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EDITED BY
Theolis Barbosa,
Oswaldo Cruz Foundation (Fiocruz), Brazil

REVIEWED BY
LAKSHMI VEMU,
Kamineni Hospitals, India
Chuan Wang,
Sichuan University, China

*CORRESPONDENCE
Shui-Hua Lu
lushuihua66@126.com
Xu-Hui Liu
liuxuhui666@126.com

†These authors have contributed equally to this work

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Diagnostic accuracy of loop-mediated isothermal amplification for pulmonary tuberculosis in China

Jing-Jing Lin[1†], Xiu-Hong Xi[2†], Lu Xia[2†], Yao-Ju Tan[3], Yu Chen[4], Hong-Qin Di[5], Zhen-Hua Chen[6], Ting Yu[7], Jian-Hao Wei[2], Peng Fang[5], Xiao-Ma Lin[6], Bi-Yi Su[3], Ming-Zhe Yan[7], Yong-Min Yu[4], Kosuke Okada[8], Norihisa Noguchi[9], Toshimitsu Annaka[9], Xu-Hui Liu[10*] and Shui-Hua Lu[11,12*]

[1]Minhang Hospital, Fudan University, Shanghai, China, [2]Shanghai Public Health Clinical Center, Fudan University, Shanghai, China, [3]Pulmonary Disease Institute, Guangzhou Chest Hospital, Guangzhou, China, [4]Department of Clinical Laboratory, The Sixth People's Hospital of Zhengzhou, Zhengzhou, China, [5]Molecular Biology Laboratory, Hebei Chest Hospital, Shijiazhuang, China, [6]Department of Tuberculosis, Hunan Chest Hospital, Hunan, China, [7]Tuberculosis and Respiratory Department, Wuhan Jinyintan Hospital, Hubei, China, [8]Japan Anti-Tuberculosis Association (JATA), Tokyo, Japan, [9]Biochemical Research Laboratory, Eiken Chemical Co. Ltd, Ohtawara, Japan, [10]Department of pulmonary medicine, Shenzhen Third People's Hospital, Shenzhen, China, [11]Department of pulmonary medicine, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital, Shenzhen, China, [12]Department of pulmonary medicine, The Second Affiliated Hospital, School of Medicine, Southern University of Science and Technology, Shenzhen, China

Objectives: To evaluate the diagnostic accuracy of Loop-Mediated Isothermal Amplification Platform (LAMP) in detecting pulmonary tuberculosis (PTB).

Methods: This multicenter prospective study was conducted at six sites in China from June, 2018 to December, 2019. Patients with suspected PTB were consecutively recruited and respiratory samples were collected from all patients. LAMP, Xpert MTB/RIF assay (Xpert), fluorescence smear microscopy, and BACTEC MGIT 960 liquid culture (*Mtb* culture) were performed for each sample. Diagnostic accuracy indices were calculated against *Mtb* culture results.

Results: A total of 845 participants were enrolled, but only 799 were included in the analysis. The sensitivities of LAMP, Xpert, and smear microscopy were 78.6% (239/304), 82.2% (250/304), and 63.8% (194/304), respectively, and their specificities were 88.7% (439/495), 86.1% (426/495), and 94.9% (470/495), respectively. The LAMP assay showed substantial agreement with other tests (kappa 0.64–0.79).

Conclusion: The LAMP assay performs as well as Xpert MTB/RIF assay and *Mtb* culture in tertiary-care hospitals. It can be used as an alternative test for

detecting PTB with the advantages of being fast, inexpensive, and easy to operate.

KEYWORDS

tuberculosis, diagnosis, Xpert MTB/RIF assay, smear microscopy, loop-mediated isothermal amplification

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is a global public health challenge. In, 2018, only 55% of pulmonary tuberculosis (PTB) cases were bacteriologically confirmed (1). In high-income countries with access to the most sensitive diagnostic tests, approximately 80% of PTB cases are bacteriologically confirmed, while the rate of bacteriological confirmation is only 30% in China (1). Rapid molecular tests, such as the Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, CA, USA), have not been generalized to marginalized areas until now, partly because of the relatively high cost and requirement for ongoing specialist technical support; therefore, other methods are used in these areas. For example, fluorescence and acid-fast smear microscopy are still widely used in China, but they are insensitive and do not distinguish between *Mtb* and non-tuberculous mycobacteria (NTM) (2). BACTEC MGIT 960 liquid culture (Becton Dickinson Biosciences, Sparks, MD, USA) and Lowenstein–Jensen medium culture are the gold standard for *Mtb* detection. However, even the fastest culture methods are too slow to help with initial TB management, as results are reported after weeks rather than hours or days. Loop-mediated isothermal amplification for TB (TB-LAMP; Eiken Chemical Company, Tokyo, Japan) is a novel nucleic acid amplification assay endorsed by the World Health Organization (WHO) for the detection of PTB. Compared with the Xpert MTB/RIF assay, LAMP is less expensive (50–70% less cost than Xpert in China), easier to operate (result could be read by the naked eye), and comparably fast (3, 4). Some studies have reported the diagnostic accuracy of LAMP in China, but the results have been inconsistent (5–7). Therefore, we conducted a multicenter study with a large sample size to determine the diagnostic accuracy of LAMP in this region.

Abbreviations: CI, confidence interval; LAMP, loop-mediated isothermal amplification; *Mtb*, *Mycobacterium tuberculosis*; NPV, negative predictive value; NTM, non-tuberculous mycobacteria; PPV, positive predictive value; PTB, pulmonary tuberculosis; TB, tuberculosis.

Materials and methods

Study design and population

This multicenter prospective diagnostic accuracy study was conducted in six tertiary-care hospitals (Shanghai Public Health Clinical Center, Guangzhou Chest Hospital, Hunan Chest Hospital, Hebei Chest Hospital, Zhengzhou Sixth People's Hospital, and Wuhan Jinyintan Hospital) from June, 2018 to December, 2019 (ChiCTR-DDD-17013146). The study protocol was reviewed and approved by the ethics committee of the Shanghai Public Health Clinical Center (2018-S013-02), and the study was performed in accordance with domestic clinical study guidelines. Written informed consent was obtained from the participants (or their parents or guardians for minors). Patients of all ages and both sexes were consecutively enrolled if they met one or more of the following suspected PTB criteria (8): (1) household TB contact in the previous 3 months; (2) fever or cough for more than two weeks; (3) weight loss or failure to gain weight in the previous 3 months; (4) a positive tuberculin skin test or T-SPOT; and (5) chest radiography suggestive of TB (required). In this study, confirmed TB was defined as *Mtb* culture-positive. Clinically, diagnostic criteria were established according to the diagnostic consensus and domestic guidelines (2, 8, 9). Exclusion criteria included 1) inadequacy or invalid samples for all tests; 2) incomplete clinical data or an indeterminate clinical diagnosis; and 3) exposure to anti-TB drugs for ≥4 weeks (including carbapenems, fluoroquinolones, macrolides, and aminoglycosides).

Sample processing

Sputum or induced sputum was used for diagnostic testing. Samples were processed according to the manufacturer's instructions (10–12). A 60 μL sample of sputum was used for the LAMP assay(Loopamp PURE DNA Extraction Kit; Eiken Chemical Company, Tokyo, Japan), and the remainder of the sample was divided into three for smear microscopy, *Mtb* culture (BACTEC MGIT 960 system, BD Biosciences), and Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, CA, USA). Cultures that were positive for acid-fast bacilli were confirmed as *Mtb* complex

(MTBC) organisms using MPT64/MPB64 antigen detection according to the manufacturer's instructions (Capilia, Hangzhou, China) (2). NTM strains were confirmed by Sanger sequencing. The Limit of Detection (LoD) of Xpert is 131 cfu/mL.The LoD of LAMP assay is 1.28copies/μL when 5μL sample solution is used for reaction. It was performed according to the WHO guidelines (4): (1) A sample of 60 μL of sputum was transferred to a heating tube containing extraction solution; (2) the sample was mixed by inverting it 3–4 times, and the heating tube was placed on the heating block at 90°C for 5 min to inactivate and lyse mycobacteria; (3) take out the heating tube and place it at room temperature for 2 minutes, then invert it 3-5 times; (4) the heating tube was attached to an adsorbent tube, mix the contents thoroughly to ensure no white powder residue; (5) 30 μL of the resulting solution were squeezed from the adsorbent tube to the reaction tube, and invert reaction tube for 2 min to mix the solution and the reagent which is in the lid, then shake the mixture to the bottom; (6) the temperature on the digital display on the incubator was confirmed to be 67°C; (7) the reaction tubes were loaded into the heating block; (8) the sample was amplified for 40 min, and the amplification was stopped automatically after 40 min; (9) a fluorescence detector (Loopamp LF-160; Eiken Chemical Co., Ltd., Tokyo, Japan) was used to check the reaction tubes, and the results were judged with the naked eye, green indicates positive.

Data analysis and statistical methods

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to evaluate diagnostic accuracy. Categorical variables were compared using Pearson's chi-square, McNemar's (paired), or Fisher's exact test. Statistical p-value of ≤0.05 was considered significant. The 95% confidence intervals (CIs) were estimated for the data with binomial distributions. Kappa values were assessed to determine the agreement between categorical variables (13). Statistical analyses were performed using SPSS software version 22 (IBM Corp, Armonk, NY, USA), Excel for Windows 10 (Microsoft, Redmond, WA, USA), and GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA).

Results

Study participants

We enrolled 845 patients with suspected PTB. Of these, 10, 15, 6, and 15 cases were excluded due to culture contamination, incomplete data, repetitive testing, and NTM infection, respectively. Thus, 799 cases were analyzed, of which 549 (68.7%) patients were male, and the median age was 53 years (interquartile range; 33, 64). *Mtb* culture was positive in 304 (38%) samples. Additionally, 332 (41.6%) samples were categorized as *Mtb* culture-negative clinically diagnosed TB, and 163 (20.4%) as other respiratory diseases. (Figure 1, Table 1).

Diagnostic accuracy of LAMP assay for PTB detection

The diagnostic indices of all assays against *Mtb* cultures are shown in Table 2. LAMP showed an overall sensitivity of 78.6% (95% CI, 73.8–82.9%), similar to that of Xpert (82.2%, p=0.126), and higher than that of smear microscopy (63.8%, p<0.001). In smear-positive and culture-positive cases, the sensitivity of the LAMP assay was 90.7% (176/194), and in smear-negative and culture-positive cases, the sensitivity was 57.3% (63/110). The specificities of LAMP, Xpert, and smear microscopy were 88.7% (95% CI: 85.7–91.3), 86.1% (95% CI: 82.8–88.9), and 94.9% (95% CI: 92.8–96.6), respectively. Furthermore, when stratified by age, the sensitivities of LAMP between adolescent (aged 10–19 years) and adults (aged 20 years or over) were 78.6% (11/14), 78.6% (228/290) (Supplement 1 Table 1).

Compared with clinical diagnosis, the sensitivities of LAMP, Xpert, *Mtb* culture, and smear microscopy were 46.4%, 50.2%, 47.8%, and 34.4%, respectively, and the specificity was 100% (Table 3).

If the 15 NTM cases had been included in the analysis, smear microscopy and *Mtb* culture would show lower specificity than nucleic acid assays, with 92.7% (95% CI: 88.2–95.8) and 91.6% (95% CI: 86.8–95.0) specificity when compared with clinical diagnosis, respectively (Supplement 2 Table 2). When against *Mtb* cultures, the sensitivities of smear microscopy, LAMP and Xpert were 64.9%, 78.4%.75.2% (Supplement 2 Table 2). Sixty-five "false positive" cases (LAMP-positive and *Mtb* culture-negative) were reviewed, and all cases were categorized as clinically diagnosed TB.

Consistency between LAMP and other assays

In a single-sample test, the diagnostic consistency between LAMP and other assays was substantially high, with kappa values of 0.789, 0.677, and 0.637 for Xpert, *Mtb* culture, and smear microscopy, respectively.

Discussion

TB has been inadequately controlled in China in recent decades. To eliminate the TB epidemic in China, any improvement in TB diagnostics, including cost and turnaround time reduction, is desirable. The use of the *gyrB* gene as a target for identification of *Mtb* and the extent to which fluoroquinolone resistance, lineage markers, or other

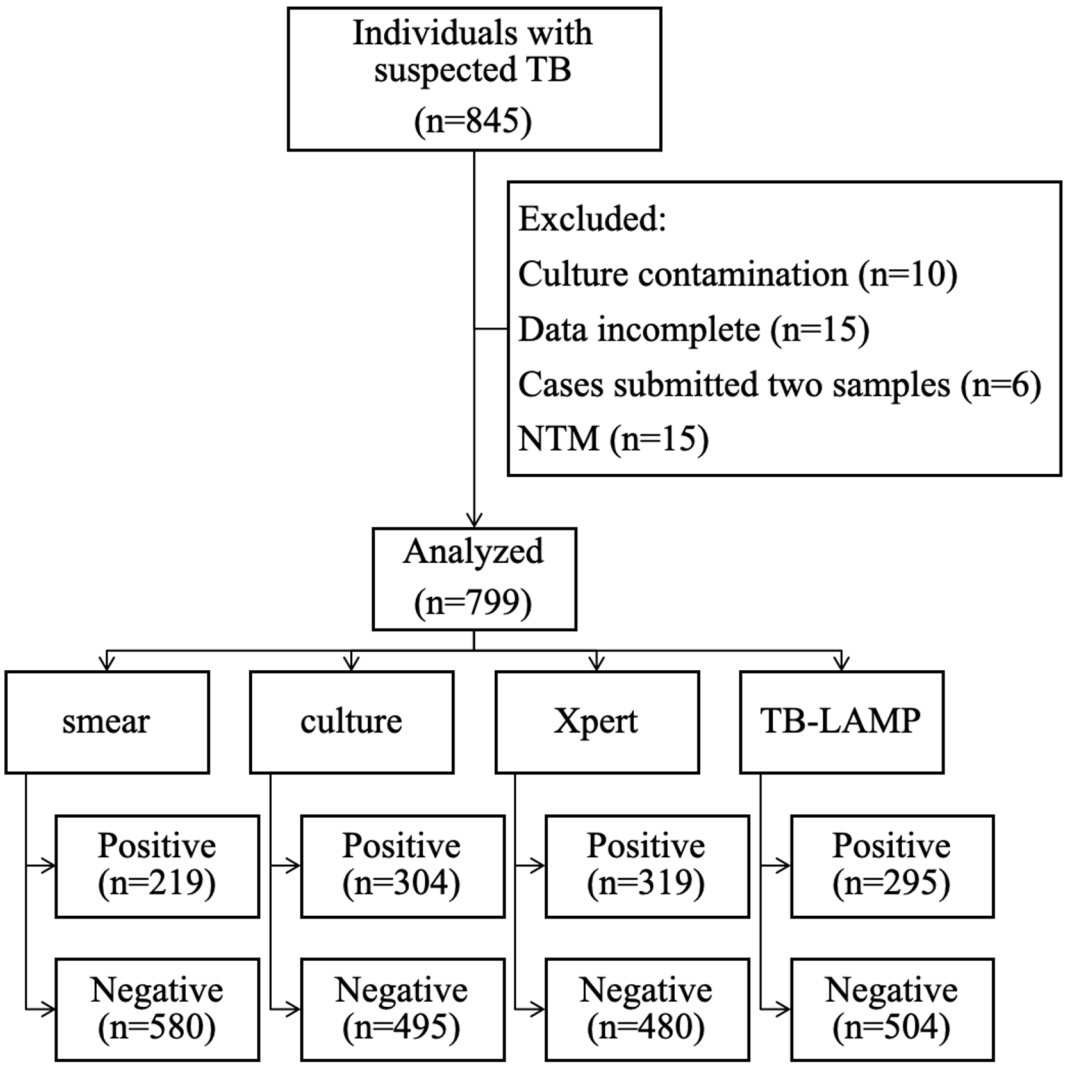

FIGURE 1
Flow chart of patient enrolment and the results of the diagnostic tests. Abbreviations: NTM, non-tuberculous mycobacteria.

mutations in this locus would impact the diagnostic accuracy of assays, but LAMP, targets the *gyrB* and *IS6110* regions, may reduce the impact.Our study confirmed that the performance of the TB-LAMP assay in PTB diagnosis is non-inferior to the Xpert assay and *Mtb* culture. Additionally, this assay showed substantial agreement with Xpert, *Mtb* culture, and smear microscopy, indicating that it can be used as an adjunctive test. The results of this study are comparable with those of another multicenter study conducted in Peru, South Africa, Brazil, and Vietnam (14). In clinical practice, especially in resource-limited settings, LAMP may facilitate the early diagnosis of PTB, as it requires less infrastructure, has a shorter turnover time, and is cheaper (7).

In a systematic review, nine TB-LAMP studies yielded summary estimates of sensitivity of 80.9% and specificity of 96.5% (15). Our study reported a similar sensitivity (78.6%) but a lower specificity (88.7%) against the culture. Ou et al. also conducted a multicenter study in three tertiary hospitals in China and reported a similar sensitivity of 74.88% and specificity of 86.50% (6). We reviewed the database and attributed this lower specificity to four reasons: first, over 60% of the participants were early stage cases, who were asymptomatic or presented mild symptoms with single-lobular infiltration; second, as this study was conducted in tertiary hospitals, a proportion of smear-positive participants (who were also more likely to be culture-positive) were pre-screened

TABLE 1 Demographic characteristics of the study participants and diagnostic test results according to the recruitment center.

	Shanghai (N=163)	Guangzhou (N=175)	Hunan (N=129)	Wuhan (N=40)	Zhengzhou (N=150)	Shijiazhuang, Hebei (N=142)	All patients (N=799)
Demographic characteristics							
Mean Age, years	49.37 (6–87)	50.55 (13–92)	53.90 (15–87)	49.23 (17–77)	44.13 (12–70)	50.20 (12–91)	49.53 (6–92)
Male sex	106/163 (65.0%)	123/175 (70.3%)	93/129 (72.1%)	25/40 (62.5%)	108/150 (72.0%)	94/142 (66.2%)	549/799 (68.7%)
Clinically diagnosed TB	127/163 (77.9%)	125/175 (71.4%)	112/129 (86.8%)	36/40 (90.0%)	138/150 (92.0%)	98/142 (69.0%)	636/799 (79.6%)
Culture positive	75/163 (46.0%)	59/175 (33.7%)	45/129 (34.9%)	16/40 (40.0%)	59/150 (39.3%)	50/142 (35.2%)	304/799 (38.0%)
Smear positive	70/163 (42.9%)	56/175 (32.0%)	16/129 (12.4%)	9/40 (22.5%)	34/150 (22.7%)	34/142 (23.9%)	219/799 (27.4%)
Xpert positive	77/163 (47.2%)	68/175 (38.9%)	41/129 (31.8%)	22/40 (55.0%)	57/150 (38.0%)	54/142 (38.0%)	319/799 (39.9%)
LAMP positive	74/163 (45.4%)	61/175 (34.9%)	36/129 (27.9%)	18/40 (45.0%)	52/150 (34.7%)	54/142 (38.0%)	295/799 (36.9%)

TABLE 2 Accuracy of assays compared with *Mtb* culture for diagnosis of PTB.

Tests	Sensitivityn/N, %	Specificityn/N, %	PPV%	NPV%
Smear microscopy	194/304, 63.8 (95% CI, 58.3–69.1)	470/495, 94.9 (95% CI, 92.8–96.6)	88.6 (95% CI, 83.9–92.3)	81.0 (95% CI, 77.7–84.1)
Xpert	250/304, 82.2 (95% CI, 77.6–86.2)	426/495, 86.1 (95% CI, 82.8–88.9)	78.4 (95% CI, 73.6–82.6)	88.8 (95% CI, 85.7–91.3)
LAMP	239/304, 78.6 (95% CI, 73.8–82.9)	439/495, 88.7 (95% CI, 85.7–91.3)	81.1 (95% CI, 76.3–85.2)	87.1 (95% CI, 84.0–89.8)

TABLE 3 Accuracy of assays compared with clinical diagnosis.

Tests	Sensitivityn/N, %	Specificityn/N, %	PPV%	NPV%
MGIT culture	304/636, 47.8 (95% CI, 43.9–51.7)	163/163, 100	100	32.9 (95% CI, 28.9–37.2)
Smear microscopy	219/636, 34.4 (95% CI, 30.8–38.2)	163/163, 100	100	28.1 (95% CI, 24.6–31.9)
Xpert	319/636, 50.2 (95% CI, 46.3–54.0)	163/163, 100	100	34.0 (95% CI, 29.8–38.3)
LAMP	295/636, 46.4 (95% CI, 42.5–50.3)	163/163, 100	100	32.3 (95% CI, 28.4–36.5)

NPV, negative predictive value; PPV, positive predictive value

and treated before visiting our sites; third, LAMP, nucleic acid amplification testing, could detect uncultured or dead bacteria, but culture could only detect live bacteria. Patients known to have PTB who are mid-treatment may only remain dead, non-viable bacteria in their sputum; fourth, sample contamination, or harsh sample decontamination procedures might be harmful for viability of *Mtb* but a little impact on existence of DNA.

This TB-LAMP assay, showed high specificity (100%) when compared with clinical diagnosis, and was not affected by NTM infection in this study. This result is similar to those of previous studies in Gambia (16) and India (17).

This study had limitations. First, owing to the lack of clinical data available on subjects, such as HIV status and history of TB, the sensitivity and specificity of LAMP in these populations have not been well-demonstrated. Second, most participants were enrolled in tertiary hospitals and could have been treated for <4 weeks. Thus, the results of this study are not representative of primary care services. Third, infant group (aged under 1 year) was not enrolled, and child group (aged under 10 years) only included 1 patient who was culture-negative, and the number of cases of adolescent group was significantly smaller than that of adult group, so it was hard to

conclude that no significant difference in the efficiency of LAMP between different age groups. Further studies about diagnosis accuracy of LAMP need to be done in AIDS patients, infants and adolescents.

Conclusion

LAMP, a cheap and simple assay, holds promise as a rapid, highly sensitive, and specific test for TB case detection in China and other developing countries with a high burden of TB.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The ethics committee of the Shanghai Public Health Clinical Center (2018-S013-02). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

S-HL and X-HL were responsible for the study design and manuscript revision; J-JL, LX, and X-HX participated in conducting the study, collecting data, and writing the manuscript; J-JL, J-HW, YC, Z-HC, H-QD, PF, X-ML, B-YS, Y-JT, M-ZY, TY, and Y-MY enrolled participants in six hospitals; and KO, NN, and TA contributed to the site investigation and provided technical support. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author NN and TA are employed by Eiken Chemical Co. Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2022.1046948/full#supplementary-material

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