Diagnostic accuracy of the LAMP assay for Neisseria meningitidis

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Abstract

Background

Neisseria meningitidis is a major cause of bacterial meningitis, and these infections are associated with a high mortality rate. Rapid and reliable diagnosis of bacterial meningitis is critical in clinical practice. However, this disease often occurs in economically depressed areas, so an inexpensive, easy to use, and accurate technology is needed. We performed a meta-analysis to assess the potential of the recently developed loop-mediated isothermal amplification (LAMP) assay for detection of meningococcus.

Methods

Pubmed, Embase, and Web of Science were searched to identify original studies that used the LAMP assay to detect meningococcus. After pooling of data, the sensitivity and specificity were calculated, a summary receiver operating characteristic (SROC) curve was determined, and the area under the SROC curve was computed to determine diagnostic accuracy. Publication bias was assessed using Deek's funnel plot. Results We examined 14 studies within 6 publications. The LAMP assay had high sensitivity (94%) and specificity (100%) in the detection of meningococcus in all studies. The area under the SROC curve (0.980) indicated high overall accuracy of the LAMP assay. There was no evidence of publication bias.

Conclusion

The LAMP assay has accuracy comparable to bacterial culture and PCR for detection of meningococcus, but is less expensive and easier to use. We suggest adoption of the LAMP assay for detect meningococcus, especially in economically depressed areas.

Background

*Neisseria meningitidis* is a Gram-negative bacterium that only infects humans, and is a significant cause of meningitis. Based on capsular polysaccharides, there are 13 serogroups of this species (A, B, C, D, E, H, I, K, L, W, X, Y, and Z)\textsuperscript{[1]} Serogroups A, B, C, Y, and W–135 are mainly responsible for human diseases\textsuperscript{[2]}, and serogroups A and C are mainly responsible for meningitis. The incidence of bacterial meningitis is greatest among infants under 1 year-old, followed by adolescents\textsuperscript{[3]}. The overall incidence of meningitis has declined in recent decades due to the increasing use of meningococcal vaccines\textsuperscript{[4]}, but the incidence of meningitis from bacterial strains not covered by vaccines has increased, and bacterial meningitis remains a significant public health problem. About 200,000 patients worldwide die from bacterial meningitis every year, and the mortality can be up to 60% in parts of sub-Saharan Africa and in poor and developing countries\textsuperscript{[5,6]}. Patients who receive treatment have a mortality rate of about 10%, but survivors often experience serious sequelae, including limb amputation, neurological deficits, and other serious disabilities\textsuperscript{[7]}. 
The clinical detection of *Neisseria meningitidis* in many countries currently uses PCR, ELISA, and bacterial culture\[^8\], and PCR is considered the most authoritative standard. However, these technologies have limited applicability in poor and developing countries. For example, PCR and ELISA can be time-consuming, difficult to perform, and relatively expensive\[^9\]. Identification by bacterial culture can take many days, has low sensitivity, and has a reduced detection rate for patients who were pretreated with antibiotics\[^5\].

Loop-mediated isothermal amplification (LAMP) is a relatively new nucleic acid amplification technology that can simultaneously detect six meningococcal groups of *N. meningitidis* (A, B, C, W, X, and Y). It is simple to perform, rapid, has high sensitivity, and is inexpensive\[^10\], making it especially suitable for developing countries and regions with limited resources. No previous studies have comprehensively analyzed the sensitivity and specificity of the LAMP assay for detection of *Neisseria meningitidis*. The present meta-analysis evaluated the diagnostic accuracy and feasibility of using LAMP to identify meningitis due to *Neisseria meningitidis*.

**Methods**

**Search strategy and study selection**

The phrases “Neisseria meningitidis”, “Meningococcus”, “LAMP” and “Loop-mediated isothermal amplification” were used in combination to systemically search the literature from January 1997 to February 7, 2019 in 5 databases (Pubmed, Embase, Medline, Web of Science, and Cochrane Library) for identification of original studies that used LAMP to detect *Neisseria meningitidis*. The bibliographies of all publications were also reviewed to identify additional studies.

All literature titles and abstracts were independently screened, and five researchers (including Fan Shujin and Tan Hongkun) read the full text of each article that had extractable data to determine its eligibility for inclusion. Differences regarding eligibility were resolved through negotiation. Each included study used LAMP for detection of *Neisseria meningitidis* and a gold standard test (PCR or culture); provided data that were extractable and included true positivity (TP), false positivity (FP), false negativity (FN), and true negativity (TN) of the LAMP assay; and was original research written in English or Chinese. Studies that were animal experiments, from conferences or literature reviews, or did not have extractable data were excluded.

**Data extraction and quality assessment**

Five researchers independently extracted the data from each included study, including author, publication year, sample size, sample type, gold standard test results, TP, FP, FN, and TN. Regarding patient selection, index test, reference standard, flow and timing were used to evaluate the diagnostic accuracy of the LAMP assay.
Statistical analysis

MetaDisc version 1.4.0.0 was used to calculate sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR). A summary receiver operating characteristic (SROC) curve was plotted for calculations of sensitivity and specificity. A random effects model was used to summarize all data. Deek’s funnel plot was drawn using STATA, and a bivariate box plot and a post-test probability plot were used to detect literature bias. Review Manager version 5.2 was used for plotting study quality evaluation.

Results

Search results and study characteristics

Our initial literature search identified 83 publications and no additional (gray) literature (Figure S1). After application of the inclusion and exclusion criteria, we identified 6 publications for inclusion in the meta-analysis, all of which were published between 2011 and 2018 (Table 1). These studies were performed in Ireland, the United Kingdom, South Korea, and Japan. Because some of these publications used multiple types of sampling, we categorized these 6 publications as 14 studies with 3185 samples.

Diagnostic accuracy of LAMP assay

We used a random effects model to determine the diagnostic accuracy of the LAMP assay. Overall, the sensitivity was 0.94 (95% CI = 0.89 to 0.98, $I^2 = 24.7\%$; Figure 1), the specificity was 1.00 (95% CI = 0.99 to 1.00, $I^2 = 58\%$; Figure 2), the PLR was 69.45 (95% CI = 30.65 to 157.37, $I^2 = 66.8\%$; Figure 3), the NLR was 0.13 (95% CI = 0.08 to 0.21, and $I^2 = 0.0\%$; Figure 4), the DOR was 929.06 (95% CI = 339.22 to 2544.53, $I^2 = 18.9\%$; Figure 4), and SROC analysis indicated the AUC was 0.9805 (Figure 6).

Quality Evaluation

We assessed the quality of the studies by analysis of patient selection, the index test, the reference standard, and study flow and timing (Figures 7 and 8). The results indicated that patient selection had a high risk of bias in 3 studies, an unclear risk of bias in 3 studies, and a low risk of bias in 8 studies. Analysis of patient flow and timing indicated that only 1 study had a high risk of bias and 2 studies had an unclear risk of bias. Analysis of the index test indicated an unclear risk of bias in 1 study, and low risk of bias in the other 13 studies. Analysis of reference standards indicated an uncertain risk of bias in 4 studies and low risk of bias in 9 studies. Analysis of applicability concerns indicated low concerns in all three categories.

Publication bias
Deek’s funnel plot (Figure 9) showed that publication bias was not significant ($P = 0.07$). A bivariate box plot of sensitivity and specificity showed that there was no significant heterogeneity among the included studies (Figure 10). The post-test probability nomogram showed that for samples with a predicted probability of 50%, the post-test probability of a positive result was 100%, and the post-test probability of a negative result was 1% (Figure 11).

**Discussion**

Bacterial meningitis caused by *Neisseria meningitidis* has high global incidence and mortality rates, and is an especially severe problem in undeveloped regions. At present, routine laboratory diagnosis of bacterial meningitis employs bacterial culture and Gram staining, but this method is limited because it has a low sensitivity and is very time-consuming\[11,12\]. PCR is a widely accepted laboratory test for diagnosis of bacterial meningitis, and many studies have confirmed its high sensitivity and specificity. Unfortunately, the current PCR applications used to detect bacterial meningitis are too complicated and expensive for clinicians in undeveloped regions\[12,13\]. Thus, existing PCR tests are difficult to apply in areas with the highest incidences of bacterial meningitis. A rapid, low-cost, easy to use, and highly sensitive technique is needed for these at-risk populations.

LAMP is a simple technique developed in recent years that targets 4 specific primers in 6 regions of the target gene and does not require a thermocycler. Under the action of a strand displacement DNA polymerase (Bst DNA polymerase), genes are amplified at a constant temperature of 60 to 65°C, and amplification can be $10^9$-fold to $10^{10}$-fold in 15 to 60 min. Its main advantages are that it is simple, fast, and inexpensive\[14,15\].

We performed a meta-analysis of 14 independent studies reported in 6 publications. These studies were performed in diverse geographic regions and used a variety of different samples. Thus, the study of McKenna et al.\[16\] was from Ireland and had 139 throat swab samples, 141 plasma samples, 72 blood samples, 22 cerebrospinal fluid samples, 16 respiratory secretion samples, and 4 stool samples. The study of Bourke et al.\[17\] was from the United Kingdom and had 141 nasopharyngeal swab samples and 144 blood samples. The study of Lee et al.\[18\] was from South Korea and examined 31 cerebrospinal fluid samples. The study of Lee et al.\[19\] was also from South Korea and examined 1574 cerebrospinal fluid samples (568 from Vietnam, 536 from China, and 470 from South Korea). The study of Takahashi et al.\[20\] was from Japan and examined 836 throat swab samples. The study of Higgins et al.\[21\] was from Ireland and examined 65 blood samples. The study of Lee et al.\[18\] used culturing as the reference standard, and all the other studies used PCR as the reference standard. Our meta-analysis results indicated that LAMP had an overall sensitivity of 94% (95% CI = 0.89 to 0.98) and an overall specificity of 100% (95% CI = 0.99 to 1.00) for the detection of *N. meningitidis*, and that the accuracy was high regardless of the sample type and geographic origin of the patients. Therefore, LAMP is highly suitable for the rapid, accurate, and inexpensive clinical detection of *N. meningitidis* in economically impoverished
areas. Further prospective studies are needed to verify the safety and most suitable samples for the clinical use of LAMP.

In conclusion, our research showed that the LAMP assay had high sensitivity and specificity in detecting bacterial meningitis due to *N. meningitidis*. This assay thus provides reliable information for clinical laboratory tests, and is suitable for detecting bacterial meningitis. However, further research is needed to verify the clinical feasibility and accuracy of this test in the impoverished areas where it is most needed.

**Declarations**

**Abbreviations**

LAMP: loop-mediated isothermal amplification; SROC: summary receiver operating characteristic; PCR: Polymerase Chain Reaction; ELISA: Enzyme Linked Immuno Sorbent Assay; TP: true positivity; FP: false positivity; FN: false negativity; TN: true negativity; PLR: positive likelihood ratio; NLR: negative likelihood ratio; DOR: diagnostic odds ratio; SROC: summary receiver operating characteristic

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**Author contributions**

FSJ, THK, XYC, CYZ, XTA, PZY, YSO, LQ, LXY and LZX participated in the design of the project, formulation of the search strategy, and determination of inclusion of exclusion criteria. All authors participated in the literature search, data extraction and processing, and quality evaluation. FSJ created the figures and table. FSJ and THK wrote the manuscript. All researchers read and approved the final version of the manuscript.

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**Table**

Due to technical limitations, Table 1 is only available for download from the Supplementary Files section.

**Figures**
Figure 1

Sensitivity of the included studies.
Figure 2

Specificity of the included studies.
Figure 3

Positive LR of the included studies.
Figure 4

Negative LR of the included studies.
Figure 5

Diagnostic OR of the included studies.
Figure 6

SROC curve of LAMP for diagnosis of N. meningitidis.

Figure 7
Overall quality assessment of the included studies.

**Figure 8**

Quality assessment of the individual studies.
Figure 9

Deek's funnel plot asymmetry test.
Figure 10

Bivariate boxplot of the relationship of sensitivity and specificity.
Figure 11

Fagan nomogram of disease probabilities based on Bayes' theorem.

Supplementary Files

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