Original Article

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Performance of reversed transcription loop-mediated isothermal amplification technique detecting EV71: A systematic review with meta-analysis

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Summary **Human enterovirus 71 (EV71) is the major etiological agent of hand, foot and mouth disease (HFMD), which is a common infectious disease in young children. Studies in the past have shown that reversed transcription loop-mediated isothermal amplification (RT-LAMP) was a rapid approach for the detection of EV71 in HFMD. This meta-analysis study is to evaluate the diagnostic role of RT-LAMP in detecting EV71 infection. A comprehensive literature research of PubMed, Embase, Wan Fang Data, and Chinese National Knowledge Infrastructure databases was conducted on articles aiming at the diagnostic performance of RT-LAMP in EV71 detection published before February 10, 2014. Data from selected studies were pooled to yield the summary sensitivity, specificity, positive and negative likelihood ratio (PLR, NLR), diagnostic odds ratio (DOR), and receiver operating characteristic (SROC) curve by using STATA VERSION 12.0 software. Ten studies including a total of 907 clinical samples were of high quality in this meta-analysis. Overall, the pooled sensitivity, specificity, PLR, NLR, DOR, and the area under the SROC curve was 0.99 (0.97, 1.00), 0.97 (0.94, 1.00), 5.90 (95% CI: 3.90-8.94), 0.20 (95% CI: 0.14-0.29), and 1.00 (95% CI: 0.99-1.00), respectively. The univariate analysis of potential variables showed some changes in the diagnostic performance, but none of the differences reached statistical significance. Despite inter-study variability, the test performance of RT-LAMP was consistent with real-time RT-PCR in detecting EV71. This meta-analysis suggests that RT-LAMP is a useful diagnostic tool with high sensitivity and specificity for detecting EV71.**

Keywords: Human enterovirus 71 (EV71), reversed transcription loop-mediated isothermal amplification (RT-LAMP), detection, meta-analysis

1. Introduction

Human enterovirus 71 (EV71) is the major etiological agent of hand, foot, and mouth disease (HFMD), which is a febrile exanthematous disease mostly prevalent in children younger than ten years old (*1,2*). In recent years, EV71 associated outbreaks have been reported worldwide and its infection has become serious threat to the health of infants and young children (*3-7*). EV71 infection can cause various clinical manifestations and has been associated with severe neurological and cardiopulmonary complications, such as aseptic meningitis, encephalitis, and poliomyelitis-like paralysis, resulting in higher mortality rates (*8-11*).

Traditional methods for EV71 detection primarily depend on virus culture and identification, and serodiagnosis, which are either time-consuming or have a high false positive rate. Recently, reverse transcription-PCR (RT-PCR) and real-time quantitative RT-PCR (qRT-PCR) assays have been developed to detect EV71 with high specificities and sensitivities (*12-14*). However, these methods require sophisticated instrumentations and expensive reagents, which lead a result that the methods are difficult to be applied in developing countries or in field situations (*15-17*). Therefore, a rapid, reliable, and cost-effective molecular

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test should be developed to be content with the growing demand.

Loop-mediated isothermal amplification (LAMP), a nucleic acid amplification method, was first established in 2000, which has been considered as a powerful nucleic acids amplification tool because of its simplicity, speed, specificity, and cost-effectiveness (*18*). Since then, this method has been used widely in rapid detection for viruses, such as dengue virus, West Nile virus, Japanese encephalitis virus, Ebola virus, H1N1 influenza virus, and so on (*19-23*). The detection of EV71 by RT-LAMP with RNA extraction was developed recently (*15-17, 24-30*), however, results from a single study did not have sufficient power to demonstrate the role of RT-LAMP in the detection of EV71 infection. Therefore, we performed systematical meta-analysis to provide a more comprehensive and reliable analysis of the diagnostic accuracy of RT-LAMP for the diagnosis of EV71 infection.

2. Materials and Methods

2.1. *Study protocol*

This analysis was conducted with a predetermined protocol following the recommendations of Deeks *et al*. (*31*). Data was collected and reported according the Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement (Table S1, *http://www.biosciencetrends.com/docindex.php?year=2014&kanno=2*) (*32*).

2.2. *Search strategy*

Relevant studies published before February 10, 2014 were identified from searching PubMed, Embase, Wan Fang Data, and Chinese National Knowledge Infrastructure databases using the following terms: ("enterovirus 71" OR "EV71" AND ("detection" OR ("Reverse transcription loop-mediated isothermal amplification method" OR "RT-LAMP" OR "LAMP"). Languages were not restricted.

2.3. *Inclusion and exclusion criteria*

Studies included in this meta-analysis had to meet the following criteria: (1) Studies detecting the nasopharyngeal swab, stool, throat swabs, and rectal swabs specimens were included; (2) Patient samples were detected using qRT-PCR method, RT-PCR or virus isolation; (3) The literature data should contain sensitivity and specificity and detection limit; (4) For the studies using the same or overlapping data by the same authors, the most recent or largest population were selected. Exclusion criteria: (1) unqualified data; (2) small scale studies with fewer than 30 patients; (3) works designated as conference abstract, letters, case reports, editorials or reviews; (4) duplicated publications.

2.4. *Data extraction and quality assessment*

All selected manuscripts were reviewed by two authors (L.Z and HL.W), independently. Any disagreement was resolved by a third person (XJ.Y). The following information was extracted from every eligible work: the first author, year of publication, country, control method, number of patients, detection limit, the number of true positive (TP), false positive (FP), false negative (FN), and true negative (TN). The qualities of all the manuscripts were assessed according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) (Table S2, *http://www.biosciencetrends.com/docindex.php?year=2014&kanno=2*) (*33*). Fourteen items related to quality appraisal were used in this meta-analysis. Each of these items was scored "yes" (1 score), "no" (0 score) or "unclear" (-1 score). Disagreements were resolved by discussions and consensus.

2.5. *Statistical analysis*

For each selected publication, the pooled sensitivity, pooled specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR), and their 95% confidence intervals (CI) were calculated. A bivariate mixed model was adjusted to obtain a summary receiver operating characteristic (SROC) curve by summarizing the joint distribution of sensitivity and specificity with Moses linear model and the corresponding area under the curve (AUC) was calculated as a global measurement of test performance (*34,35*). The closer the AUC was to 1, the better the test performance. Publication bias was assessed visually using a funnel plot and tested with Egger's tests with $p < 0.10$ being considered statistically significant (*36*). Empty cells were handled using a 0.5 continuity correction. Meta-regression was analyzed to identify possible sources of heterogeneity. Values of $p < 0.05$ and $I2 > 50\%$ were considered to be statistically significant. Subgroup analyses and sensitivity analysis were also performed where required. All analyses were performed with STATA 12.0 (Stata Corp LP, College Station, Texas, United States).

3. Results

3.1. *Literature search*

A total of 219 potential relevant manuscripts were retrieved after the primary search of the electronic databases for published work on the subject. One hundred and ninety five of these manuscripts were excluded after further review of the title and abstract for irrelevant topics, and an additional 10 manuscripts

were excluded for duplication of the reports. Then the remaining 14 manuscripts were undergoing full text review. Finally, 10 studies were included for further meta-analysis. The detailed process of this literature search is shown in Figure 1.

3.2. *Study quality assessment*

QUADAS list of questions were used to review the test quality of the included studies. Most of the studies satisfied a majority of the items on the list, and reports of intermediate results and withdraw cases were the most common missing items in the studies in this analysis (Table S2, *http://www.biosciencetrends.com/docindex.php?year=2014&kanno=2*). The Egger's test revealed no significant publication bias among the included reports ($p = 0.067$).

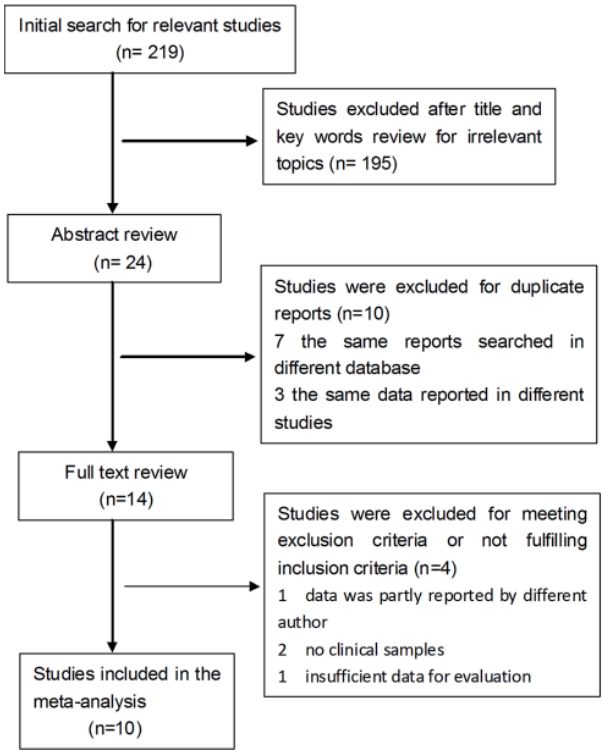

Figure 1. Flow chart describing the literature search conducted for this meta-analysis.

3.3. *Overall diagnostic performance of RT-LAMP*

As a result, 10 eligible studies with a total of 907 samples were included in this meta-analysis, which were published from 2011 to 2012. All the samples were also detected by qRT-PCR or RT-PCR, or even virus isolation for conformation, however, the units of the detection limit of RT-LAMP varied in different studies (Table 1). The basic characteristics (TP, FP, TN, and FN) were also listed in the table.

The forest plot of sensitivity, specificity, PLR, and NLR for RT-LAMP method in the detection of EV71 infection was shown in Figures 2 and 3. The pooled sensitivity and specificity were 0.99 (95% CI: 0.97-1.00) and 0.97 (95% CI: 0.94-1.00), respectively (Figures 2 and 3). By heterogeneity analysis, I2 of sensitivity, specificity, PLR, and NLR was 0.0% ($p = 0.739$), 47.5% ($p = 0.047$), 0.0% ($p = 0.994$), and 0.0% ($p = 0.998$), respectively, implicating that there was no significant heterogeneity among the samples. The pooled PLR was 5.90 (95% CI: 3.90-8.49); the pooled NLR was 0.20 (95% CI: 0.14-0.29).

The pooled DOR and the SROC curves based on summary sensitivity and specificity across all data sets were shown in Figure 4. The pooled DOR was 843.27 (95% CI: 294.19-2417.15), with individual DORs ranging from 312.00 to 41600. The results of DOR showed consistency across the included studies, without noticeable heterogeneity ($p = 0.602$, $I^2 = 0.0\%$). The point size in the SROC curve represented the proportional study weight. All the data gathered near the top left corner where both the sensitivity and specificity were the highest. The area under the curve (AUC) was 1.00 (95% CI: 0.99-1.00) indicating a high diagnostic accuracy.

In addition, we systematically removed one data set at a time, and recalculated the DOR and AUC values for the remaining studies. The changes of pooled DOR and

Table 1. Summary of the studies included in the meta-analysis

Reference number	Author	Publication year	Country	Conformation method	Samples	Detection limit	Test results				QUADAS
							TP	FP	FN	TN	
15	Shi *et al.*	2011	China	qRT-PCR	123	1PFU	56	2	0	65	
16	Nie *et al.*	2011	China	qRT-PCR	47	0.33$TCID_{50}$	25	0	0	22	
17	Nie *et al.*	2012	China	qRT-PCR	145	1.6 $TCID_{50}$	124	0	5	16	
24	Geng *et al.*	2011	China	qRT-PCR	58	10-5 dilution	25	8	0	25	
25	He *et al.*	2012	China	qRT-PCR	33	160 copies	24	0	0	9	
26	Jiang *et al.*	2011	China	qRT-PCR	40	0.01 PFU	26	0	2	12	
27	Li *et al.*	2012	China	qRT-PCR	41	100 copies	27	0	0	14	
28	Wang *et al.*	2012	China	RT-PCR	252	10 copies	52	0	0	200	
29	Xia *et al.*	2011	China	qRT-PCR	108	5 copies	101	0	3	4	
30	Zhao *et al.*	2011	China	RT-PCR	60	10 copies	31	4	0	26	

Abbreviations: qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; PFU, plaque forming unit; TCID50,50% tissue culture infective dose; TP, true positive; FP, false positive; FN, false negative; TN, true negative.

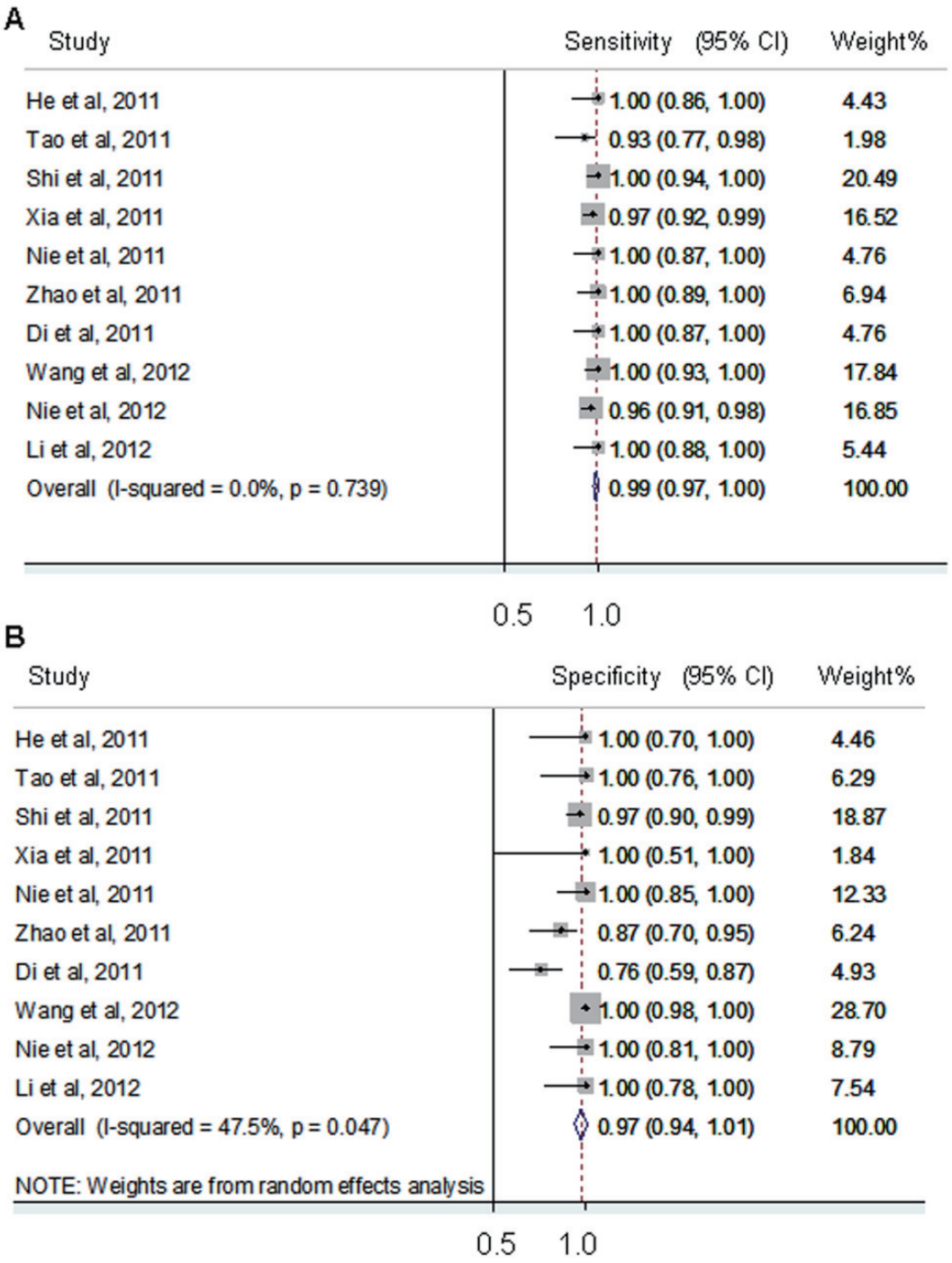

Figure 2. Forest plots of sensitivity and specificity of RT-LAMP for the detection of EV71 infection. (A) Pooled sensitivity. **(B)** Pooled specificity. Effect sizes were pooled by fixed-effect models. The point estimates from each study were shown as solid squares. The pooled estimates were shown as an empty diamond. Error bars represented 95%CIs.

the corresponding changes in AUC values did not show significant difference (data not shown). These results suggested that no single data set carry enough weight to significantly influence the pooled test performance reported for the ability of RT-LAMP in detection of EV71 infection.

3.4. *Univariate analysis*

For exploring the potential variables that may have influenced the results, the following variables were chosen for subgroup analysis: publication year, sample size and confirmation method. In the subgroup analysis of publication year, the specificity was 0.96 for 2011, and 1.0 for 2012, thus, there was significant heterogeneity of specificity between studies published in 2011 and that published in 2012 (p = 0.027). Differences in the sensitivity, the PLR and NLR in this subgroup and differences in other subgroups were not statistically significant (Table 2).

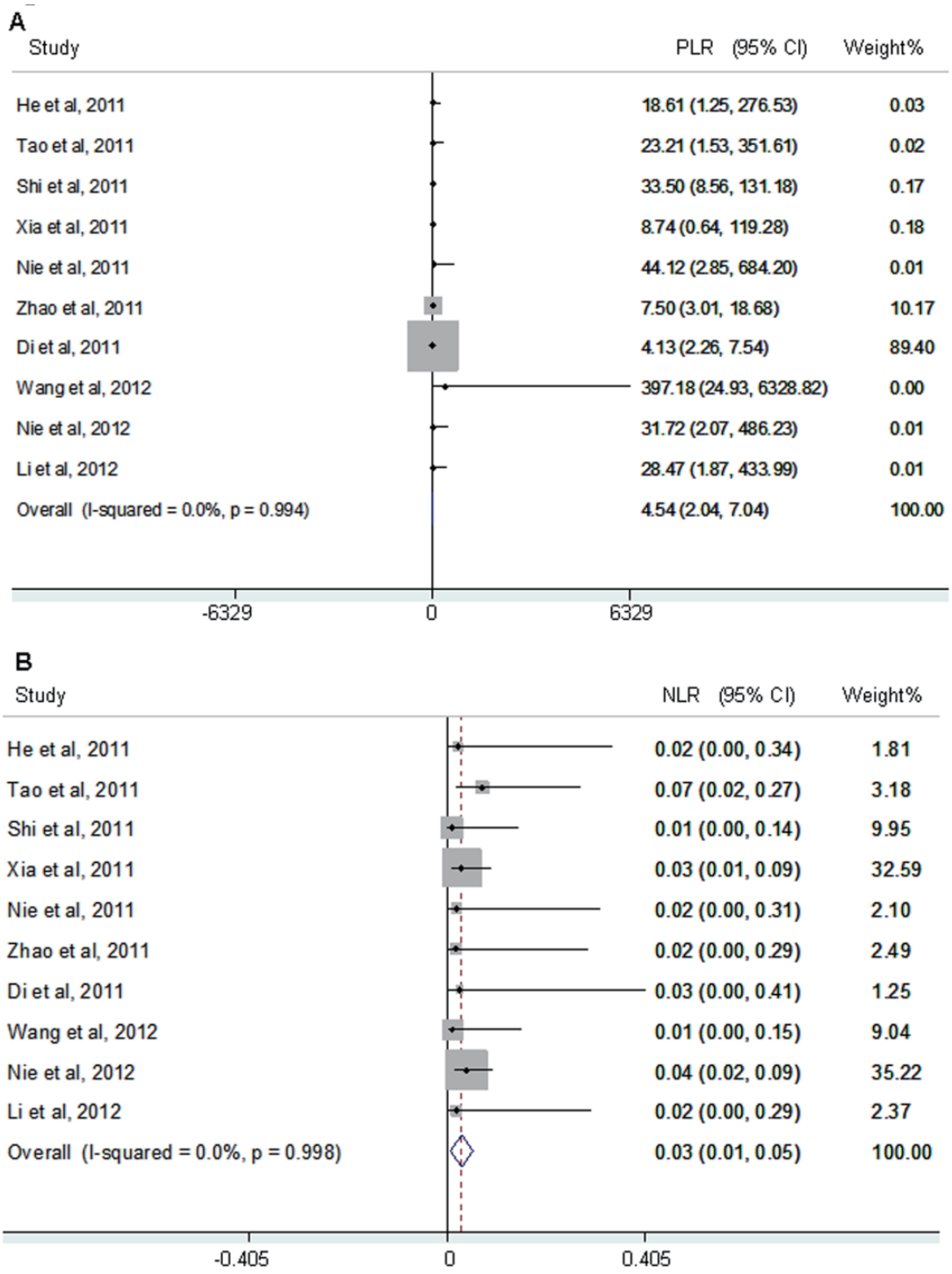

Figure 3. Forest plots of positive and negative likelihood ratios of RT-LAMP for the detection of EV71 infection. (A) Pooled positive likelihood ratio. **(B)** Pooled negative likelihood ratio. Effect sizes were pooled by fixed-effect models. The point estimates from each study were shown as solid squares. Error bars represented 95% CIs.

4. Discussion

A rapid expansion of HFMD has occurred in many provinces in China since 2008, especially in spring and summer every year. Although most of the HFMD cases without sever complications are mild and do not need much medical attention, cases infected by EV71 often lead to serious complications, or even death (*37-39*). Children around 3 years old are infected the most commonly and the cases infected by EV71 may cause severe neurological disease within 2-5 days, which led to an international demand for a rapid, simple, sensitive and accurate molecular method for the diagnosis of EV71 infection. qRT-PCR and RT-PCR have been proved to be reliable nucleic acid-based methods for the detection of EV71 in laboratories, however, both methods require specialized equipment and are not cost effective. RT-LAMP is a useful molecular technique for

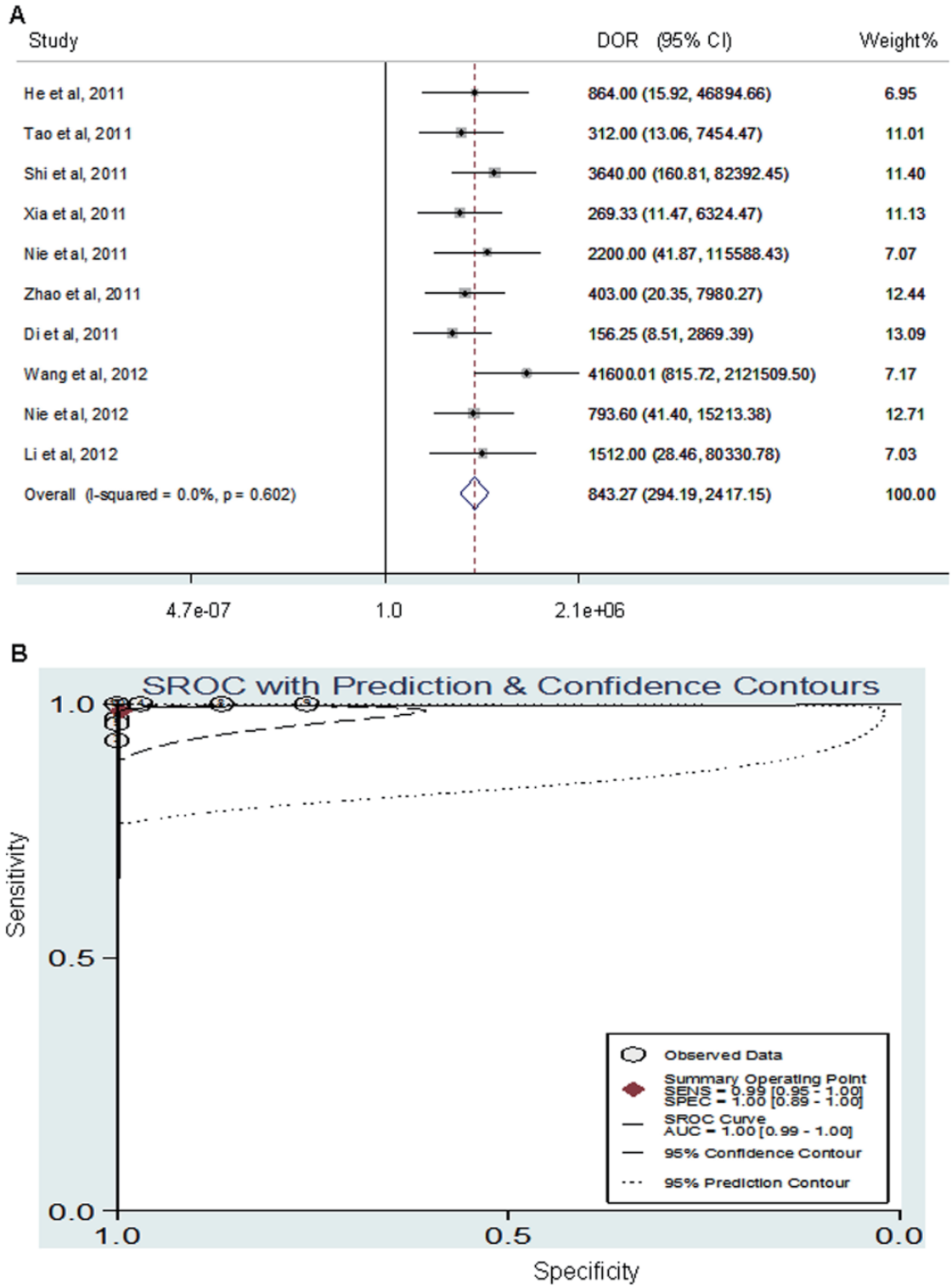

Figure 4. Overall DOR and SROC curve for all data sets describing the diagnostic performance of RT-LAMP in detecting EV71 infection. (A) Overall diamond. **(B)** The SROC curves for all data sets. Effect sizes were pooled by fixed-effect models. The pooled DOR is shown as an empty diamond. Each square in the SROC cure represents one study. Sample size was indicated by the size of the square.

nucleic acid research (*18*). To our knowledge, this is the first pooled estimation of the RT-LAMP method in detecting EV71 infection.

In this meta-analysis, 10 relevant studies with a total of 907 samples were included. Although results were not consistent across different studies, the overall diagnostic performance of detecting EV71 RNA with RT-LAMP showed pooled sensitivity and specificity of 0.99 (95% CI: 0.97, 1.00) and 0.97 (95% CI: 0.94, 1.00), respectively. The pooled DOR and AUC of the SROC curves for all data sets were 843.27 and 1.00, respectively. The results were consistent with previous

Table 2. Subgroup analysis of potential variables influencing the test performance of RT-LAMP

Variables	Subgroup	Number of studies	Sensitivity (95%CI)	P_{h1}	Specificity (95% CI)	P_{h2}	PLR(95%CI)	P_{h3}	NLR(95%CI)	P_{h4}
Publication year										
	2011	7	0.99 (0.97, 1.00)	0.692	0.96 (0.93, 0.99)	0.027	4.54 (2.04, 7.03)	0.749	0.03 (-0.004, 0.06)	0.803
	2012	3	0.98 (0.96, 1.00)		1.00 (0.99, 1.00)		30.87 (-130.11, 191.86)		0.03 (-0.001, 0.07)	
Sample size										
	< 100	6	1.00 (0.97, 1.00)	0.513	0.96 (0.91, 1.00)	0.076	4.48 (1.98, 7.00)	0.438	0.03 (-0.03, 0.10)	0.912
	≥ 100	4	0.98 (0.97, 1.00)		1.00 (0.99, 1.00)		21.11 (-20.88, 63.10)		0.03 (0.01, 0.05)	
PCR techniques										
	RT-PCR	2	1.00 (0.97, 1.00)	0.325	1.00 (0.99, 1.00)	0.14	7.50 (-0.33, 15.33)	0.434	0.01 (-0.06, 0.08)	0.582
	qRT-PCR	8	0.99 (0.97, 1.00)		0.97 (0.94, 1.00)		4.21 (1.57, 6.84)		0.03 (0.01, 0.06)	

Abbreviations: CI, confidence interval; P_{h1}, P value for heterogeneity between subgroups in sensitivity; P_{h2}, P value for heterogeneity between subgroups in specificity; PLR, positive likelihood ratio; P_{h3}, P value for heterogeneity between subgroups in PLR; NLR, negative likelihood ratio; P_{h4}, P value for heterogeneity between subgroups in NLR.

studies, representing that RT-LAMP method was highly efficient in EV71 detection, regardless of the sample origin variation. In addition, the virus strains of EV71 and the sequences of EV71 were not uniform, which could be potential source of variation that may have influenced the test performance.

Infections caused by EV71 and Human coxsackievirus A16 (CVA16) shared similar clinical symptoms, and indeed, the protein sequences of the two viruses were in high similarity, therefore, frequent mis-diagnosis of EV71 and CVA16 infections happened because of the difficulty in distinguishing the two viruses (*40*). Exact nucleotide sequences of RT-LAMP products can be derived from target DNA and primers, and restriction enzymes recognition sites were often introduced, thus, it is possible to predict the specific digestion outcomes of the samples (*15*). Therefore, RT-LAMP assay can provide high specificity for the detection of EV71 infection.

Heterogeneity between studies, which exists widely and could not be considered to be attributed to variances, is a potential problem in results interpretation for meta-analysis. As a result, pooled sensitivity is usually applied to assess whether the meta-analysis is influenced by any individual study. When the selected studies were removed one by one and the heterogeneity did not show significant change, the results of the analysis did not depend on one particular study absolutely. According the provided information, the univariate analysis was also carried out. Three subgroups were categorized and subgroup analysis of potential variables influencing the performance of RT-LAMP was performed. As most of the results did not show significant difference, it suggested that the conclusions are reliable.

There are several limitations in this meta-analysis. First, only 10 manuscripts met the inclusion criteria in this analysis. The sample size was so small that it limited the generalization of the results. Also, qualities of the selected manuscripts were not uniform. For example, the essential demographical data like age and gender distributions were not presented in most studies. These factors might be potential heterogeneity sources in the analysis. In addition, the detection limits were not uniformed in the same unit in primary study. As the experiments were done by different manipulators, and the EV71 virus strain used in the studies were also different from each other, the results were not comparable with each other.

In conclusion, RT-LAMP is a simple, rapid, cheap, specific and sensitive nucleic acid detecting method and has great value in diagnosis of EV71 infection for on-site application in early stage, which could contribute to control of EV71 infection.

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References

1. Bruu AL. Enteroviruses: polioviruses, coxsackieviruses, echoviruses and newer enteroviruses. In: A practical guide to clinical virology, second edition (Haaheim LR, Pattison JR, Whitley RJ, eds.). John Wiley & Sons, Ltd, Chichester, UK, 2003; pp. 44-45.
2. Zhu D, Zhao XY, Yao Y, Dai FF, He H, Li RQ, Jin RH, Liang LC, Li N. A new factor influencing pathogen detection by molecular assay in children with both mild and severe hand, foot, and mouth disease. Diagnostic microbiology and infectious disease. 2013; 76:162-167.
3. Kim KH. Enterovirus 71 infection: An experience in Korea 2009. Korean J Pediatr. 2010; 53:616-622.
4. Lan YC, Lin TH, Tsai JD, Yang YC, Peng CT, Shih MC, Lin YJ, Lin CW . Molecular epidemiology of the 2005 enterovirus 71 outbreak in central Taiwan. Scand J Infect Dis. 2011; 43:345-349.

5. Li Wei A, Benjamin KW K, Kwai Peng C, Chua LT, James L, Goh KT. Epidemiology and control of hand, foot and mouth disease in Singapore, 2001-2007. Ann Acad Med Singapore. 2009; 38:106-112.
6. Schuffenecker I, Mirand A, Antona D, Henquell C, Chomel JJ, Archimbaud C, Billaud G, Peigue-Lafeuille H, Lina B, Bailly JL. Epidemiology of human enterovirus 71 infections in France, 2000-2009. J ClinVirol. 2011; 50:50-56.
7. Wen HL, Si LY, Yuan XJ, Hao SB, Gao F, Chu FL, Sun CX, Wang ZY. Complete genome sequencing and analysis of six enterovirus 71 strains with different clinical phenotypes. Virol J. 2013; 10:115.
8. Chong CY, Chan KP, Shah VA, Ng WY, Lau G, Teo TE, Lai SH, Ling AE. Hand, foot and mouth disease in Singapore: a comparison of fatal and non-fatal cases. Acta Paediatr. 2003; 92:1163-1169.
9. Wang SM, Liu CC, Tseng HW, Wang JR, Huang CC, Chen YJ, Yang YJ, Lin SJ, Yeh TF. Clinical spectrum of enterovirus 71 Infection in children in Southern Taiwan, with an emphasis on neurological complications. Clin Infect Dis. 1999; 29:184-190.
10. Yang Y, Wang H, Gong E, Du J, Zhao X, McNutt MA, Wang S, Zhong Y, Gao Z, Zheng J. Neuropathology in 2 Cases of fatal enterovirus type 71 infection from a recent Epidemic in the People's Republic of China: A histopathologic, immunohistochemical, and reverse transcription polymerase chain reaction study. Hum Pathol. 2009; 40:1288-1295.
11. Weng KF, Chen LL, Huang PN, Shih SR. Neural pathogenesis of enterovirus 71 infection. Microbes Infect. 2010; 12:505-510.
12. Fujimoto T, Yoshida S, Munemura T, Taniguchi K, Shinohara M, Nishio O, Chikahira M, Okabe N. Detection and quantification of enterovirus 71 genome from cerebrospinal fluid of an encephalitis patient by PCR applications. Jpn J Infect Dis. 2008; 61:497-499.
13. Tan EL, Yong LLG, Quak SH, Yeo WCA, Chow VTK, Poh CL. Rapid detection of Enterovirus 71 by real-time TaqMan RT-PCR. J ClinVirol. 2008; 42:203-206.
14. Xiao XL, He YQ, Yu YG, Yang H, Chen G, Li HF, Zhang JW, Liu DM, Li XF, Yang XQ, Wu H. Simultaneous detection of human enterovirus 71 and coxsackievirus A16 in clinical specimens by multiplex real-time PCR with an internal amplification control. Arch Virol. 2009; 154:121-125.
15. Shi W, Li K, Ji Y, Jiang Q, Shi M, Mi Z. Development and evaluation of reverse transcription-loop-mediated isothermal amplification assay for rapid detection of enterovirus 71. BMC infectious diseases. 2011; 11:197.
16. Nie K, Zhang Y, Luo L, Yang MJ, Hu XM, Wang M, Zhu SL, Han F, Xu WB, Ma XJ. Visual detection of human enterovirus 71 subgenotype C4 and Coxsackievirus A16 by reverse transcription loop-mediated isothermal amplification with the hydroxynaphthol blue dye. J Virol Methods. 2011; 175:283-286.
17. Nie K, Qi SX, Zhang Y, Luo L, Xie Y, Yang MJ, Zhang Y, Li J, Shen H, Li Q, Ma XJ. Evaluation of a direct reverse transcription loop-mediated isothermal amplification method without RNA extraction for the detection of human enterovirus 71 subgenotype C4 in nasopharyngeal swab specimens. PloS one. 2012; 7:e52486.
18. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000; 28:E63.
19. Parida M, Posadas G, Inoue S, Hasebe F, Morita K. Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of West Nile virus. J Clin Microbiol. 2004; 42:257-263.
20. Parida M, Horioke K, Ishida H, Dash PK, Saxena P, Jana AM, Islam MA, Inoue S, Hosaka N, Morita K. Rapid detection and differentiation of dengue virus serotypes by a real-time reverse transcription-loop-mediated isothermal amplification assay. J Clin Microbiol. 2005; 43:2895-2903.
21. Parida MM, Santhosh SR, Dash PK, Tripathi NK, Saxena P,Ambuj S, Sahni AK, Lakshmana Rao PV, Morita K . Development and evaluation of reverse transcription-loop-mediated isothermal amplification assay for rapid and real-time detection of Japanese encephalitis virus. J Clin Microbiol. 2006; 44:4172-4178.
22. Kurosaki Y, Takada A, Ebihara H, Grolla A, Kamo N, Feldmann H, Kawaoka Y, Yasuda J. Rapid and simple detection of Ebola virus by reverse transcription-loop-mediated isothermal amplification. J Virol Methods. 2007; 141:78-83.
23. Kubo T, Agoh M, Mai LQ, Fukushima K, Nishimura H, Yamaguchi A, Hirano M, Yoshikawa A, Hasebe F, Kohno S, Morita K. Development of a reverse transcription-loop-mediated isothermal amplification assay for detection of pandemic (H1N1) 2009 virus as a novel molecular method for diagnosis of pandemic influenza in resource-limited settings. J Clin Microbiol. 2010; 48:728-735.
24. Geng YZ, Yao WQ, Guo JQ, Yu W, Mao LL, Chen JY, An CL. Establishment and application of RT-LAMP technique in detection of enterovirus 71 gene. Chinese journal of Zoonoses (with English abstract). 2011; 27:176-179.
25. He YQ, Zong WP, Zhiyi Y, Xionghu W, Yu SY, Hong Y, Yingchun D, Guifang H. Detection of human enterovirus 71 reverse transcription loop-mediated isothermal amplification (RT-LAMP). Lett Appl Microbiol. 2012; 54:233-239.
26. Jiang T, Liu J, Deng YQ, Xu LJ, Li XF, Han JF, Cao RY, Qin ED, Qin CF. Development and evaluation of a reverse transcription-loop-mediated isothermal amplification assay for rapid detection of enterovirus 71. J Clin Microbiol. 2011; 49:870-874.
27. Li K, Shi WF, Shi D, Luo GH, Shi M, Li X, Hou XL. Rapid detection of EV71 with reverse transcription loop-mediated isothermal amplication. J Mol Diagn Ther (Chinese with English abstract). 2012; 4:26-29.
28. Wang X, Zhu JP, Zhang Q, Xu ZG, Zhang F, Zhao ZH, Zheng WZ, Zheng LS. Detection of enterovirus 71 using reverse transcription loop-mediated isothermal amplification (RT-LAMP). J Virol Methods. 2012; 179:330-334.
29. Xia JF, Yan XF, Yu H, Qu D, Long JE. Simple and rapid detection of human enterovirus 71 by reverse-transcription and loop-mediated isothermal amplification: cryopreservation affected the detection ability. Diagn Microbiol Infect Dis. 2011; 71:244-251.
30. Zhao GP. Development and application of loop-mediated isothermal amplification methods for enterovirus 71 and coxsackievirus A16 detection. Dissertation, 2011, Hebei Medical University.
31. Deeks J. Systematic reviews of evaluations of diagnostic and screening tests. In: Systematic Reviews in Health

Care: Meta-analysis in context (Egger M, Davey Smith G, Altman D, eds.). BMJ Publishing Group, London, 2001.
32. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA Statement. PLoS Med. 2009; 6:e1000097.
33. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol. 2003; 3:25.
34. Moses LE, Shapiro D, Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: Data-analytic approaches and some additional considerations. Stat Med. 1993; 12:1293-1316.
35. Walter S. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. Stat Med. 2002; 21:1237-1256.
36. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997; 315:629-634.
37. Shimizu H, Utama A, Onnimala N, Li C, Li-Bi Z, Yu-Jie M, Pongsuwanna Y, Miyamura T. Molecular epidemiology of enterovirus 71 infection in the Western Pacific Region. Pediatr Int. 2004; 46:231-235.
38. Li L, He Y, Yang H, Zhu J, Xu X, Dong J, Zhu Y, Jin Q. Genetic characteristics of human enterovirus 71 and coxsackievirus A16 circulating from 1999 to 2004 in Shenzhen, People's Republic of China. J ClinMicrobiol. 2005; 43:3835-3839.
39. Li P, Zhang Y, Yue YY, Song NN, Li ZH, Meng H. Complete genome sequence analysis of enterovirus 71 JN200804 strain isolated in Shandong Province. Journal of Shandong University (Health Science) (Chinese with English Abstract). 2011; 49:113-117.
40. Oberste MS, Peñaranda S, Maher K, Pallansch MA. Complete genome sequences of all members of the species Human enterovirus A. J Gen Virol. 2004; 85:1597-1607.

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