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REVIEW

Efficacy of LAMP assay for Mycobacterial spp. detection to prevent treatment delays and onset of drug resistance: a systematic review and meta-analysis

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ABSTRACT

Background: Tuberculosis (TB) *re*mains a deadly disease affecting one-third population globally. Long turnaround time and poor sensitivity of the conventional diagnostics are the major impediments for faster diagnosis of *Mycobacterial spp* to prevent drug resistance. To overcome these issues, molecular diagnostics have been developed. They offer enhanced sensitivity but require sophisticated infrastructure, skilled manpower and remain expensive.
Methods: In that context, loop-mediated isothermal amplification (LAMP) assay, recommended by the WHO in 2016 for TB diagnosis, sounds as a promising alternative that facilitates visual read outs. Therefore, the aim of the present study is to conduct a meta-analysis to assess the diagnostic efficiency of LAMP for the detection of a panel of *Mycobacterium spp*. following PRISMA guidelines using scientific databases. From 1600 studies reported on the diagnosis of *Mycobacterium spp*., a selection of 30 articles were identified as eligible to meet the criteria of LAMP based diagnosis.
Results: It was found that most of the studies were conducted in high disease burden nations such as India, Thailand, and Japan with sputum as the most common specimen to be used for LAMP assay. Furthermore, *IS6110* gene and fluorescence-based detections ranked as the most used target and method respectively. The accuracy and precision rates mostly varied between 79.2% to 99.3% and 73.9% to 100%, respectively. Lastly, a quality assessment based on QUADAS-2 of bias and applicability was conducted.
Conclusion: LAMP technology could be considered as a feasible alternative to current diagnostics considering high burden for rapid testing in low resource regions.

Keywords: Diagnosis, LAMP, Meta-analysis, Mycobacteria, Therapeutics, Tuberculosis

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) remains a deadly disease affecting millions of people worldwide. It is estimated to affect approximately one-third of the global population and is becoming one of the most fatal infectious diseases. MTB usually attacks the lungs, but TB bacteria can infect any part of the body such as kidney, spine, or brain (1). Worldwide, TB is the 13th leading cause of death and the second raging infectious killer after human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (2). In 2020, an estimated 10 million people got ill with TB worldwide, the infection being divided as 5.6 million men, 3.3 million women, and 1.1 million children. TB affects most of the countries among all age groups and can be fatal if not treated properly. Moreover, the emergence of drug-resistant strains has further complicated the problem and has become a rising obstacle against efficient therapeutics (3). Therapeutics are available but the effective control of the disease is impeded due to the lack of rapid and accurate diagnostics. Under such significant circumstances, there is an urgent need for rapid, accurate, and cost-effective diagnostic test for TB to identify new cases and reduce the time-to-treatment and prevent its further transmission.

The current available methods are primarily based on smear microscopy (acid-fast staining), culture, and nucleic

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acid amplification. Although methods based on acid-fast staining are sensitive, they pose problems in low-resource places and are time-consuming (4). The solid culture method requires around 4-8 weeks, while liquid-based culture methods also require around 10-14 days (4). Nucleic acid amplification techniques are based on polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP). Although hemi-nested PCR based on GeneXpert for MTB detection is rapid, sensitive, and specific, it also poses challenges of high cost and high end equipment dependency, which limits its implementation in low-resource regions (5). LAMP is an isothermal DNA amplification method that relies on four or six pairs of primers to amplify minute quantities of DNA within a shorter period with simple operation, making it more suitable for low-resource regions (6). Thus, research in TB diagnostics aims to find an efficient, reproducible, cost-effective tool with minimal infrastructure requirements. LAMP is a popularly adopted new age technology for rapid nucleic acid amplification which is widely used for pathogen (virus, bacteria, and malaria) detection including severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) (7-9). LAMP-based detection methods have been proved to be more sensitive than GeneXpert assay. In fact, the World Health Organization (WHO) has endorsed LAMP for TB as a replacement for smear microscopy for peripheral settings (10).

In pursuit of developing better diagnostics, which are crucial for achieving global elimination of TB, we performed a systematic review and meta-analysis to access the diagnostic accuracy of LAMP to detect mycobacteria. Even if couple of studies have depicted the efficacy of LAMP during the last decade, an updated version is missing. Moreover, most of these studies were specific to either pulmonary or extrapulmonary TB. Therefore, the present study not only offers an up-to-date diagnostic performance of LAMP for TB detection but also covers other *Mycobacterium* spp. The pooled sensitivity and specificity of LAMP were analyzed against different references. Further, diagnostic efficiency was determined based on reference methods, target genes, and detection methods of LAMP. Taken together, we aimed to evaluate the diagnostic potency of LAMP as a tool for detection of mycobacteria to address the current TB diagnosis burden in low-resource places.

Methods

The Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines (11) were followed for identification of eligible studies in the present systematic review and meta-analysis.

Search strategy

Diverse scientific databases, for example, PubMed, Google Scholar, Science Direct, Scopus, BioRxiv, and MedRxiv, were searched to screen for studies performed using LAMP for TB diagnostics from the year 2000 till March 2022. The terms such as LAMP, Tuberculosis, *Mycobacterium* and mycobacteria were used in various combinations during our research without any limitations: "LAMP + Tuberculosis" or "LAMP + *Mycobacterium*" or "LAMP + mycobacteria" or "LAMP + TB" or "LAMP + Tuberculosis + *Mycobacterium*" or "LAMP + Tuberculosis + mycobacteria" for PubMed, Science Direct, and Google Scholar without using any language restriction. The retrieved results were screened for duplication and conformity with the prespecified eligibility criteria.

Study eligibility criteria

Inclusion criteria

This systematic review and meta-analysis included: (1) both peer-reviewed and preprint original articles on LAMP technology used for detection of any mycobacterial species such as MTB, *M. bovis*, and *M. africanum*; (2) only full-text articles written in English language; and (3) articles that contain data on true-positive (TP), false-positive (FP), false-negative (FN), and true-negative (TN) values for the assay or have sufficient data so that the number of TP, FP, FN, and TN (performed on clinical samples) could be determined.

Exclusion criteria

Exclusion was made for: (1) studies based on non-isothermal amplification; (2) studies where data are irretrievable; (3) review articles, editorials, commentaries, proceedings, etc.; (4) foreign language articles (other than English) based on LAMP-mediated detection of mycobacteria.

Data extraction

Potential articles after reviewing titles and abstracts followed by full text for inclusion were extracted by two authors (G.S.B. and Z.H.). Consultation from two independent authors (S.J. and S.H.) was made to eliminate the doubt about any discrepancy. The extracted information from included studies had authors, year of publication, location of study, sample size, types of specimens, target genes, detection method, and standard reference method. The data extracted for evaluation of diagnostic accuracy for LAMP were performed by using either respiratory or non-respiratory specimens with any of the reference methods such as smear microscopy, culture, and GeneXpert. The important parameters in this meta-analysis such as TP, TN, FP, and FN of all studies were either extracted or calculated to provide their sensitivity and specificity values. The included studies (n = 30) were then assessed for their methodological quality to reduce systematic biases and inferential errors from the collected data.

Statistical analysis

The quantitative analysis of the included studies (n = 30) from the data extracted such as the values of TP, FP, TN, FN and sample size was performed. Furthermore, the values of sensitivity and sensitivity were mined or calculated from the available data. Moreover, pooled sensitivity and specificity of LAMP associated with 95% confidence interval (CI) were estimated. To maintain the accuracy and precision, the formulas: Accuracy = [TP + TN/TP + TN + FP + FN]* 100 and Precision = [TP/TP+FP]* 100 (12,13) were used. Accuracy and precision are important characteristics of any measurement.

Accuracy is the degree of closeness of measured value to a standard value. However, precision provides the information regarding the closeness of multiple measured values to each other. Accuracy and precision are independent of each other. Forest plot for sensitivity and specificity were plotted using R-software along with summary receiver operating characteristic (SROC) for the given study.

Quality assessment

Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool was used to assess the methodological quality of the eligible studies. The risk of bias in the included studies (n = 30) was assessed from four areas of bias, for example, patient selection, index test, reference standards, and flow timing (14,15). For each QUADAS-2 domain specific yes/no questions were tailored. Following these criteria, the eligible studies were then refereed for low, unclear, or high risks of bias. Furthermore, we also judged to generate low, unclear, or high-risk applicability.

Results

Literature survey

We followed the PRISMA guidelines (11) to search the literature for the present study (Fig. 1). The major scientific databases viz. PubMed, Science Direct, Scopus, BioRxiv, and MedRxiv have been extensively searched applying the above inclusion criteria and around 1,600 articles were extracted. From the 1,600 articles, we included the ones that were published after the year 2000 since the inception of LAMP technology (6) and thus excluded 22 articles. Further, only articles written in English language were considered and thus excluded 44 articles. Reading the titles and abstracts of these studies allowed to exclude further 1,029 articles comprising the review articles, editorials, proceedings etc. Following this exclusion, we removed the duplicated articles and further excluded 390 articles. Additional 73 articles were irrelevant as they didn't use LAMP technology for the diagnosis of any mycobacterial species and were excluded, leaving a panel composed of 42 eligible studies. Lastly, from the 42 included articles, further 12 articles were also eliminated because their TP, FP, TN, and TN values were either not specified in these articles or the sensitivity and specificity values could not be calculated. Altogether, we observed that only 30 articles were eligible for detailed meta-analysis (Fig. 1) considering all the exclusion criteria.

Study characteristics and meta-analysis

Table I shows the data extracted from the eligible studies mentioning the details of authors, year of publication, country of study, types of specimens, target genes, detection method, and reference methods. Figure 2 shows the country-wise distribution of 30 identified articles included in the present study. Most of the studies (43.3%; n = 13) were conducted in the high TB burden nations such as India followed

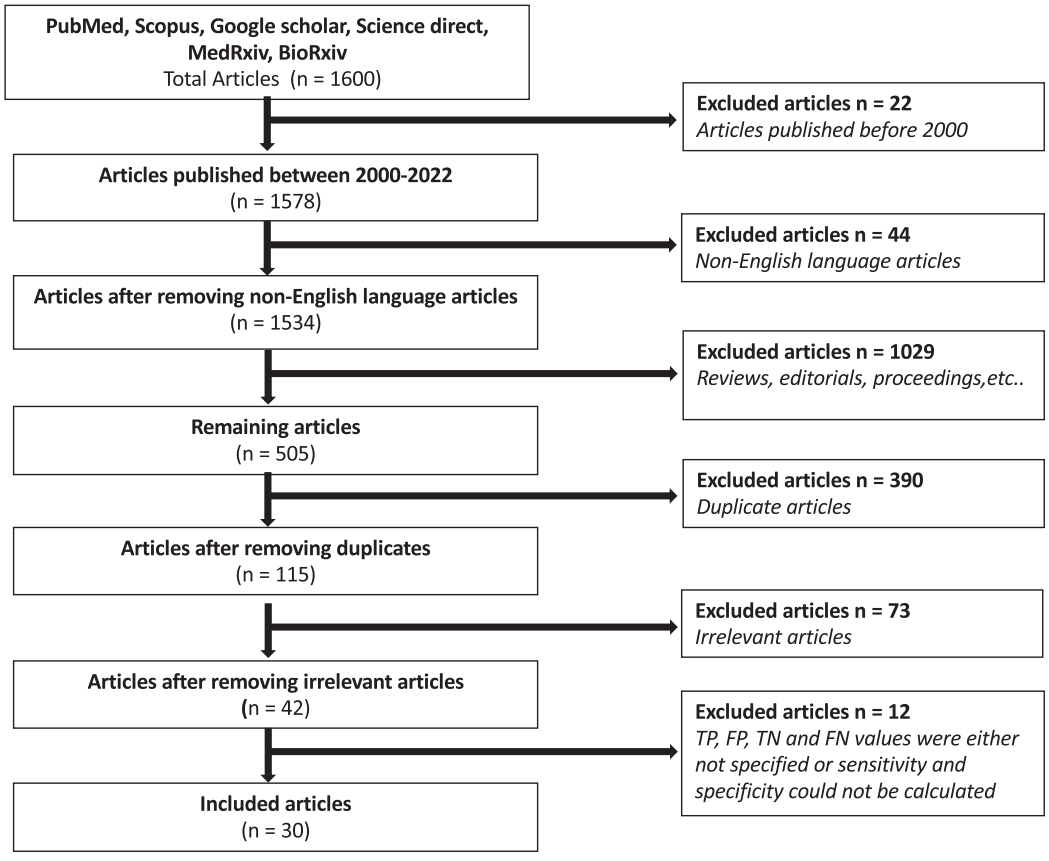

Fig. 1 - Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) flowchart depicts search of the literature and screening strategy for meta-analysis.

Table I - Characteristics and outcomes of the included studies (n = 30)

S. No.	Author	Journal	Country	Reference Method	Specimen	Target Gene	Detection Method	TP	TN	FP	FN	Size	Sensitivity	Specificity
1	Boehme et al (2007)	J Clin Microbiol	Switzerland	Culture, smear microscopy	Sputum	gyrB	Fluorescence, turbidity	173	500	4	5	682	97.70%	99%
2	Pandey et al (2008)	J Med Microbiol	Japan	Acid-fast staining, bacterial culture, radiology	Sputum	16S rRNA	Fluorescence	90	98	6	6	200	94%	94.20%
3	Poudel et al (2009)	Kathmandu Univ Med J (KUMJ)	Nepal	Smear microscopy, culture, radiology	Sputum	16S rRNA	Fluorescence	97	96	6	3	202	97%	94.12%
4	Geojith et al (2011)	J Microbiol Methods	India	Culture, PCR reverse-hybridization line probe assay, genotype MTBE assay	Sputum	rimM	Colorimetry, gel electrophoresis	17	17	1	21	56	44.70%	94.40%
5	George et al (2011)	PLoS One	India	Fluorescence smear microscopy, culture	Sputum	rimM	Colorimetry, gel electrophoresis	31	36	2	2	71	93.90%	94.70%
6	Mitarai et al (2011)	Int J Tuberc Lung Dis	Japan	Culture, smear microscopy, nucleic acid amplification (NAA)	Sputum	gyrB	Fluorescence, turbidity	196	88	9	27	320	87.90%	90.70%
7	Nagdev et al (2011)	J Clin Microbiol	India	PCR	Cerebrospinal fluid	IS6110	Turbidity	15	8	2	2	27	88.23%	80%
8	Sethi et al (2013)	J Clin Lab Anal	India	Smear microscopy, culture, PCR	Sputum	16S rRNA, IS6110	Colorimetry, gel electrophoresis	87	30	0	16	133	84.50%	100%
9	Cao et al (2015)	J Microbiol Methods	China	Smear microscopy, culture, PCR	Sputum	IS6110	Fluorescence	98	18	5	2	123	98.00%	78.30%
10	Joon et al (2015)	Int J Tuberc Lung Dis	India	Smear microscopy, culture, PCR	Endometrial fluid, urine, blood, semen, cerebrospinal fluid, pleural fluid, pus, pericardial fluid, peritoneal fluid, intestinal and lymph node biopsy tissue	IS6110, MPB64, sdaA	Colorimetry	28	262	23	2	315	93.30%	91.90%
11	Moon et al (2015)	J Med Microbiol	Korea	Culture, smear microscopy	Sputum	hspX	Colorimetry, turbidity, gel electrophoresis	32	255	3	13	303	71.10%	98.80%
12	Bojang et al (2016)	J Infect	Gambia	Smear microscopy, culture, GeneXpert MTB/RIF	Sputum	16S rRNA	Fluorescence	98	157	10	1	266	99.00%	94.00%
13	Gray et al (2016)	J Clin Microbiol	Switzerland	Culture, smear microscopy	Sputum	gyrB	Fluorescence	331	###	52	61	1777	84.40%	96.60%
14	Kaku et al (2016)	Jpn J Infect Dis	Japan	Smear microscopy, culture	Sputum	gyrB, IS6110	Fluorescence	134	312	5	21	472	86.50%	98.40%
15	Modi et al (2016)	Int J Tuberc Lung Dis	India	Culture, radiology, staining, PCR	Cerebrospinal fluid	IS6110, MPB64	Fluorescence, gel electrophoresis, turbidity	144	100	0	6	250	96.00%	100.00%

(*Continued*)

Table I - (*Continued*)

16	Sharma et al (2016)	Tuberculosis (Edinb)	India	PCR, culture, smear microscopy	Needle aspirate	IS6110, MPB64	Fluorescence, gel electrophoresis, turbidity	108	50	0	12	170	90.00%	100.00%
17	Sharma et al (2016)	J Orthop Res	India	Culture, staining, PCR	Synovial fluid, pus	IS6110, MPB64	Fluorescence, gel electrophoresis, turbidity	81	50	0	9	140	90.00%	100.00%
18	Joon et al (2017)	J Microbiol Methods	India	PCR, culture, smear microscopy	Sputum	IS6110, MPB64	Colorimetry, gel electrophoresis	17	212	6	1	236	94.40%	97.20%
19	Reddy et al (2017)	Int J Tuberc Lung Dis	South Africa	Culture, smear microscopy, Xpert	Sputum	gyrB	Fluorescence	119	514	17	45	695	72.60%	96.80%
20	Yadav et al (2017)	Int J Tuberc Lung Dis	India	Culture, smear microscopy, GeneXpert	Sputum	gyrB, IS6110	Fluorescence	82	368	3	0	453	100.00%	99.20%
21	Kim et al (2018)	Ann Lab Med	Korea	Culture, smear microscopy, PCR	Sputum	gyrB, IS6110	Fluorescence, turbidity	87	186	0	17	290	83.60%	100.00%
22	Nguyen et al (2018)	Diagn Microbiol Infect Dis	Vietnam	Smear microscopy, culture, Xpert MTB/RIF	Sputum	gyrB, IS6110	Colorimetry, fluorescence	15	445	23	18	501	45.50%	95.10%
23	Perera et al (2018)	Ceylon Med J	Sri Lanka	Smear microscopy, culture	Culture isolates	rimM	Colorimetry	31	10	5	0	46	100.00%	66.67%
24	Joon et al (2019)	J Microbiol Methods	India	Culture, smear microscopy, GeneXpert MTB/RIF assay, PCR, LAMP-LFD assay	Sputum	sdaA	Colorimetry	13	92	2	0	107	100.00%	97.87%
25	Phetsuksiri et al (2019)	Jpn J Infect Dis	Thailand	Culture, immuno-chromatographic test	Sputum	mpt64	Colorimetry	144	5	1	1	151	99.31%	83.33%
26	Punati et al (2019)	Braz J Microbiol	India	Culture, PCR	Fecal samples	IS900	Turbidity, gel electrophoresis, colorimetry, lateral flow device	86	294	9	0	389	100.00%	97.02%
27	Rajput et al (2019)	J Microbiol Methods	India	Culture, smear microscopy, PCR	Fluids, urine, pus	IS6110, Pab, MPB64	Colorimetry, fluorescence	90	32	9	23	154	79.65%	78.05%
28	Han et al (2020)	BMC Infect Dis	China	Xpert MTB/RIF, SAT-TB assay	Pleural fluids	gyrB, IS6110	Fluorescence	59	41	1	164	265	26.50%	97.60%
29	Phetsuksiri et al (2020)	Jpn J Infect Dis	Thailand	Microscopy, culture, PCR, radiology	Sputum	16S rRNA	Colorimetry, fluorescence, gel electrophoresis, immuno-chromatography	119	102	24	7	252	94.44%	80.95%
30	Phetsuksiri et al (2020)	Rev Inst Med Trop Sao Paulo	Thailand	Xpert MTB/RIF, culture, smear microscopy	Sputum	16S rRNA	Colorimetry, fluorescence, gel electrophoresis	126	71	0	7	204	94.74%	100.00%

LAMP = loop-mediated isothermal amplification; LFD = lateral flow dipstick; PCR = polymerase chain reaction.

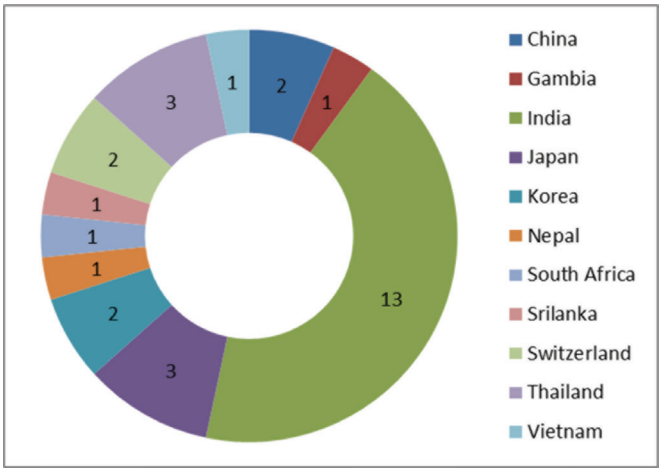

Fig. 2 - Country-wise distribution of included studies (n = 30) reported in the present investigation.

by Thailand and Japan (each 10%; n = 3). Two studies each were also conducted in countries such as China, Korea, and Switzerland (6.3%; n = 2). Apart from this, one study each, that is, 3.3%, was from countries included such as Gambia, Nepal, South Africa, Sri Lanka, and Vietnam. Although most of the included articles do not mention about the patient details, the type of specimen (Fig. 3) used in most of the studies was sputum (42.8%; n = 21). In addition, some studies have been tested on other specimens such as cerebrospinal fluid (n = 4), fecal samples (n = 1), urine (n = 3), blood (n = 2), and pleural fluid (n = 3) for the detection of mycobacteria by using LAMP. Furthermore, the standard smear microscopy, culture assay, and PCR-based methods were used as references either alone or in combination (n = 30). Of note, radiology was also used (n = 4) to validate LAMP results as a reference standard (Tab. I), with one study using immunochromatography (16). Next, we examined the various target genes used for the eligible studies. Ten different types of target genes including hspX, *IS900*, mpt64, Pab, sdaA, rimM, 16SrRNA, *MPB64*, gyrB, and *IS6110* were used in the included studies (n = 30). IS6110 gene was most frequently used in the included studies (n = 14; 31.18%) followed by gyrB (n = 9, 20.45%), 16SrRNA (n = 6, 13.63%), and *MPB64* (n = 6, 13.63%) genes (Fig. 4). Furthermore, while analyzing detection methods used for these 30 studies, fluorescent method (n = 19, 32.39%) was the most frequently performed followed by colorimetry (n = 14, 25.35%), gel electrophoresis (n = 11, 20.00%) and turbidity (n = 9, 16.36%) methods (Fig. 5). In 53.33% (n = 16) of studies, more than one detection method was used. In 16.66% (n = 5) of studies, combination of three methods was used while in only two studies (6.66%), combination of four different methods was reported (17,18).

Among all the eligible studies, 4 studies showed 100% sensitivity, while for 16 studies this parameter was higher than 90%. Similarly, 6 studies exhibited 100% specificity while 90% or more specificity was observed in 24 studies (Tab. I). Furthermore, upon analysis of sensitivity and specificity using forest plot at 95% CI, we found that the sensitivity values varied between 0.26 and 1.00 and the specificity values ranged from 0.67 to 1.00 (Fig. 6). A total of 27 out of the 30 included studies showed pooled sensitivity greater than 70%. Only three studies reported sensitivity values of 26% and 45% each (19-21). In terms of FP rate (1-specificity), 27 included studies showed a pooled FP rate higher than 80% (Fig. 7). Additionally, the accuracy and precision rates of included studies were calculated and varied between 37.73% and 99.33%. The analysis proved that 22 studies displayed more than 90% accuracy with only 4 studies depicting less than 80% accuracy (Tab. II). Likewise, the precision rates varied between 39.47% and 100%. The analysis showed that 21 studies exhibited more than 90% precision rate with only 3 studies depicting less than 80%. Of note, we observed that six studies displayed 100% precision rate.

Table II - Accuracy and precision of the included studies (n = 30)

S. No.	Study	Accuracy	Precision
1	Boehme et al (2007)	98.68	97.74
2	Pandey et al (2008)	94.00	93.75
3	Poudel et al (2009)	95.54	94.17
4	Geojith et al (2011)	60.71	94.44
5	George et al (2011)	94.36	93.93
6	Mitarai et al (2011)	88.75	95.60
7	Nagdev et al (2011)	85.18	88.23
8	Sethi et al (2013)	87.96	100.00
9	Cao et al (2015)	94.30	95.14
10	Joon et al (2015)	92.06	54.90
11	Moon et al (2015)	94.71	91.42
12	Bojang et al (2016)	95.86	90.74
13	Gray et al (2016)	93.64	86.42
14	Kaku et al (2016)	94.49	96.40
15	Modi et al (2016)	97.60	100.00
16	Sharma et al (2016)	92.94	100.00
17	Joon et al (2017)	97.03	73.91
18	Reddy et al (2017)	91.07	87.50
19	Sharma et al (2016)	93.57	100.00
20	Yadav et al (2017)	99.33	96.47
21	Kim et al (2018)	94.13	100.00
22	Nguyen et al (2018)	91.81	39.47
23	Perera et al (2018)	89.13	86.11
24	Joon et al (2019)	98.13	86.66
25	Phetsuksiri et al (2019)	98.67	99.31
26	Punati et al (2019)	97.68	90.52
27	Rajput et al (2019)	79.22	90.90
28	Han et al (2020)	37.73	98.33
29	Phetsuksiri et al (2020)	87.69	83.21
30	Phetsuksiri et al (2020)	96.56	100.00

Quality assessment of the study

Almost two-thirds of the included studies (22 out of 30 studies) have a high risk of patient selection bias due to

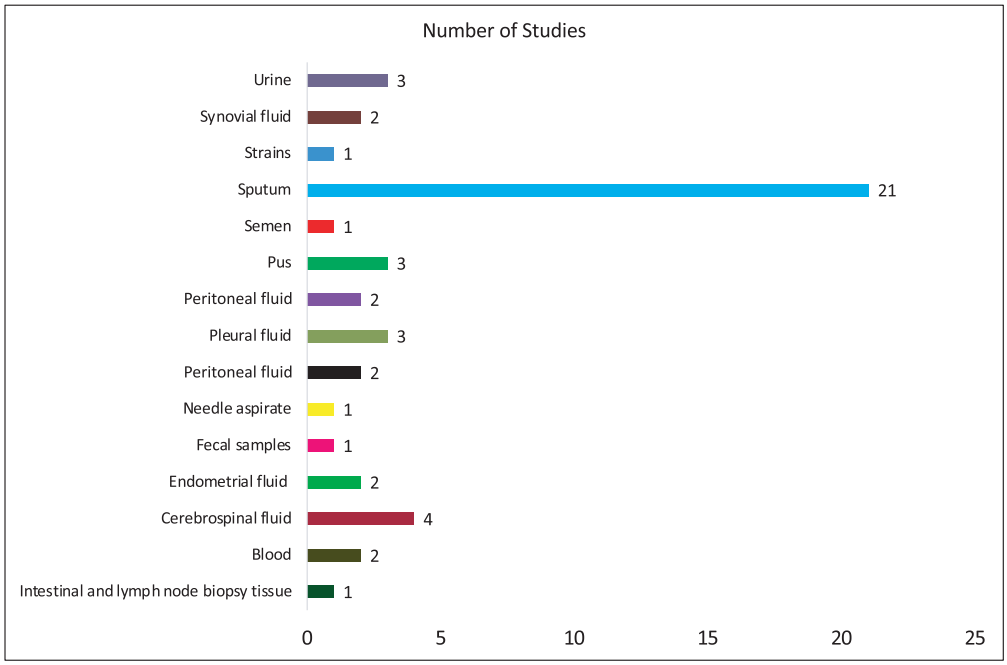

Fig. 3 - Distribution of type of specimen for detection of mycobacteria in the included studies (n = 30).

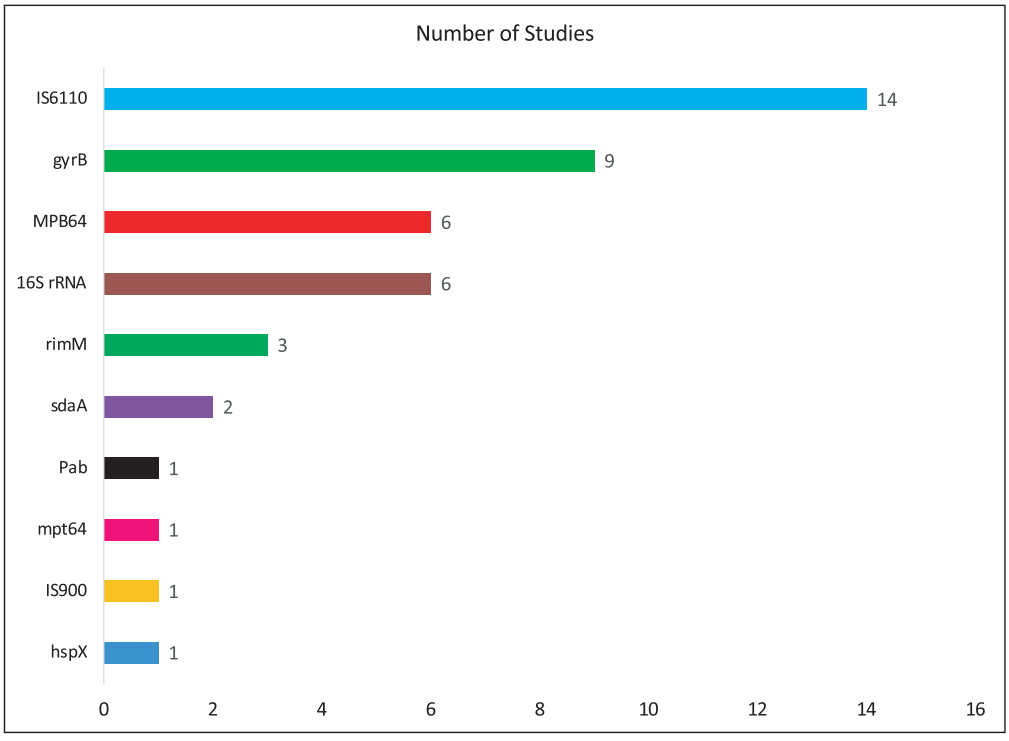

Fig. 4 - Distribution of target genes reported in the included studies (n = 30).

non-random patient selection and case-control study design (Fig. 8, Tab. I). Around 26% (8 out of 30) of the included studies have low risk of patient selection bias because these studies provided sufficient details about patient inclusion/exclusion criteria; 86% of included articles (26 out of 30 studies) present low risk of index test bias because these tests clearly stated the quantitative detection read-outs with reported thresholds. Moreover, these studies explicitly declared that their index and reference tests were done simultaneously in parallel to each other or that testing was blinded from each other. Two studies (19,22) were reported without defined detection thresholds. One study (23) had unclear risk of index test bias as the quantitative detection thresholds were not explained. It was either unclear whether index test results were interpreted with knowledge of reference test results or if only qualitative read-out was used for

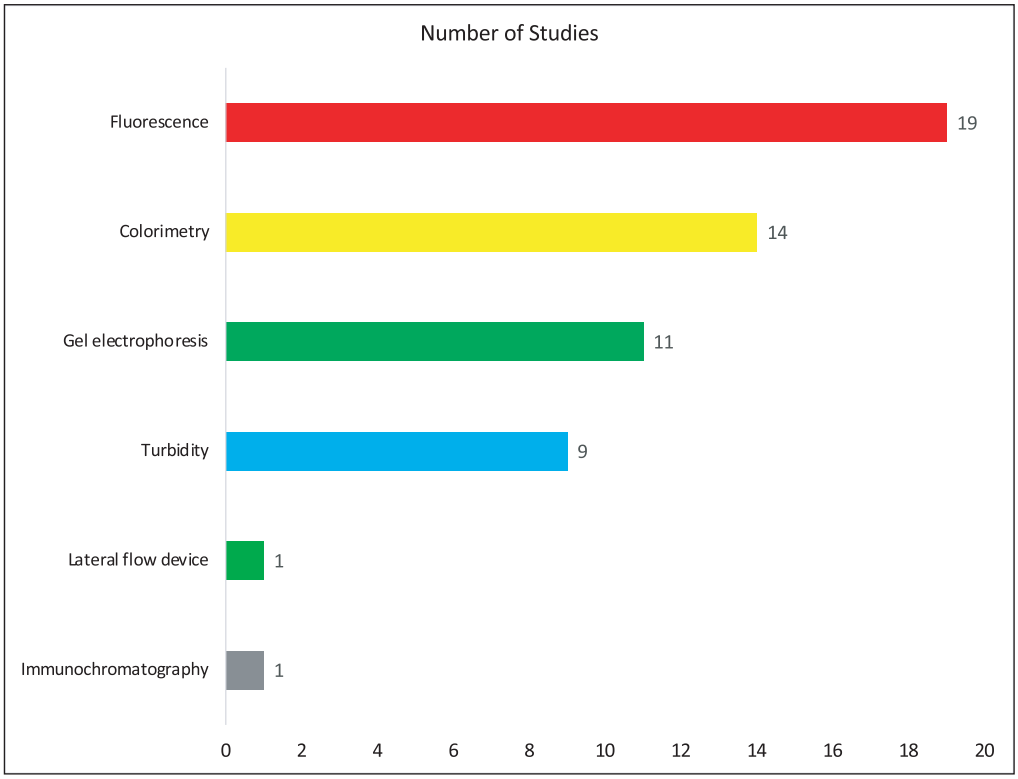

Fig. 5 - Distribution of type of detection method for mycobacteria in the included studies (n = 30).

Study	TP	FP	FN	TN	Sens (95% CI)	Spec (95% CI)
Boehme et al.,2007	173	4	5	500	0.97 (0.93-0.99)	0.99 (0.98-1.00)
Pandey et al.,2008	90	6	6	98	0.94 (0.87-0.97)	0.94 (0.88-0.97)
Poudel et al., 2009	97	6	3	96	0.97 (0.92-0.99)	0.94 (0.88-0.97)
Geojith et al.,2011	17	1	21	17	0.45 (0.30-0.61)	0.94 (0.74-0.99)
George et al., 2011	31	2	2	36	0.94 (0.80-0.98)	0.95 (0.83-0.99)
Mitarai et al., 2011	196	9	27	88	0.88 (0.83-0.92)	0.91 (0.84-0.95)
Nagdev et al., 2011	15	2	2	8	0.88 (0.65-0.97)	0.80 (0.49-0.94)
Sethi et al., 2013	87	0	16	30	0.84 (0.76-0.90)	1.00 (0.89-1.00)
Cao et al., 2015	98	5	2	18	0.98 (0.93-0.99)	0.78 (0.58-0.90)
Joon et al., 2015	28	23	2	262	0.93 (0.78-0.98)	0.92 (0.88-0.95)
Moon et al., 2015	32	3	13	255	0.71 (0.57-0.82)	0.99 (0.97-1.00)
Bojang et al., 2016	98	10	1	157	0.99 (0.95-1.00)	0.94 (0.89-0.97)
Gray et al., 2016	331	52	61	1333	0.84 (0.80-0.87)	0.96 (0.95-0.97)
Kaku et al., 2016	134	5	21	312	0.86 (0.80-0.91)	0.98 (0.96-0.99)
Modi et al., 2016	144	0	6	100	0.96 (0.92-0.98)	1.00 (0.96-1.00)
Sharma et al., 2016	108	0	12	50	0.90 (0.83-0.94)	1.00 (0.93-1.00)
Joon et al., 2017	17	6	1	212	0.94 (0.74-0.99)	0.97 (0.94-0.99)
Reddy et al., 2017	119	17	45	514	0.73 (0.66-0.79)	0.97 (0.95-0.98)
Sharma et al., 2016	81	0	9	50	0.90 (0.82-0.95)	1.00 (0.93-1.00)
Yadav et al., 2018	82	3	0	368	1.00 (0.96-1.00)	0.99 (0.97-1.00)
Kim et al., 2018	87	0	17	186	0.84 (0.76-0.90)	1.00 (0.98-1.00)
Nguyen et al., 2018	15	23	18	445	0.45 (0.29-0.62)	0.95 (0.93-0.97)
Perera et al. , 2018	31	5	0	10	1.00 (0.89-1.00)	0.67 (0.42-0.85)
Joon et al., 2019	13	2	0	92	1.00 (0.77-1.00)	0.98 (0.93-0.99)
Phetsuksiri et al., 2019	144	1	1	5	0.99 (0.96-1.00)	0.83 (0.43-0.97)
Punati et al., 2019	86	9	0	294	1.00 (0.96-1.00)	0.97 (0.94-0.98)
Rajput et al., 2019	90	9	23	32	0.80 (0.72-0.86)	0.78 (0.63-0.88)
Han et al., 2020	59	1	164	41	0.26 (0.21-0.32)	0.98 (0.88-1.00)
Phetsuksiri et al., 2020	119	24	7	102	0.94 (0.88-0.97)	0.81 (0.73-0.87)
Phetsuksiri et al., 2020	126	0	7	71	0.95 (0.90-0.98)	1.00 (0.95-1.00)

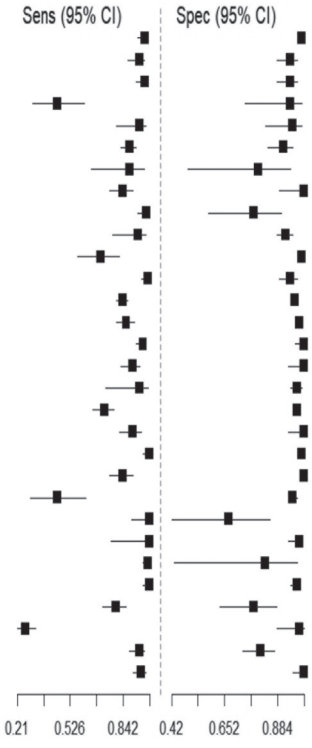

Fig. 6 - The Forest plot of sensitivity and specificity of included studies (n = 30) on the diagnostic performance of loop-mediated isothermal amplification (LAMP) technique.

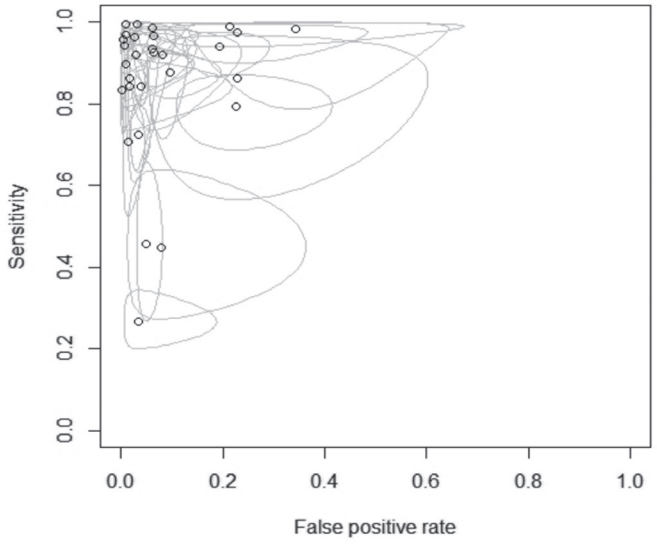

Fig. 7 - Summary receiver operating characteristic (SROC) depicts loop-mediated isothermal amplification (LAMP) diagnostic performance in mycobacteria diagnosis.

reading the results. Hence, index test bias of these studies was unclear. For the rest of the included studies, almost all (n = 30) have low risk of reference standard bias because they provided enough information about the standard reference test used in the study.

Half of the studies (15 out of 30) have an unclear risk of flow and timing bias as there is not enough information, whether reference standard results were interpreted with the knowledge of the results of the index test. One study (24) was at high risk as it did not provide any information on whether the samples for a reference test and the index test were taken at the same time. Our review question did not focus on any patient demographics. None of the included studies attempted to exclude patients based on demographics and thus had no "concern of patient selection applicability" (Fig. 8, Tab. I). Index tests of all studies have generally been used for Point-of-care test (POCTs) and thus have low concern of index test applicability. Reference standard tests of nearly all studies were culture, smear microscopy, Xpert test, PCR, or combinations of them. Thus, we graded these studies as having low concern of standard test applicability.

Discussion

Early and correct diagnosis of all the TB forms is pertinent for effective treatment of the disease and prevention of the spread of infection, particularly in nations which have high burden. The currently available diagnostics rely mostly on smear microscopy, culture, and PCR-based methods which are not only time-consuming and low sensitive but cumbersome and costly (25,26). LAMP assay provides a faster and innovative point-of-care diagnostic alternative as it is cost-effective, sensitive, and gives results in less than 1 hour due to amplification under isothermal condition by strand displacement activity of Bst DNA polymerase and visual readouts (27-30). In fact, the efficiency of LAMP in diagnosis of pulmonary TB is evident from wide ranges of studies (31-35). Additionally, LAMP has been successfully deployed for diagnosis of other forms of TB such as tuberculous meningitis (36,37), osteoarticular TB (38), and tubercular lymphadenitis (39). Although a few studies have evaluated the diagnostic validity of LAMP by meta-analysis for diagnosis of MTB (40),

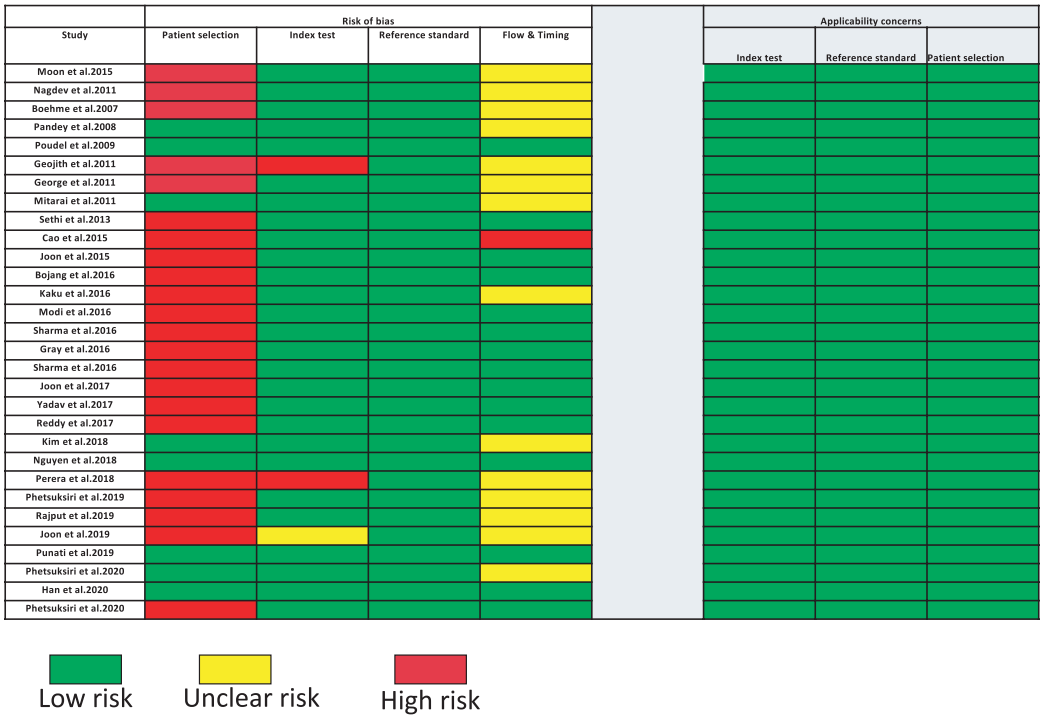

Fig. 8 - Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) summary of items for risk of bias and applicability in included studies (n = 30). Green color depicts the low risk of biasedness, yellow color depicts the unclear risk of biasedness, and red color depicts the high risk of biasedness.

pulmonary TB (41), and extrapulmonary TB (42), an updated meta-analysis covering all forms of mycobacteria was still missing. Hence, the aim of the present study was to systematically review and perform the meta-analysis to assess the diagnostic accuracy of the LAMP assay for detection of all forms of mycobacteria.

This meta-analysis revealed that most of the studies were conducted in high TB burden countries such as India, Thailand, and Japan (Fig. 2). We observed that for the detection of mycobacteria sputum could be considered as the most chosen sample (Fig. 3). When considering the target genes, we found a variety of genes that were used in the included studies. However, IS6110 ranked first among all evaluated genes in the included studies (Fig. 4). This occurrence could be due to the presence of multiple copies of IS6110 present in the MTB genome (43). However, other target genes such as 16s rRNA and gyrB were also prominent. Next, we considered the detection method that was used for assessing the LAMP results. Most of the studies used fluorescence-based methods followed by colorimetry, gel electrophoresis, and turbidity, with no justification of their choices (Fig. 5). The prominence of fluorescence methods could be due to their increased sensitivity for the detection. Exceptionally, only one study mentioned lateral flow-based detection method despite market applicability.

Forest plot was used to calculate the sensitivity and specificity. The pooled sensitivity values of meta-analysis ranged between 0.26 and 1.0 (Fig. 6) and forest plot and SROC curve revealed a pooled specificity value between 0.67 and 1.0 (Fig. 7) with 95% CI. The accuracy and precision were calculated for the included studies and for 16 studies we found that the accuracy rate was higher than their corresponding precision rates and vice versa for 14 articles upon intra-comparison of accuracy with precision (Tab. II).

The current study also exhibited few limitations. Firstly, we observed high risk of patient selection bias or index test bias in almost two-thirds of the eligible studies (Fig. 8). Therefore, the use of unbiased patient cohorts and double-blinded index test may be recommended for future studies. Secondly, few studies showed the highest performance with 100% sensitivity and specificity, respectively, hence displaying the lowest QUADAS risk and concerns in all the domains. Furthermore, lack of subgroup analysis and the use of solely peer-reviewed English language articles were also additional limitations. Hence, although the current meta-analysis should be interpreted with caution, however, we believe that it will not impact the robustness of the analysis leading to further improved studies and reviews. Particularly considering the growing significance of LAMP-based detection for TB comparable to other methods, such studies may be encouraged (43-45).

Conclusion

Despite suffering from few disadvantages, like false positivity due to heavy reliance on indirect detection methods such as turbidity and nonspecific dyes and not providing any additional benefits like information on mutations, drug resistance etc., the LAMP technique could be a promising molecular test to enhance case detection before conventional time-consuming culture. Its simplicity, less turnaround time, and cost-effectiveness are major attractions for clinical laboratories. Also, it will be unjust to rely on single point-of-care test for TB successfully in various kinds of populations and resource availability. Although the unit cost is higher than smear microscopy and culture-based methods, it is likely to offer good value for money relative to conventional methods. In a nutshell, the present study endorses the use of LAMP assay as a promising alternative for detection of mycobacteria, particularly in regions which are financially compromised, where drug-resistant strains are not prevalent and PCR-based tests cannot be done so frequently. The faster diagnosis through LAMP could provide an alternative solution for failed medications to current therapeutics due to delayed diagnosis and subsequent development of drug resistance, thereby providing an opportunity to employ this new information in improving treatment strategies. However, the LAMP assay still must be improved to turn to a strong and competitive alternative to other molecular diagnostic methods.

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Author contributions

G.S.B.: search, data extraction, validation. G.S.B., S.J.: data analysis. Z.F. and S.H.: supervision. G.S.B. and S.H.: writing, original draft. Z.F. and S.H. contributed to the conception and design of the study and review and editing of the manuscript.

Disclosures

Conflict of interest: The authors declare no conflicts of interest, financial or otherwise.

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