

## RESEARCH ARTICLE

# Pooled analysis of nuclear acid sequence-based amplification for rapid diagnosis of *Mycoplasma pneumoniae* infection

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**Background:** *Mycoplasma pneumoniae* (*M pneumoniae*) is a common human etiology of respiratory infections. Nuclear acid sequence-based amplification (NASBA) shows good value for the detection of *M pneumoniae* that surpasses PCR. However, the optimal detection technology still remains to be identified. The purpose of this meta-analysis was to systematically evaluate the overall accuracy of NASBA for diagnosing *M pneumoniae* infections.

**Methods:** The databases PubMed, Cochrane Library, Google Scholar, CNKI, Wang Fang, and Baidu Scholar were comprehensively searched from their initiation date to December 2017 for NASBA in the diagnosis of *M pneumoniae* infection. Meta-DiSc 1.4 statistical software was used to evaluate the sensitivity (SEN), specificity (SPE), negative likelihood ratio (-LR), positive likelihood ratio (+LR), diagnostic odds ratio (DOR), and summary receiver operating characteristic (SROC). RevMan 5.2 statistical software was used for quality evaluation of the included articles. Publication bias was evaluated by funnel plot.

**Results:** Six articles with high quality, including 10 studies, were finally included in this meta-analysis. The combined statistics results for the diagnosis of *M pneumoniae* infection by NASBA were 0.77 (SEN, 95% CI: 0.71 to 0.82); 0.98 (SPE, 95% CI: 0.98 to 0.99); 0.22 (-LR, 95% CI: 0.13 to 0.39); 50.38 (+ LR, 95% CI: 21.85 to 116.17); 292.72 (DOR, 95% CI: 95.02 to 901.75); and 0.9875 (the area under the curve of SROC).

**Conclusion:** Nuclear acid sequence-based amplification is a reliable technique to diagnose *M pneumoniae* infection. However, whether it can replace PCR and serology need to be further studied.

## KEYWORDS

meta-analysis, *Mycoplasma pneumoniae*, nuclear acid sequence-based amplification, rapid diagnosis

## 1 | INTRODUCTION

*Mycoplasma pneumoniae* is one of the primary pathogens resulting in both lower and upper respiratory infections in humans and all

age groups,<sup>1-5</sup> and is also the common respiratory system disease of pediatrics.<sup>6</sup> *M pneumoniae* infections are responsible for more than 30% of the community-acquired pneumonia (CAP) cases<sup>7-9</sup> and are also the main etiology of CAP in hospitalized patients, only

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ranking lower than *Streptococcus pneumoniae*.<sup>10,11</sup> The rates of upper respiratory infections differ across studies and may range by up to 50%.<sup>1</sup> Although *M pneumoniae* can lead to infections in any epidemiologic setting, it shows a particularly significant burden in closed community settings, where outbreaks arise. Numerous outbreaks were recorded since the 1960s in different settings, for example, hospitals,<sup>12,13</sup> institutions,<sup>14,15</sup> military bases,<sup>16,17</sup> and religious communities.<sup>18,19</sup> In many patients, the symptomatic infections are mild, steady, and continue for weeks. However, severe cases requiring hospitalization and even death may happen, particularly among aging or immunocompromised people. Up to 25% of *M pneumoniae* patients presented extrapulmonary manifestations including the central nervous system, mucous membrane, and cardiovascular system.<sup>20</sup> Some complications, for example, neurological manifestations, cause grave outcomes. Antibiotic treatment significantly moderates the symptoms and signs even before the thorough elimination of the bacteria. Early and rapid diagnosis of *M pneumoniae* infections has important clinical significance for the selection of correct antibiotics.

The clinical manifestation of patients with *M pneumoniae* infections shows no significant differences with other respiratory pathogen infections, such as *Chlamydia pneumoniae*; therefore, it is impossible to identify *M pneumoniae* infections only according to the clinical signs and symptoms, and laboratory tests for identifying *M pneumoniae* are especially important. However, there is lack of a definite standard test for diagnosis of *M pneumoniae*.<sup>21</sup>

*Mycoplasma pneumoniae* culture is time-consuming because it grows slowly (about 2 to 5 weeks for visible colonies appear).<sup>22</sup> Serological assays are the most widely used technique in the laboratory diagnosis of *M pneumoniae* infections; nevertheless, the sensitivity of serology depends on the time phase when the first serum sample is collected after the *M pneumoniae* infections and the availability of the paired serum samples collected with a 2-3-week interval, as well as the sensitivity during the acute infectious stage.<sup>23</sup> In addition, serological measurements of host immune responses did not directly measure organism load. Some infected patients have never found a detectable antibody response.<sup>24</sup> Conversely, *M pneumoniae* IgM positive is also seen in healthy children.<sup>25</sup> Thus, a nucleic acid amplification technique of was used,<sup>26</sup> which includes polymerase chain reaction (PCR) to amplify the specific DNA fragment and the nuclear acid sequence-based amplification (NASBA) using RNA templates to diagnose *M pneumoniae* infections. Compared with serology, PCR is rapid and sensitive with high specificity and is thus good for the early clinical diagnosis of *M pneumoniae* infection.<sup>27</sup> Some experts recommend PCR as the gold standard for *M pneumoniae* diagnosis.

Compared with PCR, no DNA digestion enzyme pretreatment was necessary for NASBA to amplify RNAs. Additionally, NASBA does not need an expensive nucleic acid amplification reaction device when performing constant temperature amplification. NASBA can be completed with a regular constant temperature water bath because the entire process is carried out at 42°C instead of the thermal cyclers and is thus very convenient.<sup>28</sup>

Meanwhile, only a few enzymatic cyclings are needed for NASBA to achieve the required target amount. For instance, to reach 10<sup>6</sup> amplification, PCR requires 20 cycles, while only 4-5 cycles are needed for NASBA. Moreover, the mismatching rate is low and the cycle is shorter for NASBA than RT-PCR.<sup>29</sup> Therefore, it is worth exploring the diagnostic value of NASBA for *M pneumoniae* infections. NASBA has been shown to have a good diagnostic value for *M pneumoniae* infections compared with PCR as the gold standard; however, the evidence is not sufficient due to the relatively independent studies and small sample size. The aim of the current study was to perform a systematic review and meta-analysis to evaluate the overall accuracy of NASBA to diagnose of *M pneumoniae* infections, as well as to provide powerful evidence for the possibility of using NASBA to diagnose *M pneumoniae* infections.

## 2 | MATERIAL AND METHODS

### 2.1 | Search strategy and selection criteria

Six electronic databases (PubMed, Cochrane Library, Google Scholar, CNKI, Wang Fang, and Baidu Scholar; three databases in English and three in Chinese) were comprehensively searched by two researchers independently from their initiation date to December 2017 with the following search terms: (i) NASBA OR Nucleic Acid Sequence-Based Amplification; (ii) *Mycoplasma pneumoniae* OR *M pneumoniae* OR *pneumonia mycoplasma*. For the included studies, citation and reference lists were screened.

### 2.2 | Inclusion criteria

Literature screening was conducted according to the inclusion criteria for the diagnostic test study from the Collaboration Screening and Diagnostic Test Method Group in the Cochrane Library.

**Inclusion criteria:** (i) studies in Chinese or English; (ii) patient samples; (iii) prospective or retrospective studies; (iv) sample size  $\geq 30$ ; (v) PCR used as the gold standard, and all the samples were detected with both PCR and NASBA; (vi) the data in the four grid tables could be directly obtained or indirectly calculated.

**Exclusion criteria:** (i) abstract, review, systematic review, or case report; (ii) duplicated publications; (iii) incomplete raw data; (iv) unavailable full text.

**Data extraction:** A predesigned data extraction form was used for data collection. Data were retrieved by two researchers independently according to the inclusion criteria and exclusion criteria from the reports. These data included (i) basic information of the included studies (including author, year of publication, region of the publication from); (ii) research design; (iii) data from the four grid table in the included studies, including true positive (TP), false positive (FP), false negative (FN), and true negative (TN); (iv) assessment of key points for risk of bias including nucleic acid extraction techniques and detection methods, etc Any disagreement needed the consensus of a third researcher.

**Quality assessment of included studies:** The methodological quality of the included studies was evaluated using the QUADAS-2 tool recommended by the Cochrane Collaboration, and the RevMan 5.2 statistical software was used to display the quality of the study.

### 2.3 | Statistical methods

The pooled analysis was performed using Meta-DiSc 1.4 software. The ROC topographical plan was drawn, and the Spearman correlation coefficient between the logarithm of the sensitivity and logarithm of the (1-specificity) was calculated to determine whether there was a threshold effect. There was threshold effect if  $P < 0.05$ . The random-effects model was used to calculate the pooled parameters including SEN and SPE, +LR, -LR, DOR and their 95% CI for the forest plots to assess the probability of the accurate identification of both *M pneumoniae*-infected and non-infected individuals by NASBA. The heterogeneity of the eligible studies was evaluated by the  $I^2$  test. The application of the effects model depends on the heterogeneity among the studies. If no significant heterogeneity was found ( $I^2 < 50\%$ ) in the included studies, a fixed-effects model was used to analyze the results, whereas a random-effects model was applied for the meta-analysis if significant heterogeneity ( $I^2 \geq 50\%$ ) existed in the eligible studies. The SROC curve was created, and the area under the curve (AUC) was estimated. A value closer to one of the AUC indicates a higher value of NASBA for the diagnosis of *M pneumoniae* infections. The heterogeneity was analyzed and the meta-regression analysis was performed using Meta-DiSc 1.4 software to explore the source of heterogeneity. Funnel plots were made using Stata 12.0 software, and linear regression models were used to verify the symmetry of the funnel plots to identify

publication bias.  $P < 0.05$  was considered to indicate publication bias.

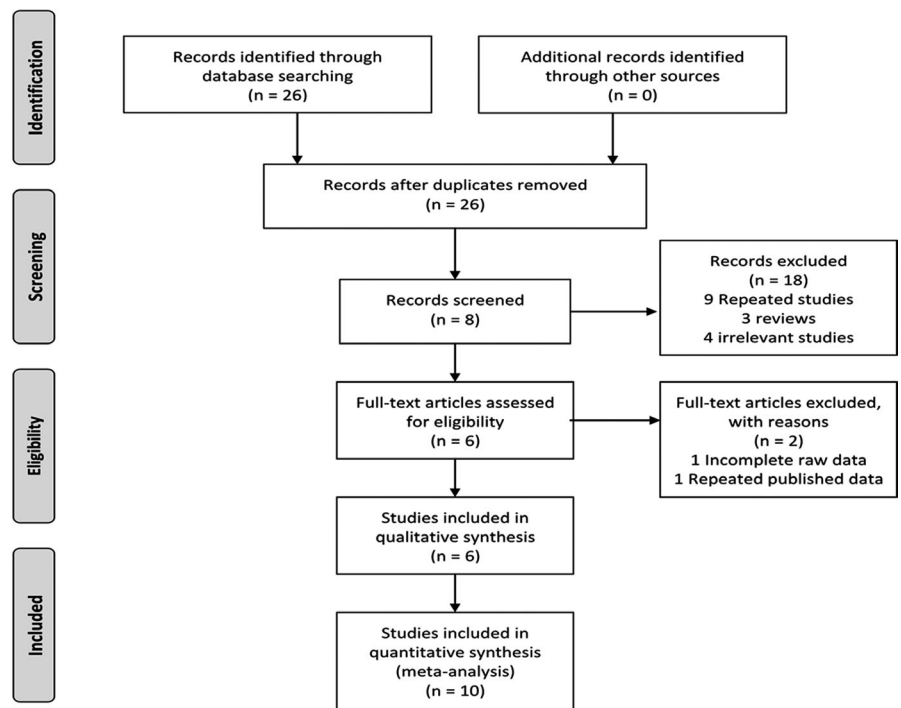
## 3 | RESULTS

### 3.1 | Literature identification and selection

The detailed process of the literature identification and selection is shown in Figure 1. A total of 26 publications were retrieved according to the search strategy described in the methods section (12 from PubMed, 0 from Cochrane Library, 5 from Google Scholar, 5 from CNKI, 2 from Wanfang, and 6 from Baidu Scholar), while nine duplicate studies, three reviews, four unrelated articles, and three articles without full text were excluded after reviewing the titles and abstracts. One article with incomplete original data and one article with data published repeatedly were further excluded after reading the full text. Finally, six articles<sup>30-35</sup> meeting the inclusion criteria were included in this meta-analysis.

### 3.2 | Characteristics and quality evaluation of the included studies

Ten groups of relatively independent data were included in the six included studies. The characteristics of the six included studies are shown in Table 1. The methodological quality of the articles was evaluated using the QUADAS-2 tool. As shown in Figure 2, our results showed that the quality of the six included studies was high and that therefore the included studies were representative. Four studies were from Belgium, one study was from the Netherlands and one from France. Four studies were prospective and two studies did not report the study design method.

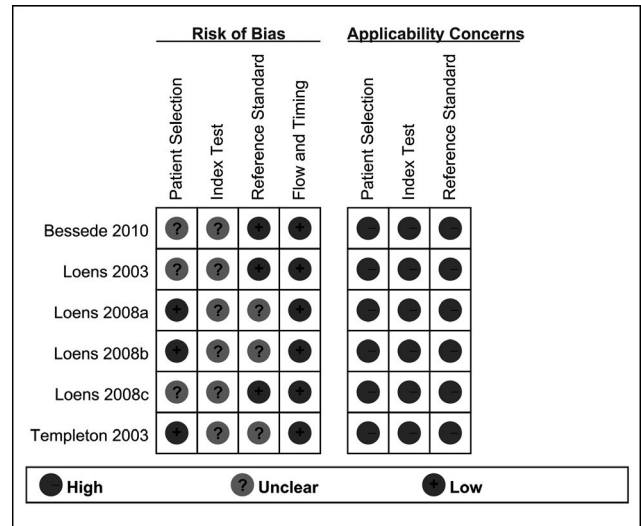


**FIGURE 1** Flow diagram of study identification and inclusion

**TABLE 1** Characteristics of the included studies

Author	Year	Region	Research methods	Instrument/ Reagent	Type of NASBA	Gold standard	TP	FP	FN	TN
Templeton <sup>9</sup>	2003	Netherlands	Prospective	NucliSens basic kit	RT-NASBA	PCR	12	0	0	94
Loens <sup>10</sup>	2008	Belgium	Prospective	Manual Boom	In-house RT-NASBA	PCR	4	1	6	204
Loens <sup>10</sup>	2008	Belgium	Prospective	NucliSens MiniMAG	EasyQ RT-NASBA	PCR	9	0	1	205
Loens <sup>10</sup>	2008	Belgium	Prospective	Manual Boom	EasyQ RT-NASBA	PCR	7	0	3	205
Loens <sup>11</sup>	2003	Belgium	NA	NucliSens basic kit	RT-NASBA	PCR	16	0	1	100
Loens <sup>11</sup>	2003	Belgium	NA	NucliSens basic kit	Conventional NASBA	PCR	17	0	0	100
Bessede <sup>14</sup>	2010	France	Retrospective	NucliSens EasyMAG	EasyQ RT-NASBA	PCR and/or culture	37	0	3	37
Loens <sup>12</sup>	2008	Belgium	Prospective	NucliSens basic kit	RT-NASBA	RT-PCR	21	13	3	214
Loens <sup>13</sup>	2008	Belgium	NA	NucliSens basic kit	RT-mono-NASBA	RT-PCR	25	7	15	153
Loens <sup>13</sup>	2008	Belgium	NA	NucliSens basic kit	RT-multiplex-NASBA	RT-PCR	21	3	19	157

NA: Not Applicable



**FIGURE 2** Quality evaluation of the included studies

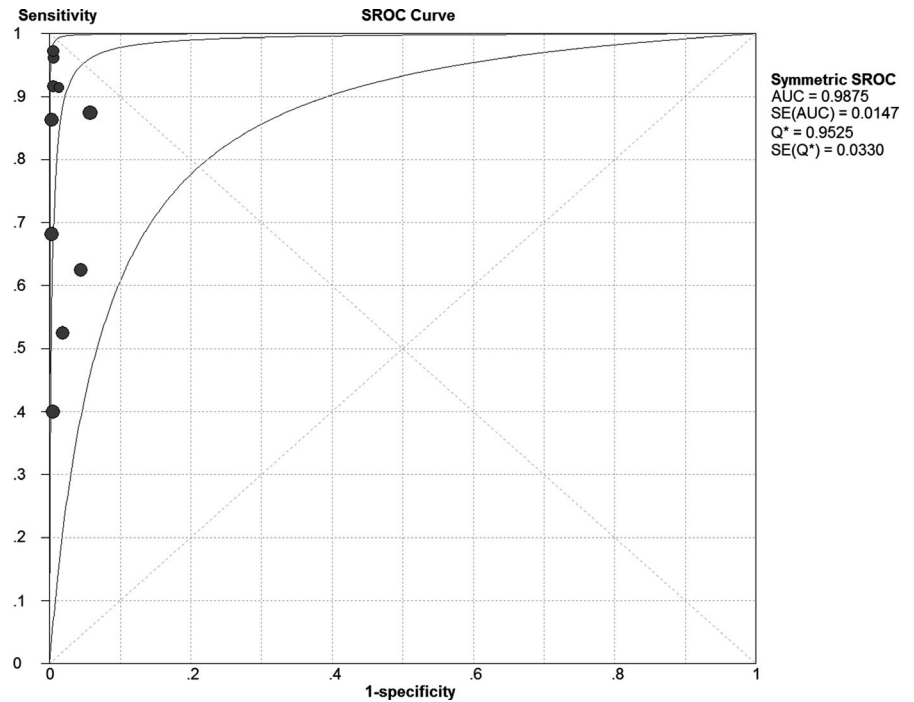
### 3.3 | Meta-analysis and overall diagnostic accuracy

Threshold effect analysis: The scatter plot of the ROC topographical plan made from Meta-Disc 1.4 software showed an atypical "shoulder-arm shape." The Spearman correlation coefficient is  $r = 0.012$  and  $P = 0.973$ , suggesting that Sen was negatively correlated with 1-Spe, with no existing threshold effect.

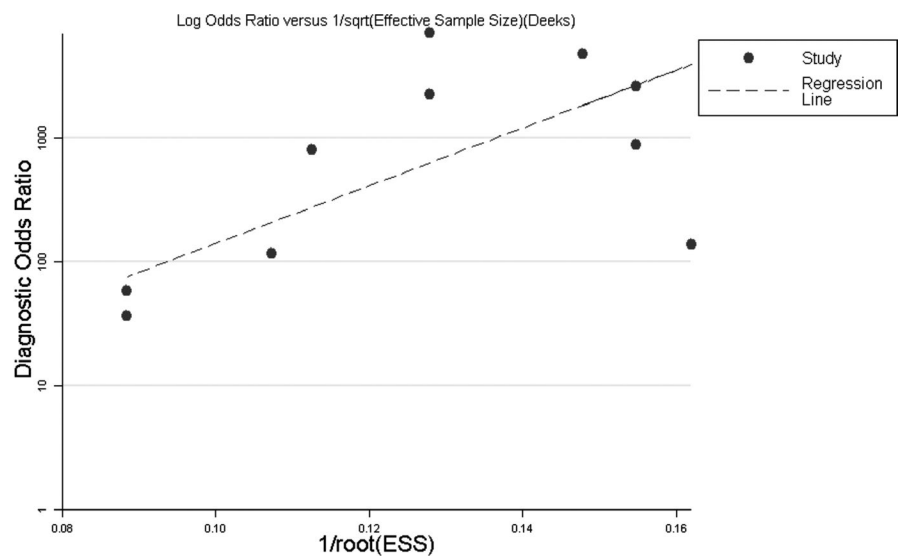
The pooled results: a meta-analysis was conducted for the pooled data extracted from the included studies using a random effects model. The results showed that SEN was 0.77 (95% CI: 0.71 to 0.82) (Figure S1); SPE was 0.98 (95% CI: 0.98 to 0.99) (Figure S2); +LR was 50.38 (95% CI: 21.85 to 116.17) (Figure S3); -LR was 0.22 (95% CI: 0.13 to 0.39) (Figure S4); DOR was 292.72 (95% CI: 95.02 to 901.75) (Figure S5); the area under the SROC curve (AUC) was 0.9875 and the  $Q^*$  index was 0.9525 (Figure 3). The results of the pooled results suggest that NASBA shows a higher accuracy in the diagnosis of *M pneumoniae* infections, in which the potential to identify individuals without infections is higher than that to identify individuals with infections.

Our results showed that heterogeneity existed in the sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio (the  $I^2$  was 82.3%, 80.6%, 62.8%, 79.5%, 62.4%, respectively). A meta-regression analysis performed using the Meta-DiSc 1.4 software for researchers, countries, nucleic acid extraction techniques, and detection methods showed that all  $P > 0.05$ , indicating that these factors are not able to explain the heterogeneity between the included studies. Other unknown factors led to the production of heterogeneity across the studies.

Publication bias assessment: Stale 12.0 software was used to make the Deeks' funnel plot to identify publication bias for the included studies, and the linear regression model was used to test the symmetry of the funnel plot. Our result showed that  $P = 0.016$ , indicating the existence of publication bias (Figure 4).



**FIGURE 3** Summary receiver operating characteristic curves of *Mycoplasma pneumoniae* infections detected by NASBA



**FIGURE 4** Deeks' funnel plot asymmetry test evaluating the publication bias based on the diagnostic odds ratio for NASBA detection of *Mycoplasma pneumoniae* infections

## 4 | DISCUSSION

A clear diagnosis of *M pneumoniae* infections as soon as possible is of great significance to guide medication, prevent complications, and control disease development. Traditional methods for detecting *M pneumoniae* infections include classical culture, serological tests, and nucleic acid detection.

However, *M pneumoniae* is difficult to culture with a low positive isolation rate and long duration; thus, *M pneumoniae* culture is not suitable for rapid clinical diagnosis.<sup>36</sup> Therefore, the diagnosis of *M pneumoniae* infections essentially relied on serology.<sup>37</sup> Unfortunately, the serological test has low sensitivity, poor

specificity, and cross-reactivity with other respiratory mycoplasmas or viruses.<sup>38-40</sup> Nucleic acid detection is rapid and sensitive with high specificity and is thus good for the rapid clinical diagnosis of *M pneumoniae* infection.

The nucleic acid detection methods that have been reported include traditional PCR, NASBA, LAMP, and others.<sup>29</sup> PCR technology has been used to detect *M pneumoniae* infections for approximately 20 years with quite a few limitations, such as the fact that the PCR inhibitors in samples may result in false-negative results; contamination can easily lead to false positives; it is relative difficult to obtain high quality samples; and the time point for sampling impacts results. The diagnostic accuracy of PCR has

been reported to decrease over 7 days after disease onset vs the serology.<sup>39-42</sup>

Nuclear acid sequence-based amplification has been reported to show a high sensitivity and specificity in the detection of *M pneumoniae* infection, as well as an even higher value than PCR in the detection of certain microbial infections, such as invasive fungal infections.<sup>43</sup> In addition, NASBA also has the advantage of no thermal cycling instruments required with high amplification efficiency,<sup>28</sup> which warrants further development and utilization. However, different diagnostic studies in this field have generated inconsistent diagnostic accuracy due to the small sample size, and the exact diagnostic accuracy of NASBA for *M pneumoniae* is difficult to establish. There are currently no systematic reviews or meta-analyses assessing NASBA for the detection of *M pneumoniae*; therefore, this systematic review and meta-analysis were conducted with the intention to provide more powerful evidence for the feasibility of NASBA in the diagnosis of *M pneumoniae* infections.

The results of our meta-analysis showed that the pooled specificity was 0.98 (95% CI: 0.98 to 0.99), suggesting that NASBA had a very low omission diagnosis rate for *M pneumoniae* infections. The pooled sensitivity was 0.77 (95% CI: 0.71 to 0.82), suggesting that NASBA may have a misdiagnosis rate for diagnosing *M pneumoniae* infections. The misdiagnosis rate may be related to the longer sample freezing and the degradation of RNA in the samples.<sup>44</sup> The area under the SROC curve is less than 0.5, which indicates that there is no diagnostic value for the tested technology. The area under the SROC curve and the Q\* index are closer to 1, indicating higher diagnostic values. Our results showed that the area under the curve of SROC (AUC) was 0.99 and Q\* index was 0.9525. +LR was 50.38 (95% CI: 21.85 to 116.17), suggesting that the positive results in *M pneumoniae*-infected individuals was 50.38-fold that of non-infected individuals; additionally, the -LR was 0.22 (95% CI: 0.13 to 0.39), suggesting a 0.22-fold greater chance of negative results in *M pneumoniae*-infected individuals than in non-infected individuals.

The DOR was 292.72 (95%CI: 95.02 to 901.75). A greater value led to a better distinguishing effect of the diagnostic tests when the DOR value is >1. Our results showed a higher value indicating a better distinguishing diagnostic effect of NASBA for *M pneumoniae* infection.

Based on the above comprehensive meta-analysis, it can be concluded that NASBA may be a reliable tool for diagnosing *M pneumoniae* infection.

The methodological qualities of the six articles included in this meta-analysis were assessed using the QUADAS-2 tool, which showed a low bias risk of the included case selection, index test, gold standard utility, good case-flow, good progression status, and high clinical applicability. Ten of the six included articles showed no threshold effect but did show heterogeneity. A meta-regression analysis was performed to explore the heterogeneous sources, and the results showed that researchers, countries, nucleic acid extraction techniques, and detection methods were not the main sources of heterogeneity, indicating that there are other unknown factors to produce heterogeneity across the included studies. Existence of the potential problem may somehow reduce

the stability of the research. Possible causes of clinical heterogeneity or methodological heterogeneity include: (i) the pathogenic conditions of the patients providing the samples is different; (ii) collection, transportation, and storage of samples are different; (iii) different experimental environments.

Deeks' funnel plots were made for the included studies. The linear regression model was used to test the symmetry of the funnel plots, and our results suggested the existence of publication bias, which may be due to some negative study results not yet having been published, which may impact our results.

There are some limitations to this study, such as: (i) unpublished data were not searched. There may be some negative results, that is, studies without statistical significance in the unpublished literature; this may result in more significant publication bias. (ii) The included studies were all from Europe, and the findings from other regions and countries are absent. (iii) The included documents are relatively old. The most recent article included in this meta-analysis was published in 2010. The development and innovation of NASBA technology in recent years has not been reflected in our research. The diagnostic value of NASBA will also increase as it is developed. (iv) The number of included studies is relatively small. (v) There are deficiencies in using PCR positive as a gold standard. For example, high sensitivity of PCR may lead to false positives.<sup>24,45</sup>

In conclusion, the present systematic review and meta-analysis suggest an important diagnostic value of NASBA for *M pneumoniae* infections based on the available evidence. The rapid clinical diagnosis of *M pneumoniae* infections using NASBA is feasible, and it can be used as an effective supplement for laboratory diagnosis of *M pneumoniae*. However, the above conclusion still needs to be further verified with a larger sample size and more involved areas.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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