment	Minimum four-fold increase in IgG titers in paired sera (collected at enrollment and 2 to 4 weeks later)	Sensitivity, specificity, PPV, NPV, PLR

Table A1: Characteristics of Included Clinical Studies

First Author, Publication Year, Country	Study Design	Patient Characteristics	Intervention(s)	Comparator(s)	Reference Standard	Outcomes
Qu 2013,[9] China	Prospective DTA	Patients aged 14 years and older with radiographically confirmed CAP; n = 125	MP detection by IgM ELISA on acute-phase sera collected at admission; positive: > 17 U/ml, borderline: 13-17 U/ml, negative: < 13 U/ml	MP detection by qPCR on throat swabs collected at admission	Four-fold or greater increase in IgG titers in paired sera collected at admission and 2 to 4 weeks later	Sensitivity, specificity, PPV, NPV, PLR
Thurman 2009,[10] United States of America	Prospective DTA with case-control design	Cases: people with fever and cough or clinical or radiologic diagnosis of CAP during a community outbreak of MP; n = 97 Controls: asymptomatic household contacts of cases and age-matched controls; n = 166	MP detection by IgM EIA on serum samples	MP detection by qPCR on oropharyngeal and/or nasopharyngeal swabs	Clinically defined case	Sensitivity, specificity, PPV
Beersma 2005,[11] The Netherlands	Prospective DTA with case-control design	Cases: patients with qPCR-confirmed acute MP infection, aged 4 to 74 years, median age 43 years; n = 27 patients; n = 46 serum specimens; 19 paired acute and convalescent stage, 8	MP detection by 13 serologic assays for IgM (11 EIAs, MAG, CFT) on acute-phase and convalescent-phase sera	IgM serologic tests compared with each other	MP detection by qPCR on throat swabs obtained at the first visit	Sensitivity, specificity, PPV, NPV

Table A1: Characteristics of Included Clinical Studies

First Author, Publication Year, Country	Study Design	Patient Characteristics	Intervention(s)	Comparator(s)	Reference Standard	Outcomes
		single serum specimens (7 acute-phase, 3/7 collected within 7 days of disease onset) Controls: patients with acute LRTIs negative for MP by PCR (n = 20; 33 serum specimens) and patients with LRTIs due to other pathogens (n = 61; 63 serum specimens)				

BU = biological unit; CAP = community-acquired pneumonia; CFT = complement fixation test; DTA = diagnostic test accuracy; EIA = enzyme immunoassay; ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; IgM = immunoglobulin M; LAMP = loop-mediated isothermal amplification; MAG = microparticle agglutination; ml = millilitre; MP = *Mycoplasma pneumoniae*; NA = not applicable; NLR = negative likelihood ratio; NPV = negative predictive value; PCR = polymerase chain reaction; PLR = positive likelihood ratio; PPV = positive predictive value; qPCR = quantitative PCR; U = unit.

Table A2: Characteristics of Included Guidelines

Objectives			Methodology			
Intended users/ Target population	**Intervention and Practice Considered**	**Major Outcomes Considered**	**Evidence Collection, Selection and Synthesis**	**Evidence Quality and Strength**	**Recommendations development and Evaluation**	**Guideline Validation**
Harris, 2011[12] – British Thoracic Society, Community-Acquired Pneumonia in Children Guideline Group						
Intended users: clinicians (nurses, physicians, respiratory care practioners) Target population: infants and children with CAP; excludes: neonates, infants with RSV bronchiolitis or children with URTI, mild fever and wheeze, specific management of children with pre-existing respiratory conditions or immunosuppression	Assessment of signs and symptoms of CAP, diagnosis (including microbiological investigations such as serology and PCR), assessment of severity, management and treatment	Diagnostic test accuracy (sensitivity and specificity), symptom improvement, morbidity and mortality, pneumonia incidence and prevalence	Electronic database searches, study selection in duplicate, systematic review with evidence tables presented	Weighted according to a provided rating scheme	Recommendations developed by expert consensus and graded according to a provided rating scheme	Internal peer review

CAP = community-acquired pneumonia; RSV = respiratory syncytial virus; URTI = upper respiratory tract infection.

APPENDIX 3: Critical Appraisal of Included Publications

Table A3: Strengths and Limitations of Diagnostic Test Accuracy Studies using QUADAS-2[4]

Strengths	Limitations
Chang, 2014[6]	
• Case-control design was avoided • No inappropriate exclusion criteria reported • Threshold for serological diagnosis was defined a priori • All patients received the same reference standard	• Unclear whether consecutive patients or a random sample of symptomatic patients were enrolled • Unclear whether results of the index tests were known when the reference tests were interpreted, and vice versa • qPCR used as reference standard while it was acknowledged that there is no accepted reference standard for MP • Samples for qPCR taken before hospital admission, serum samples for IgM testing taken after hospital admission (unclear treatment administration between sampling intervals) • Not all hospitalized patients tested for IgM
Kayuka, 2014[7]	
• Case-control design was avoided • No inappropriate exclusion criteria reported • Reference standard likely to correctly classify MP • Throat swab samples for the LAMP assay and acute-phase sera for the IgM tests were taken at the same time (first visit after onset of fever) • All patients were assessed with the same index tests and reference standards • All enrolled patients were included in the analysis	• Unclear whether consecutive patients or a random sample of symptomatic patients were enrolled • The threshold for IgM test positivity was a subjective measure (colour change) • Unclear whether results of the index tests were known when the reference tests were interpreted, and vice versa
Medjo, 2014[8]	
• Case-control design was avoided • Appropriate exclusion criteria were used • Threshold for serological diagnosis was defined a priori • Reference standard likely to correctly classify MP • Throat swab samples for qPCR and acute-phase sera for the IgM and IgG tests were taken at the same time (enrollment) • All patients were assessed with the same index tests and reference standards • All enrolled patients were included in the analysis	• Unclear whether consecutive patients or a random sample of symptomatic patients were enrolled • Unclear whether results of the index tests were known when the reference tests were interpreted, and vice versa
Qu, 2013[9]	
• Eligible patients were enrolled consecutively • Case-control design and inappropriate patient exclusions avoided • Threshold for serological diagnosis was	• Unclear whether results of the index test were known when the reference tests were interpreted, and vice versa

Table A3: Strengths and Limitations of Diagnostic Test Accuracy Studies using QUADAS-2[4]

Strengths	Limitations
defined a priori • Reference standard likely to correctly classify MP • Throat swab samples for qPCR and acute-phase sera for the IgM and IgG tests were taken at the same time (admission) • All patients were assessed with the same index tests and reference standards • All enrolled patients were included in the analysis	
Thurman, 2009[10]	
• All enrolled patients were included in the analysis	• Case-control design used • Clinically defined cases (reference standard) all assumed to be positive for MP due to outbreak situation, even though there are other pathogens that can cause pneumonia • Unclear whether patient status (case or control) was known when index test results were interpreted • Samples collected at any time after symptom onset and results pooled • Reporting of PPV is inappropriate (case-control design)
Beersma, 2005[11]	
• Test thresholds pre-specified	• Case-control design used • Unclear time interval between collection of throat swab sample for qPCR and acute-phase serum for IgM test • Unclear whether patient status (case or control) was known when index test results were interpreted • Unclear whether qPCR is an appropriate reference standard • Not all patients received the same reference standard (controls included those who were not tested by qPCR for MP but were assumed to be negative) • Not all patients tested with all index tests (due to large serum sample requirements) • Reporting of PPV is inappropriate (case-control design)

IgG = immunoglobulin G; IgM = immunoglobulin M; MP = *Mycoplasma pneumoniae*; PPV = positive predictive value; qPCR = quantitative polymerase chain reaction.

Table A4: Strengths and Limitations of Guidelines using AGREE II[5]	
Strengths	**Limitations**
Harris, 2011[12]	
• Guideline objectives, health questions, and relevant population are specifically described • Members from relevant professional groups were included in guideline development • Target users in general (medical professionals) defined in the guideline disclaimer • Systematic search of electronic databases performed • Health benefits, side effects, and risks have been considered in formulating the recommendations • Explicit link between the evidence base and the recommendations • Key recommendations are easily identifiable • Audit tool provided on the British Thoracic Society website • No external funding involved in guideline development, no competing interests declared	• Patient input was not sought during guideline development • No mention of grey literature search, selection limited to English language studies • Inclusion criteria for study selection not clearly defined • Evidence summarized but strengths and limitations of included studies leading to assigned evidence level not clearly described • Methods for formulation of recommendation not clearly described • Draft guideline internally reviewed by the British Thoracic Society; individual reviewers not specified • Timeline for guideline update provided but methodology for update not described • Recommendations for microbiological diagnostic methods are ambiguous regarding specific tests, indications, and timing • No discussion of guideline implementation provided

APPENDIX 4: Main Study Findings and Author's Conclusions

Table A5: Summary of Findings of Included Diagnostic Test Accuracy Studies

<table>
<tr><th>Main Study Findings</th><th>Author's Conclusions</th></tr>
<tr><td>Chang, 2014[6]</td><td></td></tr>
<tr><td>
• 54/290 patients with a clinical diagnosis of pneumonia were MP+ by qPCR
• 244/290 patients were hospitalized, 41/244 hospitalized patients were MP+ by qPCR
• 182/244 hospitalized patients were tested for IgM

MP detection in hospitalized patients with qPCR as reference standard:
<table>
<tr><th>Diagnostic Test Result</th><th>MP (n = 37)</th><th>No MP (n = 145)</th><th>Total (n = 182)</th></tr>
<tr><td>IgM+</td><td>23</td><td>21</td><td>44</td></tr>
<tr><td>IgM–</td><td>14</td><td>124</td><td>138</td></tr>
</table>
• In 17 of 23 IgM and qPCR positive patients, antibody testing was performed ≥ 7 days after fever onset

DTA results:
<table>
<tr><th></th><th>IgM (qPCR reference standard)</th><th>qPCR (IgM reference standard)</th></tr>
<tr><td>Sensitivity (%)</td><td>62.2</td><td>52.3</td></tr>
<tr><td>Specificity (%)</td><td>85.5</td><td>89.9</td></tr>
<tr><td>PPV (%)</td><td>52.3</td><td>62.2</td></tr>
<tr><td>NPV (%)</td><td>89.9</td><td>85.5</td></tr>
</table>
• ROC curve showed an adequate IgM cutoff point of 25 BU/ml (qPCR reference standard; sensitivity = 62.2%, specificity = 90.3%, AUC = 0.76)
</td><td>
• "PCR alone is frequently insufficient for the diagnosis of M. pneumoniae infection. Clinically, interpretation of Mycoplasma IgM or PCR results should be considered with caution. A combination of PCR plus serology assays as an early diagnostic testing for patients with compatible clinical manifestations may yield the most reliable results." Page 143
</td></tr>
<tr><td>Kayuka, 2014[7]</td><td></td></tr>
<tr><td>
• Definite MP infection diagnosed in 31/191 patients (13 by culture and serology, 17 by culture only, 1 by serology only)
• Serum was collected at first visit, a mean of 3.1 ± 1.9 days after fever onset.

MP detection with IgM testing and LAMP assay compared with definite MP infection reference standard:
</td><td>
• "The LAMP assay has excellent sensitivity and specificity to detect acute MP infection…Furthermore, this assay is simple and inexpensive because less laboratory infrastructure is required. The LAMP assay is appropriate for the genetic point-of-care diagnosis of acute phase MP infection in hospital laboratories." Page 551
</td></tr>
</table>

Table A5: Summary of Findings of Included Diagnostic Test Accuracy Studies

Main Study Findings	Author's Conclusions

Diagnostic Test Result	MP (n = 31)	No MP (n = 160)
IgM+	12	37
IgM–	19	123
LAMP+	30	0
LAMP–	1	160

DTA results with positive culture and/or serology as reference standard:

	IgM	LAMP
Sensitivity, % (95% CI)	38.7 (21.8, 57.8)	96.8 (83.3, 99.9)
Specificity, % (95% CI)	76.9 (69.6, 83.2)	100 (96.6, 100)
PPV, % (95% CI)	24.5 (13.3, 38.9)	100 (83.3, 100)
NPV, % (95% CI)	99.4 (96.6, 100)	99.4 (96.6, 100)
PLR	1.67 (0.99, 2.83)	NR
NLR	0.80 (0.60, 1.07)	0.03 (0.005, 0.22)

- Part 2 (includes additional patients to the 31 LAMP+ patients initially identified): 69/117 LAMP+ patients followed over time, 24/69 changed from LAMP+ to LAMP– in a mean of 12.3 ± 9.0 days

Medjo, 2014[8]

- MP pneumonia diagnosed in 24/166 children (14.5%) by serology, qPCR, and culture (n = 18), serology alone (n = 4), or qPCR alone (n = 2)
- MP pneumonia diagnosed in 22/166 children (13.3%) by IgG
- Combined IgM and qPCR detected MP in 22/24 cases (91.7%)

MP detection with IgM and qPCR testing compared with IgG reference standard:

Diagnostic Test Result	MP (IgG positive) (n = 22)	No MP (IgG negative) (n = 144)
IgM+	18	0
IgM–	4	144
qPCR+	18	2
qPCR–	4	142

- *"detection of IgM antibodies together with [qPCR] allows for precise and reliable diagnosis of MP infections in children during the acute phases of disease, indicating a possible use of both techniques as a valid diagnostic approach in early detection of MP infection in children with CAP."* Page 6

Table A5: Summary of Findings of Included Diagnostic Test Accuracy Studies

Main Study Findings	Author's Conclusions

DTA results with IgG as reference standard:

	IgM	qPCR
Sensitivity (%)	81.82	81.2
Specificity (%)	100	98.61
PPV (%)	100	90
NPV (%)	97.30	97.26
PLR	NR	58.91

Qu, 2013[9]

- Acute MP infection diagnosed in 27/125 (21.6%) patients

MP detection with IgM and qPCR testing compared with IgG reference standard:

Diagnostic Test Result	MP (IgG positive) (n = 27)	No MP (IgG negative) (n = 98)
IgM+	2	5
IgM–	25	93
qPCR+	11	11
qPCR–	16	87

DTA results with IgG as reference standard:

	IgM	qPCR
Sensitivity (%)	7.4	40.7
Specificity (%)	94.9	88.8
PPV (%)	28.6	28.6
NPV (%)	78.8	78.8
PLR	1.45	1.45

- *"Since the sensitivity was low in all evaluated methods, the logical approach would be to incorporate PCR, culture, and serological tests for optimum diagnosis of acute M. pneumoniae infections in adults and adolescents."* Page 5

Table A5: Summary of Findings of Included Diagnostic Test Accuracy Studies

<table>
<tr><th>Main Study Findings</th><th>Author's Conclusions</th></tr>
<tr><td>Thurman, 2009[10]</td><td></td></tr>
<tr><td>
MP detection with IgM and qPCR testing compared with clinically defined cases as reference standard:
<table>
<tr><th>Diagnostic Test Result</th><th>MP (clinically defined cases) (n = 97)</th><th>No MP (non-cases) (n = 166)</th></tr>
<tr><td>IgM+</td><td>79</td><td>NR</td></tr>
<tr><td>IgM–</td><td>18</td><td>NR</td></tr>
<tr><td>qPCR+</td><td>20</td><td>3</td></tr>
<tr><td>qPCR–</td><td>77</td><td>163</td></tr>
</table>
Overall DTA results with clinically defined cases as reference standard:
<table>
<tr><th></th><th>IgM</th><th>qPCR</th></tr>
<tr><td>Sensitivity, % (95% CI)</td><td>81 (74, 89)</td><td>21 (13, 29)</td></tr>
<tr><td>Specificity, % (95% CI)</td><td>63 (55, 70)</td><td>98 (96, 100)</td></tr>
<tr><td>PPV (%)</td><td>56</td><td>87</td></tr>
</table>
Sensitivity (95% CI) of each test based on interval between symptom onset and sample collection:
<table>
<tr><th>Interval (days)</th><th>Number of patients</th><th>IgM</th><th>qPCR</th></tr>
<tr><td>0 – 21</td><td>21</td><td>76 (58, 94)</td><td>48 (26, 69)</td></tr>
<tr><td>22 – 59</td><td>17</td><td>94 (83, 100)</td><td>29 (8, 51)</td></tr>
<tr><td>> 60</td><td>29</td><td>100</td><td>12 (0, 24)</td></tr>
</table>
</td><td>
• "The present study provides insight for establishing optimal testing strategies for the accurate and timely diagnosis of M. pneumoniae infection in the context of an outbreak. Early recognition, combined with the proper sample collection, handling, and processing, increases the likelihood that M. pneumoniae will be correctly identified. Analysis of test results from 2 CAP outbreaks suggests that a combination of M. pneumoniae-specific testing modalities simultaneously performed on suspected case patients and control subjects is the most reliable strategy to determine the etiology of an outbreak, especially in the absence of other agents." Page 1248-49
</td></tr>
<tr><td>Beersma, 2005[11]</td><td></td></tr>
<tr><td>
• 11/13 immunoassays (10 microtiter EIAs, 1 MAG assay) separately detected IgM; 2 evaluated tests non-specific to IgM not reported here
• 20/27 cases (74%) IgM-positive by ≥ 1 IgM assay

Number (%) of IgM-positive patients on each immunoassay by phase of serum collection (paired samples):
</td><td>
• "Serology remains a practical and undemanding method for the diagnosis of M. pneumoniae infection, particularly in young patients with a time of disease onset of more than 1 week earlier. However, given the low sensitivities of IgM assays, particularly for adult patients, who are known to develop weak antibody responses, and the need for paired serum specimens with a rise in IgG
</td></tr>
</table>

Table A5: Summary of Findings of Included Diagnostic Test Accuracy Studies

Main Study Findings

IgM Assay	Acute-Phase Serum MP+ by qPCR (n = 19)	Convalescent-Phase Serum MP+ by qPCR (n = 19)
AniLabsystems	8 (42)	16 (84)
Biotest	5 (26)	10 (53)
ImmunoCard	5/15 (33)	7/16 (44)
ImmunoWell	3 (16)	7 (37)
Novum	8 (42)	15 (79)
Platelia	6 (32)	9 (47)
Ridascreen	4 (21)	6 (32)
Serion classic	4 (21)	10 (53)
SerodiaMycoII (MAG)	7 (37)	15 (79)
SeroMP	7 (37)	15 (79)
Virotech	3 (16)	10 (53)

Number (%) of case and control serum samples collected ≥ 7 days after symptom onset that were positive for IgM; PPV, NPV, and AUC for each immunoassay:

IgM Assay	MP+ sera by qPCR (n = 31)	MP– sera by qPCR (n = 96)	PPV (%)	NPV (%)	ROC AUC
AniLabsystems	24 (77)	88 (92)	75	93	0.85
Biotest	16 (52)	91 (95)	73	86	0.76
ImmunoCard	12/25 (48)	76 (79)	38	85	0.64
ImmunoWell	11 (35)	92 (96)	73	82	0.67
Novum	22 (71)	47 (49)	31	84	0.66
Platelia	16 (52)	94 (98)	89	86	0.87
Ridascreen	11 (35)	96 (100)	100	83	0.76
Serion classic	15 (48)	91 (95)	75	86	0.81
SerodiaMycoII (MAG)	20 (65)	84 (88)	63	88	0.83
SeroMP	22 (71)	84 (88)	65	92	0.80
Virotech	14 (45)	92 (96)	78	84	0.73

Author's Conclusions

antibody titer for the diagnosis of M. pneumoniae infection, nucleic acid amplification methods might before the preferred diagnostic procedures for the diagnosis of M. pneumoniae infections, provided that the quality of the procedures is guaranteed." Page 2284

+ = test positive; – = test negative; AUC = area under the curve; BU = biological units; CAP = community-acquired pneumonia; CI = confidence interval; EIA = enzyme immunoassay; IgM = immunoglobulin M; MAG = microparticle agglutination; ml = millilitre; MP = *Mycoplasma pneumoniae;* LAMP = loop-mediated isothermal amplification; NPV = negative predictive value; NR = not reported; PCR = polymerase chain reaction; PLR = positive likelihood ratio; PPV = positive predictive value; qPCR = quantitative PCR; ROC = receiver operating characteristic.

Table A6: Summary of Findings of Included Guidelines	
Evidence Statements	**Recommendations**
Harris, 2011[12]	
Molecular methods have shown promise but are currently most useful in identifying viral pathogens. [Ib][a]	• Microbiological diagnosis should be attempted in children with severe pneumonia sufficient to require pediatric intensive care admission or those with complications of CAP. [C][b] • Microbiological investigations should not be considered routinely in those with milder disease or those treated in the community. [C][b] • Microbiological methods should include: – Blood culture. [C][b] – Nasopharyngeal secretions and/or nasal swabs for viral detection by PCR and/or immunofluorescence. [C][b] – Acute and convalescent serology for respiratory viruses, *Mycoplasma* and *Chlamydia.* [B+][c] – If present, pleural fluid should be sent for microscopy, culture, pneumococcal antigen detection and/or PCR. [C][b]

[a] Evidence level definition: one or more rigorous studies designed to answer the question, but not formally combined.
[b] Guideline statement grade definition: formal combination of expert views.
[c] Guideline statement grade definition: one or more prospective clinical studies which illuminate, but do not rigorously answer, the question.

APPENDIX 5: Additional References of Potential Interest

Guidelines with Unclear Methodology

Toward Optimized Practice (TOP) Working Group for Nursing Home Acquired Pneumonia (NHAP). Diagnosis and management of nursing home acquired pneumonia (NHAP): clinical practice guideline [Internet]. Edmonton: Toward Optimized Practice; 2015 Mar. [cited 2015 Nov 6]. Available from: http://www.topalbertadoctors.org/download/388/NHAP_guideline.pdf
See: Serology and Invasive Testing, page 8; "Serology is not routinely recommended."

Infants and children: acute management of community acquired pneumonia. Clinical practice guideline [Internet]. North Sydney (AU): NSW Kids and Families; 2015 Mar 19. [cited 2015 Nov 6]. Available from: http://www0.health.nsw.gov.au/policies/gl/2015/pdf/GL2015_005.pdf
See: Serum, page 14

Guideline for the diagnosis and management of community acquired pneumonia: adult [Internet]. Edmonton: Toward Optimized Practice; 2008. [cited 2015 Nov 6]. Available from: http://www.topalbertadoctors.org/download/384/CAP_adult_guideline.pdf
See: Investigations, Additional Tests for Hospitalized Patients; "Serology is not routinely recommended."

Guideline for the diagnosis and management of community acquired pneumonia: pediatric [Internet]. Edmonton: Toward Optimized Practice; 2008. [cited 2015 Nov 6]. Available from: http://www.topalbertadoctors.org/download/385/CAP_pediatric_guideline.pdf
Note: "In children over 2 years of age, Mycoplasma IgM may be considered"