Systematic Review & Meta-Analysis

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PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: A systematic review & meta-analysis

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***Background & objectives*: Diagnosis for *Mycoplasma pneumoniae* usually relies on serological tests. PCR technology has some advantages but also limitations. The optimal selection for these tests still needs discussion. This paper reviews the overall diagnostic accuracy of PCR versus serological assays for diagnosis of *M. pneumoniae* infections and to identify factors associated with heterogeneity of results.**

***Methods*: MEDLINE and Embase databases were searched. Articles meeting the selection criteria were retrieved for data collection and analysis. Studies were assessed for methodological quality using QUADAS. Hierarchial summary receiver operating characteristic (HSROC) model was used to estimate summary ROC curve.**

***Results*: Initial meta-analysis showed a summary estimate of sensitivity (SEN) 0.62 (95% CI, 0.45-0.76), and specificity (SPE) 0.96 (95% CI, 0.93-0.98). Subgroup analyses were performed to identify factors associated with heterogeneity. For different gene targets, reference standards, subjects (children or adults) and different PCR types, these aspects can generate results of heterogeneity. The 16s rDNA target and adult subjects and real-time PCR may have better test results for PCR.**

***Interpretation & conclusions*: Commercial PCR tests generated consistent results with high specificity but a lower and more variable sensitivity. The findings suggest commercial PCR tests having superiorities in diagnosing *M. pneumoniae* infections but still cannot replace serology. PCR plus serology could be good screening tests for reliable and accurate diagnosis of *M. pneumoniae*.**

Key words Diagnosis - meta-analysis - *Mycoplasma pneumoniae* - PCR - serology - systematic review

Mycoplasma pneumoniae is a common cause of respiratory infections in humans. Although it is usually associated with mild acute respiratory infections such as sore throat, pharyngitis, rhinitis and tracheobronchitis, it can also cause more critical infections including pneumonia or lung abscess. *M. pneumoniae* is known to be responsible for 10 to 30 per cent of community-acquired pneumonia (CAP) cases and is also associated with acute exacerbations of asthma[1-3] and chronic obstructive pulmonary disease (COPD)[4], and even causing community outbreaks similar to influenza[5].

Correct and rapid diagnosis of *M. pneumoniae* infections is critical to initiate appropriate antibiotic treatment. Since it is impossible to diagnose this disease merely based on clinical signs and symptoms, therefore, laboratory test for detecting *M. pneumoniae*

is particularly important. As it grows slowly (requiring 2 to 5 wk for colonies to become visible), culture is time-consuming[6]. Serological assays are most widely used. But sensitivity of these assays depends on whether the first serum sample is collected early or late after the onset of disease and on the availability of paired serum samples collected with an interval of 2 to 3 wk. Immunoglobulin M (IgM) assays that are more sensitive than the complement fixation (CF) test have been developed, but the IgM response may be nonspecific[7] or absent, particularly in adults[8]. Hybridization with DNA probes proposed as a rapid and specific procedure to replace culture, lacks sensitivity[9]. Nucleic acid amplification techniques (NAATs) have the potential to generate rapid, sensitive and specific results, but proper validation and standardization are often lacking, and quality control studies have revealed frequent deficiencies resulting in both false negative and false positive results. Although studies have reported the NAATs (mainly refer to PCR tests) have high sensitivity and specificity and have been widely used clinically, these may not be available in some small hospitals especially in countries and regions of limited resources (mainly in the developing countries). The serology assays are still commonly used. Therefore, we performed this systematic review and meta-analysis to conclude the overall accuracy of PCR versus serology for diagnosing *M. pneumoniae* infections and to identify factors associated with inter-studies heterogeneity of results. We also attempted to make a suggestion for the early arrangement of screening tests.

Material & Methods

Search strategy and selection criteria: Two electronic databases were searched, MEDLINE (January 1985 to September 2009) and Embase (January 1988 to September 2009), with the following search terms: (*i*) PCR OR polymerase chain reaction OR nucleic acid amplification technique, OR direct amplification test OR ligase chain reaction OR molecular diagnostic technique; (*ii*) serologic test OR serological test, OR serodiagnose, OR immunological tests, OR *Mycoplasma pneumoniae* detection; (*iii*) diagnosis, OR diagnostic accuracy, OR (sensitivity and specificity) OR predictive value, and (*iv*) *Mycoplasma pneumoniae* infection OR *Mycoplasma pneumoniae* pneumonia. All the above were used. For included studies, citation searches and reference lists were screened.

Selection criteria were (*i*) Diagnostic accuracy studies that compared PCR tests with serological assays for *M. pneumoniae* infections; (*ii*) Reference standard: serological assays incorporating Mp-IgM/IgG/IgA; (*iii*) Outcome measures: those generated data to construct a 2·2 table for calculating the sensitivity, specificity and likelihood ratios; and (*iv*) Case-control studies with a sample size of 50 patients or above who provided sera 14 days before receiving antibiotics.

Study selection: The first two authors independently screened citations retrieved from all sources for relevance. Screening of full-text articles using pre-specified selection criteria was carried out by first four authors and checked by the last author. Disagreements were resolved by discussion.

Data extraction and quality assessment: A data extraction form was used for collecting data. Data retrieved from the reports included methodological quality, participant characteristics, test methods and outcome data. The outcome data for 2×2 table were obtained from the original reports directly or through calculation.

The quality of the studies included was assessed using a subset of criteria from the QUADAS tool (UK and Netherland), which was developed as a validated tool for diagnostic studies[10]. Data extraction and quality assessment were carried out by the first author (LZ) and checked by the other.

Statistical analysis: A hierarchical summary receiver operating characteristic (HSROC) curve model was used to summarize the paired sensitivity and specificity estimates instead of the traditional SROC model, as the traditional model has its limitations[11-13]. The HSROC model has several advantages as (*i*) it assumes an explicit formula linking sensitivity and specificity through a threshold; (*ii*) it accounts for the variability across studies; (*iii*) it can be used to estimate summaries of the data considering the threshold effects; (*iv*) it includes a summary ROC curve and average values of accuracy measures with confidence regions[11]; and (*v*) takes into account unmeasured heterogeneity between studies using random effects[12]. A bivariate random effects model of sensitivity and specificity was also used to calculate this pair of performance measures[11].

Since variability of results among different studies is expected, an investigation of heterogeneity is necessary for the meta-analysis of diagnostic tests. Stratified (subgroup) analyses were also used for variations in many aspects. Sensitivity analysis was done and publication bias was also preliminarily assessed by Deeks' funnel plot asymmetry test[14].

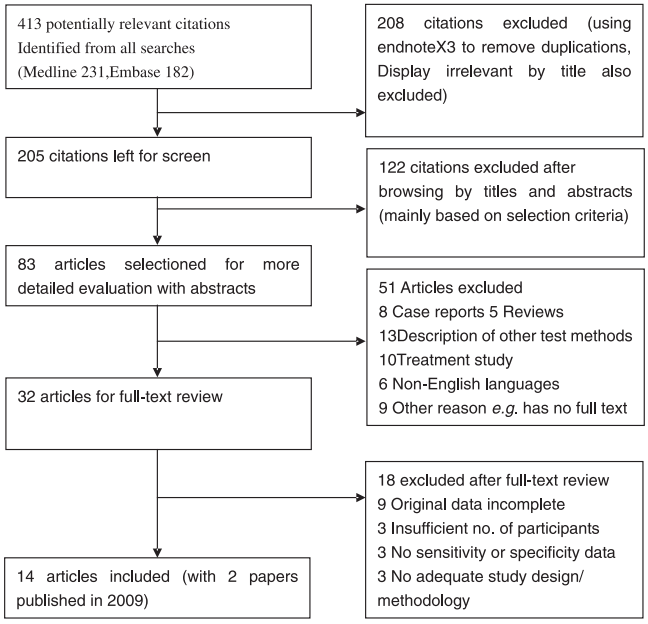


Fig. 1. Study selection scheme.

All the analyses were done using the softwares STATA version 11.0 (Stata Corporation, TX, USA) and Review Manager (RevMan) version 5.0. (Copenhagen The Nordic Cochrane Centre, The Cochrane Collaboration, 2008)

Results

Selection process of studies is outlined in Fig. 1. A total of 14 studies[15-28] were included with the characteristics of these studies listed in Table I.

Assessment of study quality: All studies included used the case-control design, five (36%) of which enrolled both children and adults with respiratory tract infections (RTI), Seven (50%) enrolled only children with CAP (n=4) or RTI (n=2) or asthma (n=1), and two (14%) only enrolled adults with CAP. Most studies (n=9, 64%) used IgM/IgG as the serology reference standard and the remaining four (36%) employed IgM/IgG/IgA (except 1 used IgM). In all 14 studies, the templates for PCR were from respiratory samples. For the target

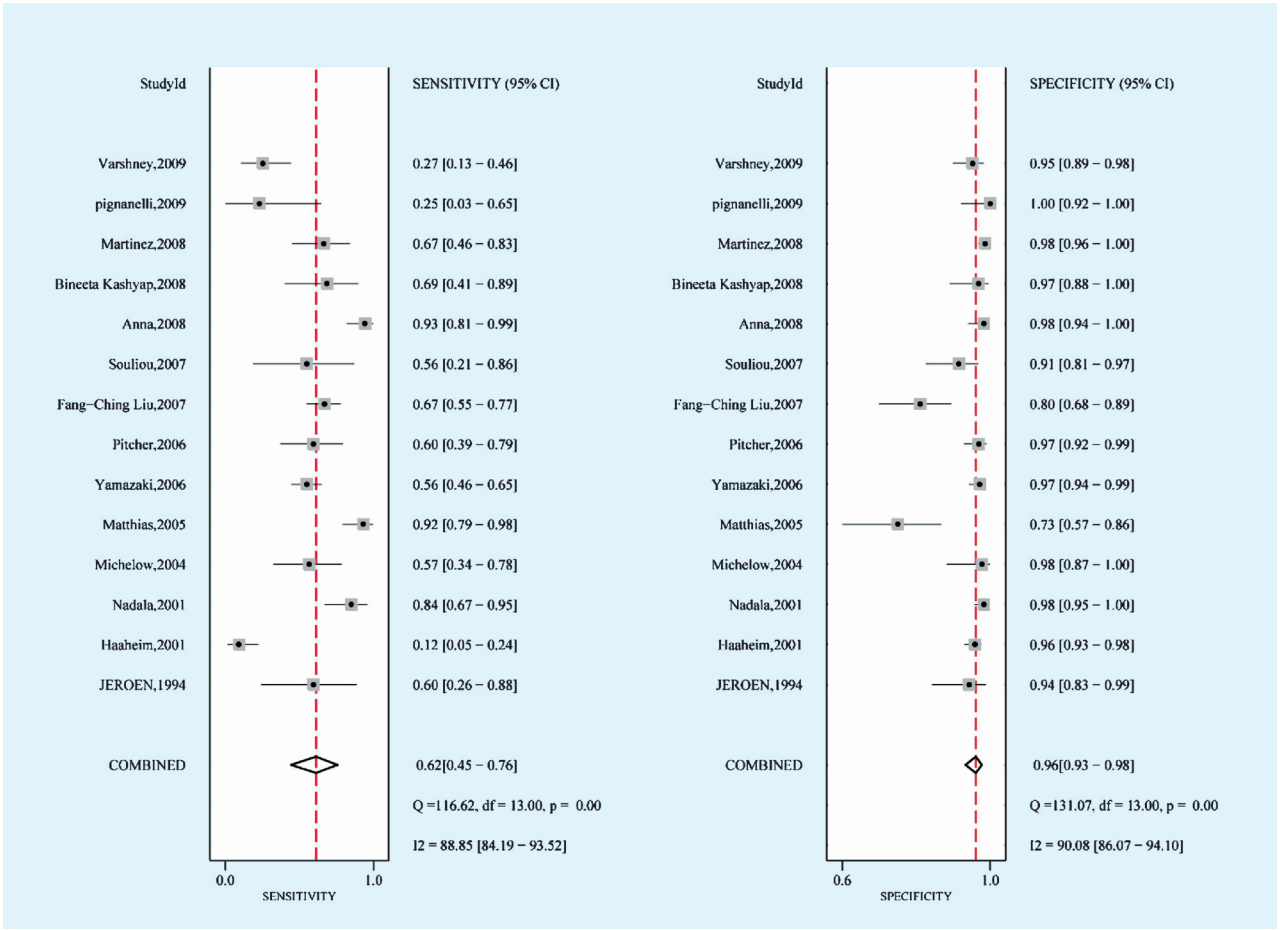


Fig. 2. Forest plot (shows initial meta-analysis results included all 14 studies).

Table I. Characteristics of included studies in the review

StudyID	Country	Patients & Clinical features*	PCR features	Samples for PCR	Reference standard
Tjhie *et al* 1994 1	The Netherlands	C+A, with RTI, not treated	Direct/conventional PCR, commercial, 16s rDNA	Respiratory tract samples, mainly throat	IgM/IgG
Haaheim *et al* 2001 2	Norway	C+A, with RTI, not treated	PCR, type NR, commercial, DNA not specified	Nasopharyngeal samples	IgM/IgG/IgA
Nadala *et al* 2001 3	Switzerland	C, with CAP, not treated	PCR, type NR, commercial 16s rDNA	Nasopharyngeal and pharyngeal samples	IgM/IgG
Michelow *et al* 2004 4	USA	C, with CAP, not treated	Real-time PCR, commercial P1 gene	Nasopharyngeal oropharyngeal samples	IgM/IgG
Beersma *et al* 2005 5	The Netherlands and Belgium	C+A, with RTI, not treated	Real-time PCR, commercial P1 gene	Throat swab samples	IgM/IgG/IgA
Yamazaki *et al* 2006 6	Japan	C, with CAP, not treated	Nested PCR, commercial P1 gene	Sputum samples	IgM/IgG/IgA
Pitcher *et al* 2006 7	UK	A, with CAP, not treated	Real-time PCR, commercial P1 gene	Respiratory tract samples mainly throat	IgM/IgG
Liu *et al* 2007 8	Taiwan,	C, with CAP, not treated	PCR, type NR, commercial P1 gene	Respiratory tract samples mainly throat	IgM/IgG
Souliou *et al* 2007 9	Greece	C, with RTI, not treated	PCR, type NR, commercial P1 gene	Throat swab samples	IgM/IgG/IgA
Nilsson *et al* 2008 10	Sweden	C+A, with RTI, most treated	Nested and real-time PCR commercial DNA not specified	Oropharyngeal samples	IgM/IgG
Kashyap *et al* 2008 11	India	C, with RTI, not treated	PCR, type NR, commercial P1 gene	Nasopharyngeal samples	IgM/IgG
Martinez *et al* 2008 12	Chile	A, with CAP, not treated	PCR, type NR, Commercial 16s rDNA and 16s rRNA	Throat swab samples	IgM/IgG
Pignanelli *et al* 2009 13	Italy	C+A, with RTI, not treated	RT-PCR, Commercial P1 gene,	BAL samples	IgM/IgG
Varshney *et al* 2009 14	India	C, whit asthma, not treated	PCR, type NR, Commercial P1 gene	Throat swab samples	IgM

All studies are case-control design and prospective data collections; *C, children; A, adult; RTI, respiratory tract infections, CAP, community-acquired pneumonia; Varshney *et al* used in house primers of P30, and published primers of P1 gene

sequence, nine studies (64%) used P1 gene and the remaining five (36%) used the 16s rDNA or other (not reported) (Table I). None of these studies reported blinded interpretation of test results.

Meta-analysis and overall diagnostic accuracy: Initial meta-analysis results included all 14 studies (Fig. 2). The overall diagnostic accuracy was 0.62 (95% CI 0.45, 0.76) by sensitivity (SEN) and 0.96 (0.93, 0.98) by specificity (SPE). Almost all studies showed nearly perfect specificity estimates. In contrast, sensitivity estimates were lower and more variable (range 0.12 -0.93). The $\chi 2$ test for heterogeneity showed very low P values (<0.001) that may suggest a substantial

heterogeneity. So the bivariate box plot was used (Fig. 3) which describes the degree of interdependence of sensitivity with specificity including the central location and identification of any outliers. The inner oval represents the median distribution of the data points and the outer oval represents the 95 per cent confidence bound. This demonstrates a skewness of the test performance measures towards a higher specificity with relative lower sensitivity, providing indirect evidence of some threshold variability. Most studies cluster within the median distribution with 4 outliers, suggesting some studies with heterogeneity. The HSROC curve further explained the summary point estimate (Fig. 4), the area under the curve (AUROC) was 0.94 (0.91, 0.96), suggesting a relative high accuracy[29].

Subgroup analyses: In order to investigate the reasons for heterogeneity, subgroup analyses were performed. One of such subgroup analyses suggested the different target sequence for PCR may be a source for heterogeneity (Table II, Fig. 5). Subgroup analysis 1 demonstrated that the 16s rDNA gene may provide a higher summary performance compared with the P1 gene or other targets used in commercial PCR for diagnosing *M. pneumoniae* infections. Subgroup analysis 2 compared the summary performance between children and children plus adults (for a subgroup at least 4 studies included). These two groups showed no significant differences, but between the children and adults, adults appeared to have better test results (Table II, Fig. 6). Since test accuracy could be influenced by the reference standard used, we compared the different reference standards as the

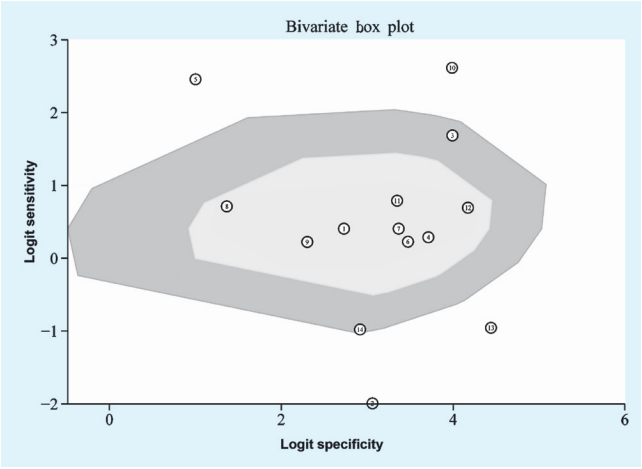


Fig. 3. Bivariate boxplot with most studies clustering within the median distribution and 4 outliers (NO. 1–14 represents studies in Table 1, X–axis: Logit specificity, Y-axis: Logit sensitivity)

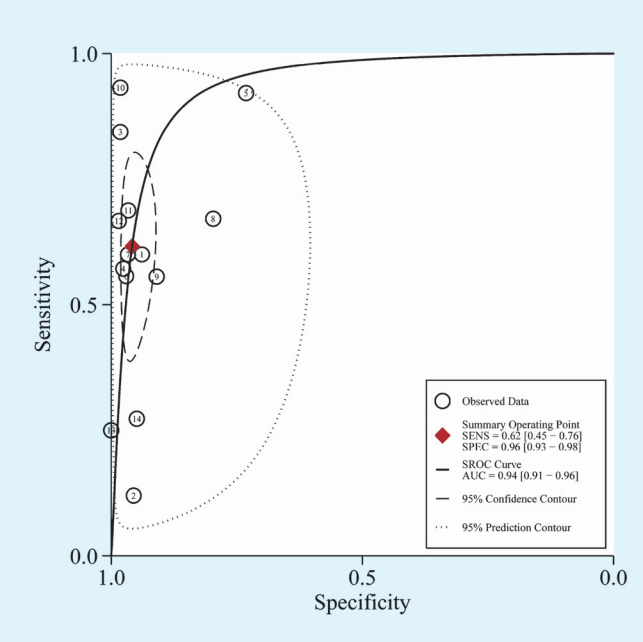


Fig. 4. HSROC curve of all 14 studies.

Table II. Summary performance estimates (Subgroup analysis 1 and 2)

Subgroup analysis 1		(P1 gene target, 9 studies)		Subgroup analysis 2		(Adults, 2 studies)	
Parameter	Estimate	95% CI	*P* value*	Parameter	Estimate	95% CI	*P* value*
Sensitivity	0.60	[0.54, 0.65]	0.224	Sensitivity	0.64	[0.49, 0.76]	0.618
Specificity	0.94	[0.92, 0.95]	0.05	Specificity	0.98	[0.96, 0.99]	0.213
		(16s rDNA target, 3studies)				(Children,7 studies)	
Sensitivity	0.74	[0.62, 0.84]	0.162	Sensitivity	0.59	[0.54, 0.65]	<0.001
Specificity	0.98	[0.97, 0.99]	0.199	Specificity	0.95	[0.93, 0.97]	<0.001
		(DNA not reported, 2studies)				(Children+Adults, 5 studies)	
Sensitivity	0.50	[0.40, 0.61]	<0.001	Sensitivity	0.62	[0.54, 0.70]	<0.001
Specificity	0.96	[0.94, 0.98]	<0.001	Specificity	0.94	[0.92, 0.96]	<0.001

*Random effects model. χ^2 test for heterogeneity

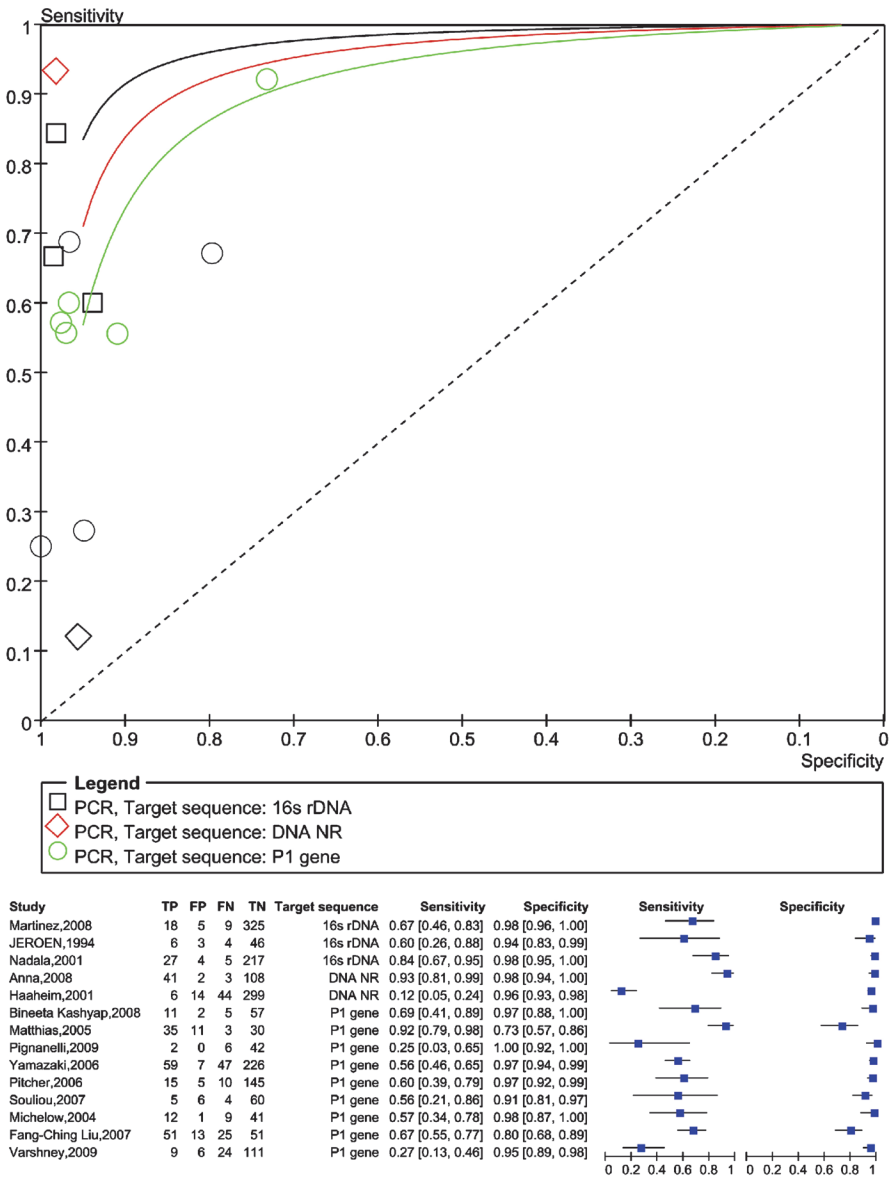
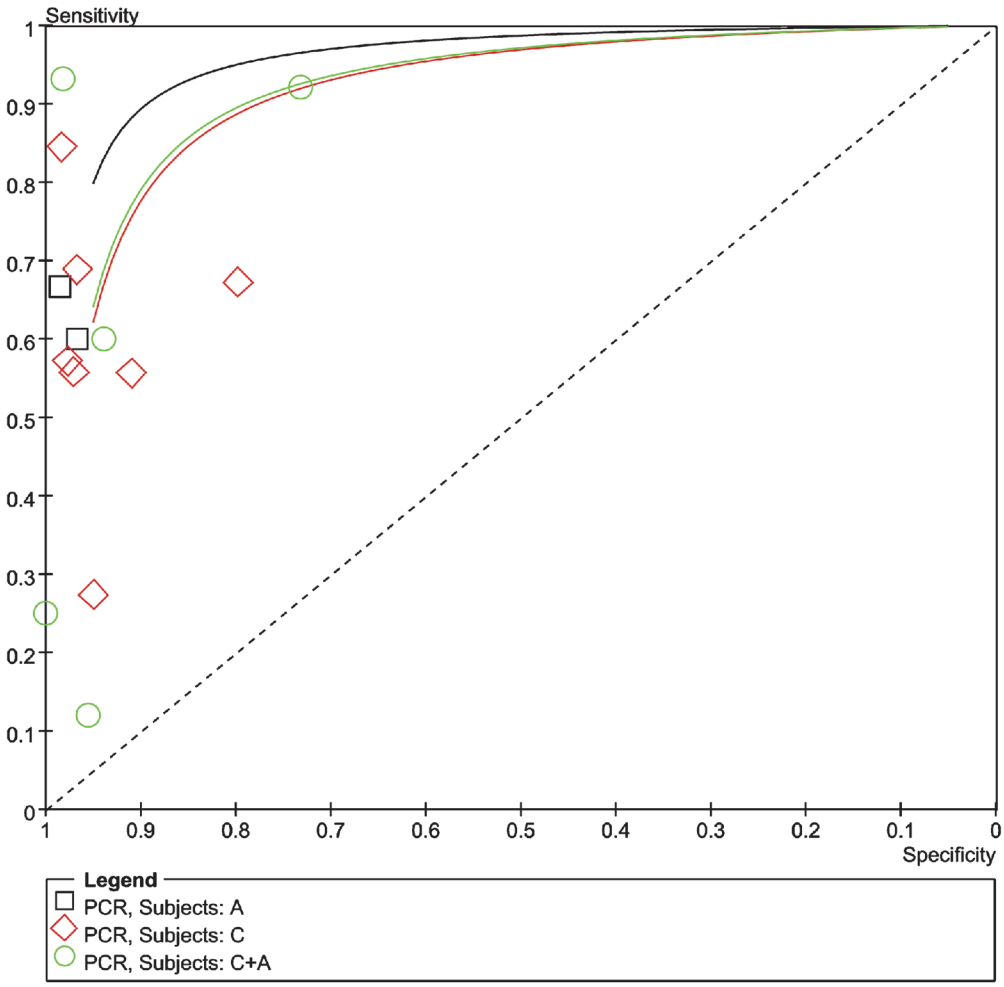


Fig. 5. Subgroup analysis 1 and forest plot for subgroup analysis 1.

Table III. Summary performance estimates (Subgroup analysis 3 and 4)

Subgroup analysis 3		(IgM/IgG ,9 studies)		Subgroup analysis 4		(Real-time PCR, 6 studies)	
Parameter	Estimate	95% CI	*P* value*	Parameter	Estimate	95% CI	*P* value*
Sensitivity	0.71	[0.66, 0.77]	<0.001	Sensitivity	0.76	[0.69, 0.83]	<0.001
Specificity	0.97	[0.96, 0.98]	<0.001	Specificity	0.95	[0.93, 0.97]	0.397
		(IgM/IgG/IgA, 4 studies)				(Conditional or type NR, 8 studies)	
Sensitivity	0.52	[0.45, 0.59]	<0.001	Sensitivity	0.53	[0.47, 0.58]	<0.001
Specificity	0.94	[0.92, 0.96]	<0.001	Specificity	0.96	[0.95, 0.97]	<0.001
		(IgM, 1 study)					
Sensitivity	0.27	[0.13, 0.46]	/				
Specificity	0.95	[0.89, 0.98]	/				

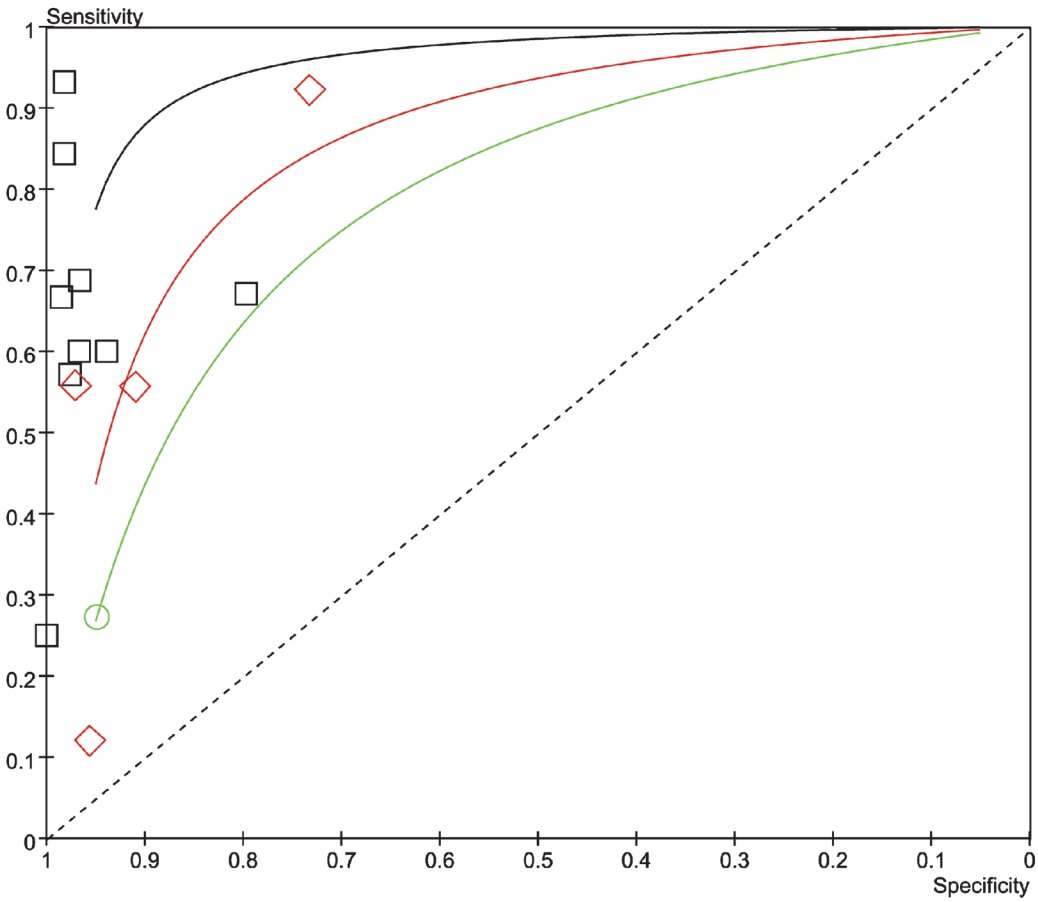


Study	TP	FP	FN	TN	Subjects	Sensitivity	Specificity
Martinez,2008	18	5	9	325	A	0.67 [0.46, 0.83]	0.98 [0.96, 1.00]
Pitcher,2006	15	5	10	145	A	0.60 [0.39, 0.79]	0.97 [0.92, 0.99]
Nadala,2001	27	4	5	217	C	0.84 [0.67, 0.95]	0.98 [0.95, 1.00]
Bineeta Kashyap,2008	11	2	5	57	C	0.69 [0.41, 0.89]	0.97 [0.88, 1.00]
Yamazaki,2006	59	7	47	226	C	0.56 [0.46, 0.65]	0.97 [0.94, 0.99]
Varshney,2009	9	6	24	111	C	0.27 [0.13, 0.46]	0.95 [0.89, 0.98]
Souliou,2007	5	6	4	60	C	0.56 [0.21, 0.86]	0.91 [0.81, 0.97]
Michelow,2004	12	1	9	41	C	0.57 [0.34, 0.78]	0.98 [0.87, 1.00]
Fang-Ching Liu,2007	51	13	25	51	C	0.67 [0.55, 0.77]	0.80 [0.68, 0.89]
JEROEN,1994	6	3	4	46	C+A	0.60 [0.26, 0.88]	0.94 [0.83, 0.99]
Pignanelli,2009	2	0	6	42	C+A	0.25 [0.03, 0.65]	1.00 [0.92, 1.00]
Anna,2008	41	2	3	108	C+A	0.93 [0.81, 0.99]	0.98 [0.94, 1.00]
Matthias,2005	35	11	3	30	C+A	0.92 [0.79, 0.98]	0.73 [0.57, 0.86]
Haaheim,2001	6	14	44	299	C+A	0.12 [0.05, 0.24]	0.96 [0.93, 0.98]

Fig. 6. Subgroup analysis 2 and forest plot for subgroup analysis 2.

subgroup analysis 3 (Table III, Fig. 7). The different serology tests used were a source of heterogeneity. Besides, the different subjects children/adults appeared to have an impact on test results. Comparisons between different types of PCR techniques showed in subgroup analysis 4 (Table III, Fig. 8). Different PCR types generated heterogeneity, but real-time PCR revealed a better performance than the others. All the samples for PCR were from clinical respiratory tract but most studies used a different sample; this aspect was also not included in the subgroup but the samples type could be another source of heterogeneity.

Sensitivity analyses and publication bias assessment: Sensitivity analysis by computing the summary measures with and without studies that [shown as

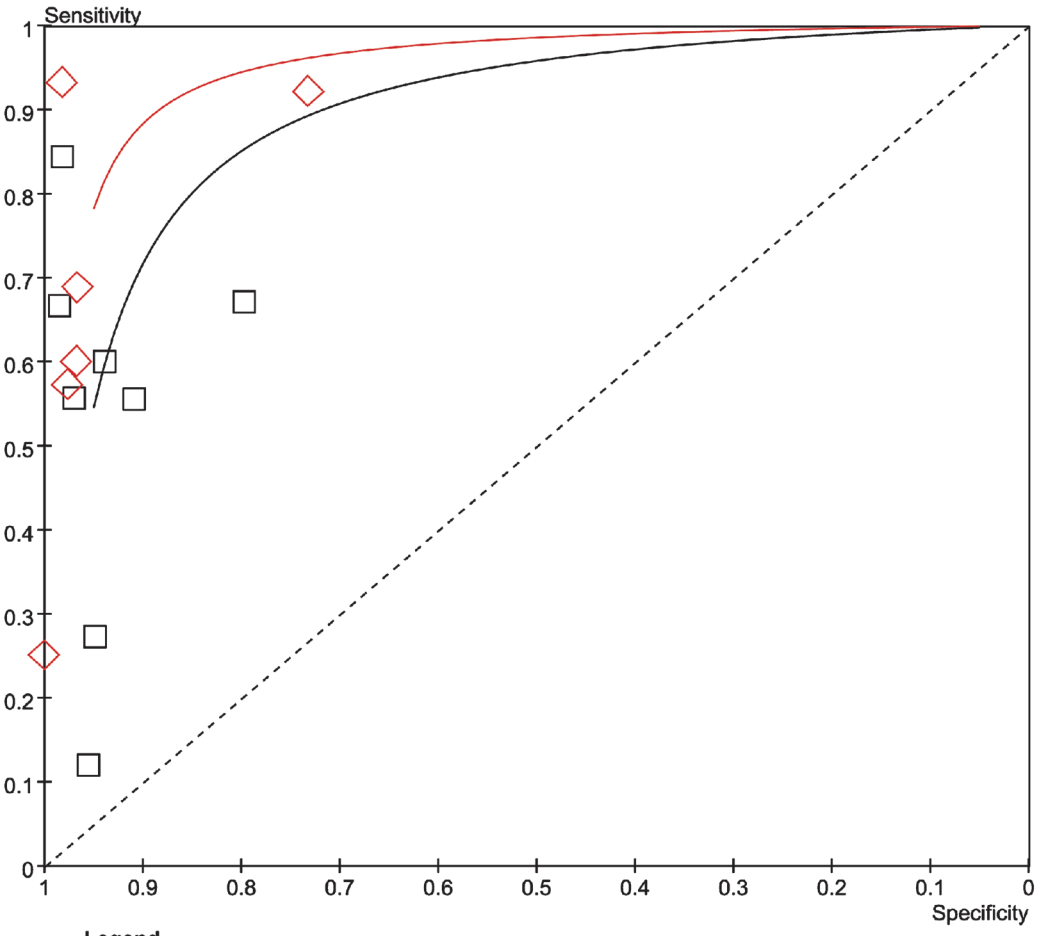


Study	TP	FP	FN	TN	reference standard	Sensitivity	Specificity
Varshney,2009	9	6	24	111	IgM	0.27 [0.13, 0.46]	0.95 [0.89, 0.98]
JEROEN,1994	6	3	4	46	IgM/IgG	0.60 [0.26, 0.88]	0.94 [0.83, 0.99]
Nadala,2001	27	4	5	217	IgM/IgG	0.84 [0.67, 0.95]	0.98 [0.95, 1.00]
Bineeta Kashyap,2008	11	2	5	57	IgM/IgG	0.69 [0.41, 0.89]	0.97 [0.88, 1.00]
Pignanelli,2009	2	0	6	42	IgM/IgG	0.25 [0.03, 0.65]	1.00 [0.92, 1.00]
Martinez,2008	18	5	9	325	IgM/IgG	0.67 [0.46, 0.83]	0.98 [0.96, 1.00]
Anna,2008	41	2	3	108	IgM/IgG	0.93 [0.81, 0.99]	0.98 [0.94, 1.00]
Pitcher,2006	15	5	10	145	IgM/IgG	0.60 [0.39, 0.79]	0.97 [0.92, 0.99]
Michelow,2004	12	1	9	41	IgM/IgG	0.57 [0.34, 0.78]	0.98 [0.87, 1.00]
Fang-Ching Liu,2007	51	13	25	51	IgM/IgG	0.67 [0.55, 0.77]	0.80 [0.68, 0.89]
Yamazaki,2006	59	7	47	226	IgM/IgG/IgA	0.56 [0.46, 0.65]	0.97 [0.94, 0.99]
Matthias,2005	35	11	3	30	IgM/IgG/IgA	0.92 [0.79, 0.98]	0.73 [0.57, 0.86]
Souliou,2007	5	6	4	60	IgM/IgG/IgA	0.56 [0.21, 0.86]	0.91 [0.81, 0.97]
Haaheim,2001	6	14	44	299	IgM/IgG/IgA	0.12 [0.05, 0.24]	0.96 [0.93, 0.98]

Fig. 7. Subgroup analysis 3 and forest plot for subgroup analysis 3.

outliers in Fig. 3 (studies 2, 5, 12, 13 in Table I)][16,19,24,27] showed that the summary measures computed after excluding 4 studies (sensitivity 0.61, specificity 0.96) were almost identical to those computed with all 14 studies (sensitivity 0.62, specificity 0.96). This suggested although the initial meta-analysis results may have heterogeneity, those results were still relatively consistent and may give a reliable overall estimates for the diagnostic accuracy of PCR versus serology for *M. pneumoniae* infections.

Publication bias was preliminarily assessed by Deeks' funnel plot asymmetry test (Fig. 9). The statistically non-significant *P* value (0.81) for the slope coefficient suggests symmetry in the data and a relative low likelihood of publication bias.



Study	TP	FP	FN	TN	PCR type	Sensitivity	Specificity
Yamazaki,2006	59	7	47	226	conditional or type NR	0.56 [0.46, 0.65]	0.97 [0.94, 0.99]
Martinez,2008	18	5	9	325	conditional or type NR	0.67 [0.46, 0.83]	0.98 [0.96, 1.00]
JEROEN,1994	6	3	4	46	conditional or type NR	0.60 [0.26, 0.88]	0.94 [0.83, 0.99]
Souliou,2007	5	6	4	60	conditional or type NR	0.56 [0.21, 0.86]	0.91 [0.81, 0.97]
Haaheim,2001	6	14	44	299	conditional or type NR	0.12 [0.05, 0.24]	0.96 [0.93, 0.98]
Fang-Ching Liu,2007	51	13	25	51	conditional or type NR	0.67 [0.55, 0.77]	0.80 [0.68, 0.89]
Varshney,2009	9	6	24	111	conditional or type NR	0.27 [0.13, 0.46]	0.95 [0.89, 0.98]
Nadala,2001	27	4	5	217	conditional or type NR	0.84 [0.67, 0.95]	0.98 [0.95, 1.00]
Bineeta Kashyap,2008	11	2	5	57	real-time PCR	0.69 [0.41, 0.89]	0.97 [0.88, 1.00]
Matthias,2005	35	11	3	30	real-time PCR	0.92 [0.79, 0.98]	0.73 [0.57, 0.86]
Pignanelli,2009	2	0	6	42	real-time PCR	0.25 [0.03, 0.65]	1.00 [0.92, 1.00]
Anna,2008	41	2	3	108	real-time PCR	0.93 [0.81, 0.99]	0.98 [0.94, 1.00]
Pitcher,2006	15	5	10	145	real-time PCR	0.60 [0.39, 0.79]	0.97 [0.92, 0.99]
Michelow,2004	12	1	9	41	real-time PCR	0.57 [0.34, 0.78]	0.98 [0.87, 1.00]

Fig. 8. Subgroup analysis 4 and forest plot for subgroup analysis 4

Discussion

M. pneumoniae is difficult to culture and diagnosis usually relies on serology in the past[30]. However, serology is not sufficiently rapid and reliable especially in specificity and is usually positive at about 7 days after the onset of disease[15,16]. PCR technology has been used for testing *M. pneumoniae* infections for about 20 yr, has several limitations, *e.g.* (*i*) PCR inhibitors in samples can lead to false-negative results; (*ii*) contamination can easily result in false-positive; (*iii*) acquiring good samples are relative difficult; and (*iv*) the time point for sampling influences results. It was reported that the diagnostic accuracy of PCR may decrease at ≥ 7 days after onset of disease in contrast to serology[15,16,24,25]. A significant increase of *M. pneumoniae* IgG titres,

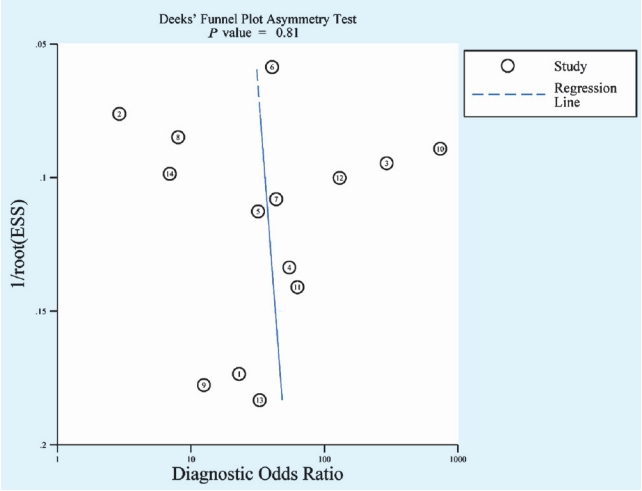


Fig. 9. Deeks' funnel plot [The statistically non-significant *P*-value (0.81) for the slope coefficient suggests symmetry in the data and a relative low likelihood of publication bias].

seroconversion in paired sera or the presence of IgM antibodies to *M. pneumoniae* were sufficient evidence of current *M. pneumoniae* infections[24].

Different diagnostic studies in this field have generated inconsistent diagnostic accuracy due to differences in threshold, test methods and PCR types. Because of various confounding factors, the exact diagnostic accuracy of PCR for *M. pneumoniae* is difficult to establish. Results from this meta-analysis indicate that commercial PCR tests generated consistent results with high specificity, but sensitivity estimates were lower and more variable than specificity. Potential explanations for these variations may include the different types of PCR and reference standard, the types of subjects, the time point for sampling and the qualities of different samples, the standard control of PCR and threshold, *etc*.

Due to relative limited research of high quality in this field and relative small number of studies included in this review, this meta-analysis has limitations and the results need to be cited with cautious. Such as, most studies included were after year 2000; different studies used different samples; the PCR assays were predominantly in paediatric populations and different methods were used.

The Infectious Disease Society of America had stated "no available diagnostic test reliably and rapidly detects *M. pneumoniae*. Thus, therapy must usually be empirical" in the Practice Guidelines for the Management of Community Acquired Pneumonia[31]. Taking the rational use of antibiotics, the costs and technical difficulty into account, the optimized strategy for the arrangement of diagnostic or screening tests on *M. pneumoniae* infections is important.

For the different subjects: adults/children, the subgroup analysis 2 suggested that the adults appeared to have better test results than children. Because of only two studies in the adult group, this meta-analysis could not give evidence that adults have good test results than children. In this review, real-time PCR revealed a better performance than the others. It has been reported that *M. pneumoniae* can be detected in patients without respiratory diseases by PCR, and real-time PCR targeting the P1 gene detects only 60 per cent of *M. pneumoniae* infections[21]. These findings need to be addressed in further research.

In conclusion, the present review and meta-analysis suggest an important role for commercial PCR tests in diagnosing *M. pneumoniae* infections with advantages, but still cannot replace serology (expensive and not feasible in routine labs; limitations for results of heterogeneity). Clinicians should select reliable PCR technology (while available) plus serology as diagnostic screening for the patients suspected. A combination of serology and PCR is recommended to provide rapid, reliable, and accurate diagnosis of *M. pneumoniae* infections.

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