Original Article

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Cytoderm metabolic-labeling SCMLP-TB for pulmonary tuberculosis diagnosis: A preliminary diagnostic accuracy study

Guangyan Liang[1,§], Guiqin Dai[1,§], Xiaorong Hu[1,§], Deliang Liu[1], Zhiqiang Lin[1], Mengru Yang[1,4], Zhuojun He[1], Peifen Chen[1], Yipeng Liu[1], Xinyun Jia[1], Xiafei Dai[1], Pengfei Zhao[1,*], Mingbin Zheng[1,2,*], Yang Zhou[1,3,*], Hongzhou Lu[1,*]

[1] National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital, Southern University of Science and Technology, Shenzhen, China;
[2] The Affiliated Dongguan Songshan Lake Central Hospital, Guangdong Medical University, Dongguan, China;
[3] Molecular Biology Research Center & Center for Medical Genetics, School of Life Sciences, Central South University, Changsha, China;
[4] School of Public Health, Shenzhen University Medical School, Shenzhen University, Shenzhen, Guangdong, China.

SUMMARY: Tuberculosis (TB) remains a significant global health issue. Early diagnosis is crucial, yet current diagnostic technologies are limited by suboptimal sensitivity. Thus, we developed a novel tuberculosis metabolic labeling probe (single cell metabolic labeling probe for tuberculosis, SCMLP-TB) and evaluated its diagnostic performance. In this retrospective study of 70 suspected TB patients, we calculated the sensitivity and specificity of SCMLP-TB and compared it with culture and Xpert MTB/RIF (Xpert) using the final clinical diagnosis as reference standard. Eligible participants were classified as confirmed TB (CT), clinically diagnosed TB (CDxT), or non-TB cases based on the diagnosis criteria for pulmonary tuberculosis (WS 288-2017). Of the 70 participants, 40 (57.0%) were diagnosed with TB, including 30 CT cases and 10 CDxT cases. The overall diagnostic sensitivity and specificity of SCMLP-TB were 97.5% and 96.7%, respectively. Notably, SCMLP-TB identified 10 CDxT cases missed by both culture and Xpert. The overall diagnostic sensitivity of culture and Xpert was 62.5% and 72.5%, respectively, while both showed a specificity of 100.0%, demonstrating that SCMLP-TB was more sensitive than culture and Xpert. Besides, the fluorescence intensity from TB patients was significantly higher than non-TB patients. The fluorescence intensity showed a significant negative correlation with the time to positivity (TTP) of culture, which suggested that SCMLP-TB could also serve as an indicator of bacterial loads in patients' samples. Consequently, SCMLP-TB demonstrated a promising tool for the sensitive and ultra-fast diagnosis of pulmonary TB suspects, particularly for paucibacillary pulmonary TB.

Keywords: Tuberculosis, SCMLP-TB, metabolic-labeling, accuracy, diagnosis

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is the leading global infectious disease in terms of morbidity and mortality (*1,2*). Early and precise diagnosis is crucial for controlling the TB epidemic (*3*). As the most common tool for TB diagnosis, sputum-smear microscopy suffered from considerable training demands, operator-dependent performance, inability to discriminate live from dead bacilli, and relatively low sensitivity (*4,5*). Culture-based assays were the gold standard of TB diagnosis, but hampered by prolonged incubation, stringent lab requirements, limited sensitivity, and contamination risk leading to inconclusive outcomes (*4,6,7*). Xpert MTB/RIF (Xpert), a polymerase chain reaction (PCR)-based test, improved TB detection yet it remained prohibitively expensive and displayed suboptimal sensitivity in paucibacillary samples, particularly in TB/HIV (human immunodeficiency virus) co-infected individuals and extrapulmonary samples (*7-10*). Therefore, developing a rapid, sensitive, specific and accurate method for diagnosing live MTB was essential for effective TB control.

Reportedly, a fluorescein-modified trehalose analog (FITC-Tre) achieved rapid and accurate detection of viable MTB in patient sputum by engaging in glycolipid biosynthesis and inserting into the bacterial cell wall (*11-13*). As the predominant lipids in the mycomembrane of MTB, trehalose monomycolates and trehalose dimycolates were essential for cellular viability (*13-15*). By antigen-85 (Ag85) complex catalysis, these

glycolipids were secured within the cytoderm (*16,17*). However, the detection efficacy of conventional fluorophores like fluorescein isothiocyanate (FITC) was limited by photobleaching and aggregation-induced quenching at high concentrations or in aggregated states (*11,18,19*).

To solve this problem, we designed SCMLP-TB (single cell metabolic labeling probe for tuberculosis), a fluorescein-tagged trehalose analog that exhibited aggregation-induced emission, which served as an exogenous substrate to selectively aggregate at MTB cytoderm and enabled fluorescent labeling and visualization of viable bacteria (*20-22*).

In this study, we retrospectively evaluated the diagnostic accuracy of SCMLP-TB using sputum or bronchoalveolar lavage fluid (BALF) samples for pulmonary TB detection. Additionally, we preliminarily explored the correlation between fluorescence (FL) intensity and bacterial load evidenced by the time to positivity (TTP) of culture. This approach showed great potential for precise MTB diagnosis and real-time bacterial viability assessment.

2. Materials and Methods

2.1. Study design and participants

This was a single-centre, retrospective study conducted at Shenzhen Third People's Hospital, which was designated as the hospital responsible for the treatment of tuberculosis (TB) patients in Shenzhen and its surrounding areas. From May 2023 to October 2024, we collected data on suspected pulmonary TB patients who were admitted to Shenzhen Third People's Hospital. The study was approved by the Ethics Committee of Shenzhen Third People's Hospital (2024-195). This retrospective study was part of a prospective project, which was registered with the Chinese Clinical Trial Registry (ChiCTR, *www.chictr.org.cn*) under identifier ChiCTR2500098704.

A total of 70 suspected pulmonary TB patients from Inpatient Departments or Outpatient Clinics were enrolled in the study through the medical database within the hospital information system. Inclusion criteria were as follows: (i) inpatients or outpatients suspected to have pulmonary TB who were registered in the hospital information system and had accessible follow-up records and (ii) patients who underwent the SCMLP-TB (single cell metabolic labeling probe for tuberculosis) test using sputum or BALF samples. Eligible participants were selected based on the diagnosis criteria for pulmonary TB (WS 288-2017), with at least one of the following: symptoms consistent with TB, chest imaging compatible with TB, or a positive interferon-gamma release assay (IGRA). An experienced infectious-disease physician made this assessment. Exclusion criteria were as follows: (i) absence of a definite diagnosis after discharge or follow-up; (ii) extrapulmonary TB or severe co-infection; (iii) empirical anti-TB therapy for ≥ 2 weeks before testing; (iv) age < 18 years, pregnancy, or psychiatric disorder. Diagnostic criteria: the attending physician made a final clinical diagnosis according to the Chinese diagnostic criteria for pulmonary TB (WS 288-2017). Patients were classified into three groups: (1) confirmed TB (CT): a biological specimen was positive by any of smear microscopy, culture, Xpert MTB/RIF (Xpert), TB-DNA and metagenomic next-generation sequencing (mNGS), with non-tuberculosis mycobacteria (NTM) excluded by NTM-DNA or mNGS, (2) clinically diagnosed TB (CDxT): when patients lacked microbiological evidence, but the attending physician strongly suspected TB after excluding other diseases by combining the patient's clinical signs, chest imaging findings and other laboratory tests, patients were diagnosed with anti-TB therapy (ATT) and confirmed to have well-responded to anti-TB treatment at months of follow-up, (3) non-TB: pneumonia, malignancy, chronic obstructive pulmonary disease, bronchiectasis or other aetiologies. Pulmonary TB cases comprised CT and CDxT cases.

Data were obtained from the medical database including patients' demographics (age, gender, smoke history, comorbidities and previous TB history), clinical symptoms (cough, fever, night sweats, loss of weight or haemoptysis), chest imaging findings, laboratory results (MTB culture, Xpert, acid-fast bacilli (AFB), TB-DNA and IGRA).

2.2. Detection procedures of SCMLP-TB

Sputum and BALF samples were collected for detection. Sputum samples, treated with NaOH-NALC (DZ0802, Leagene, Beijing, China) for 15-20 minutes, aimed to reduce viscosity, remove proteins and cellular material surrounding MTB, and eliminate other bacteria, while preserving MTB due to its dense cell wall. The samples were then filtered through a 40 μm filter (SCS402, Smtra, Hangzhou, China), centrifuged at 4,000 rpm for 15 minutes, and resuspended in 100 μL phosphate buffered saline (PBS; Sangon Biotech, Shanghai, China). Next, 100 μL of the sample was mixed with 40 μM SCMLP-TB and incubated at 37°C for 2 hours to label MTB. BALF samples followed the same processing steps post-digestion, omitting the digestion stage (*22*). After labeling, samples were centrifuged at 12,000 rpm for 5 minutes and re-suspended in PBS. The labeled MTB was visualized using confocal laser scanning microscopy (LSM 980, Carl Zeiss, Oberkochen, Germany), and the fluorescence (FL) intensities of SCMLP-TB anchored on MTB were measured at 488 nm using a multi-mode microplate reader (SH1MF-SN, Shenzhen Boxing Biotechnology Co., Ltd., Shenzhen, China) (*22*).

2.3. MTB culture

At Shenzhen Third People's Hospital, BALF and sputum samples were processed using the BACTEC MGIT 960 system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for MTB culture. All culture tubes were cultured in a 37°C incubator. The MGIT fluorescent automatic light reader was used for interpretation. All negative culture tubes continued to be cultured and read for 8 weeks. MGIT negative culture tubes should be discarded before observing the turbidity or small particles in the tube. All positive cultures detected were confirmed by acid-fast staining. Time to positivity (TTP): the interval until the first positive signal in culture (*23*).

2.4. Xpert MTB/RIF assay

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) was performed according to the manufacturer's instructions. One millilitre of sputum or BALF was subjected to centrifugal precipitation, and the resulting pellet was transferred to a leak-proof container and combined with 2 mL of processing solution. The mixture was vortexed vigorously for 10-20 times, then incubated at room temperature for 15 minutes. Exactly 2 mL was withdrawn with a pipette and loaded into the sample port of the reaction cassette. The lid was sealed firmly to initiate the assay. Approximately 2 hours after assay initiation, results were interpreted (*23*).

2.5. Statistical analysis

Baseline data were presented as frequency (%), mean ± SD, or median (IQR). Continuous variables were compared using independent *t*-tests for normally distributed data or Mann-Whitney *U* tests for non-normally distributed data. Categorical variables were analyzed using chi-square tests or Fisher's exact tests. To determine the fluorescence threshold for the SCMLP-TB assay, a receiver operating characteristic (ROC) curve was generated using the fluorescence intensity values from all 40 TB cases and 30 non-TB cases to determine the optimal diagnostic threshold. The optimal threshold was selected by balancing sensitivity and specificity, yielding a cutoff of 4,787 a.u. (Supplementary Figure S1, *https://www.ddtjournal.com/supplementaldata/294*). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were reported with two-sided 95% confidence intervals (CI). Sensitivity and specificity comparisons were performed using Fisher's exact test. A *p*-value of less than 0.05 was considered to be statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

3. Results

3.1. Study participants population

Between May 31, 2023 and October 31, 2024, 73 individuals were screened for tuberculosis (TB). The enrollment process was summarized in Figure 1. At enrollment, all participants were systematically screened for clinical symptoms and previous TB history. Of the 73 individuals, 3 were excluded: 1 with extrapulmonary TB and 2 without definitive diagnosis. Therefore, 70 individuals fulfilled the inclusion criteria and were recruited as study participants. Consequently, clinical and laboratory data were collected from these 70 screened participants at baseline (Table 1). Overall, the mean age of the study participants was 50.0 ± 16.3 years. Of the 70 participants, 29 (41.4%) were female, 41 (58.6%) were male, and 13 (18.6%) had a smoke history. Among them,

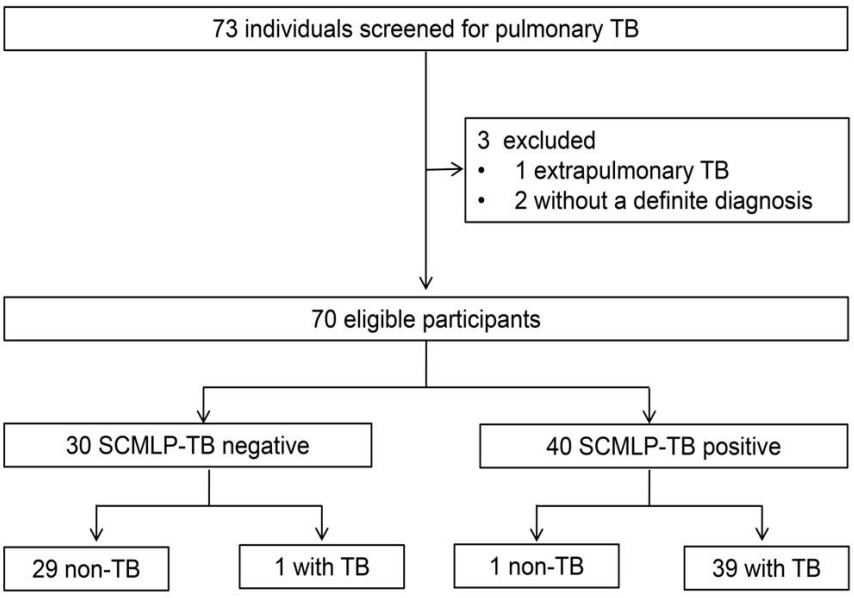

Figure 1. Flow diagram of participant enrollment. SCMLP-TB, single cell metabolic labeling probe for tuberculosis.

Table 1. Baseline characteristics of the study participants

	Number of participants with results	Overall (n = 70)	TB (n = 40)	Non-TB (n = 30)	P-value
Demographics					
Age, years	70	50.0 ± 16.3	46.5 ± 14.5	54.6 ± 17.7	0.039*
Sex	70				0.780
Female		29 (41.4%)	16 (40.0%)	13 (43.3%)	
Male		41 (58.6%)	24 (60.0%)	17 (56.7%)	
Smoke history	70	13 (18.6%)	5 (12.5%)	8 (26.7%)	0.123
Clinical					
Symptom					
Cough	70	57 (81.4%)	32 (80.0%)	25 (83.3%)	0.723
Fever	70	23 (32.9%)	11 (27.5%)	12 (40.0%)	0.264
Night sweats	70	11 (15.7%)	5 (12.5%)	6 (20.0%)	0.510
Loss of weight	70	17 (24.3%)	10 (25.0%)	7 (23.3%)	0.876
Haemoptysis	70	10 (15.7%)	6 (15.0%))	4 (16.7%)	1.000
Previous TB history	70	9 (12.9%)	6 (15.0%)	3 (10.0%)	0.723
Comorbidities					
HIV	70	13 (18.6%)	5 (12.5%)	8 (26.7%)	0.123
Diabetes	70	11 (15.7%)	10 (25.0%)	1 (3.3%)	0.011*
Haemoglobin, g/dL	70	12.2 ± 2.5	12.2 ± 2.5	12.3 ± 2.5	0.860
Positive chest imaging	68	45 (66.2%)	34 (89.5%)	11 (37.9%)	< 0.001***

Data were expressed as number (%) for categorical variables and as mean (SD) for continuous variables in case of normal distributions and median (IQR) otherwise. Positive chest imaging meant that the result of chest computed tomography was suggestive of tuberculosis. p-value: TB cases vs non-TB cases, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. TB cases were comprised of CT and CDxT. CT, confirmed tuberculosis; CDxT, clinically diagnosed tuberculosis. TB, tuberculosis.

the most frequent symptoms were cough in 57 (81.4%) and fever in 23 (32.9%), with other symptoms including night sweats in 11 (15.7%), loss of weight in 17 (24.3%), and hemoptysis in 10 (15.7%). Additionally, 9 (12.9%) of the participants had a previous history of TB prior to their enrollment in the study. Comorbidities were recorded, with HIV presenting in 13 participants (18.6%) and diabetes in 11 participants (15.7%). The mean hemoglobin level was 12.2 g/dL for the TB group and 12.3 g/dL for the non-TB group. Chest imaging findings showed that 34 out of 38 (89.5%) had abnormalities suggestive of TB in the TB group, while 11 out of 29 (37.9%) exhibited such indications in the non-TB group (Table 1).

3.2. Sensitivity and specificity analysis

We compiled detailed clinical and assay data for each participant in our study, categorizing them into confirmed TB (CT) cases, clinically diagnosed TB (CDxT) cases and non-TB cases according to the diagnosis criteria for pulmonary TB (WS 288-2017) (Figure 2A). CT and CDxT were categorized as TB cases. Since CDxT cases may introduce misclassification bias and overestimate sensitivity. To minimize this risk, final diagnoses were determined independently of SCMLP-TB results through expert adjudication. Exactly, 30 cases were CT and 10 cases were CDxT. The other 30 cases were diagnosed to be non-TB cases after follow-up visits (Figure 2A). The Venn diagram displayed 40 participants with TB detected by each diagnostic tests (culture, Xpert MTB/RIF (Xpert), and SCMLP-TB) and the overlap between tests (Figure 2B). The substantial overlap between SCMLP-TB and the other two methods indicated a strong concordance, thereby validating the reliability of SCMLP-TB alongside established diagnostic methods. Notably, the SCMLP-TB circle extended beyond the intersections, demonstrating its capacity to detect additional cases that were not identified by Xpert or culture alone. This suggested that SCMLP-TB may provide enhanced sensitivity in TB detection, complementing existing clinical tools (Figure 2B). We conducted a quantitative analysis comparing SCMLP-TB fluorescence (FL) intensity tested by microplate reader in the TB and non-TB groups (Figure 2C). 39 (97.5%) of 40 were above the detection threshold in TB group, while 1 (3.3%) of 30 in the non-TB group (Figure 2C). The median FL intensity of the TB group was 15,587 a.u., while that of the non-TB group was 2,468 a.u.. The TB group showed significantly higher FL intensity ($p < 0.001$), demonstrating robust diagnostic evidence for TB. Besides, we assessed the correlation between SCMLP-TB FL intensity and bacillary load, as measured by time to positivity (TTP). The SCMLP-TB's enhanced FL intensity showed a significant negative correlation with the TTP of culture test (Pearson's r = -0.60, p = 0.005), indicating that SCMLP-TB's FL intensity could likely serve as a reliable indicator of bacillary load in samples (Figure 2D).

Indeed, SCMLP-TB surpassed culture and Xpert assay in diagnostic performance. SCMLP-TB's sensitivity was 97.5% (95% CI 85.3-99.9; 39 of 40 cases), significantly higher than culture's 62.5% (95% CI 45.8-76.8; 25 of 40 cases) ($p < 0.05$). SCMLP-TB's specificity of 96.7% (95% CI 80.9-99.8; 29 of 30 cases) was close to culture's specificity of 100.0% (95% CI 82.8-100.0; 24 of 24 cases). Notably, SCMLP-TB detected all 10

CDxT cases missed by both culture and Xpert (Figures 2A and 2B and Table 2). This capability highlighted the potential of SCMLP-TB to serve as an ultra-sensitive diagnostic tool for paucibacillary pulmonary TB. While Xpert showed a specificity of 100% (95% CI 83.4-100; 25 of 25 cases), its sensitivity was lower at 72.5% (95% CI 55.9-84.9; 29 of 40 cases). Statistical analysis revealed that SCMLP-TB was more sensitive than Xpert ($p < 0.05$). SCMLP-TB also exhibited the optimal positive predictive value (PPV) and negative predictive value (NPV) among the three diagnostic methods, with a PPV of 97.5% (95% CI 85.3-99.9; 39 of 40 cases) and an NPV of 96.7% (95% CI 80.9-99.8; 29 of 30 cases). Culture exhibited a PPV of 100.0% (95% CI 83.4-100.0; 25 of 25 cases) and an NPV of 61.5% (95% CI 44.7-76.2; 24 of 39 cases), while Xpert showed a PPV of 100% (95% CI 85.4-100.0; 29 of 29 cases) and an NPV of 69.4% (95% CI 51.7-83.1; 24 of 35 cases), reflecting SCMLP-TB's superior diagnostic accuracy in reflecting the true TB disease status.

4. Discussion

In 2023, 10.8 million new TB cases and 1.25 million TB-related deaths occurred globally (*1*). The persistently high global incidence and mortality rates of TB highlighted the urgent need for effective diagnostic and treatment strategies. In clinical practice, Xpert MTB/RIF (Xpert),

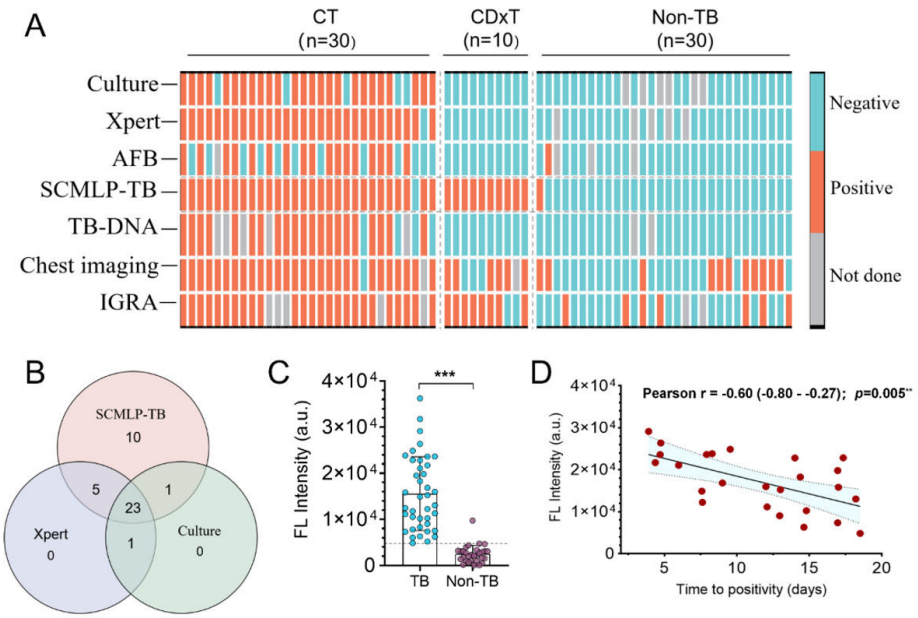

Figure 2. SCMLP-TB's diagnostic performance in participants. (**A**) Clinical and assay data for adults classified with CT cases, CDxT cases and non-TB cases. TB cases were comprised of CT and CDxT cases. (**B**) Venn diagram of overlap in TB. The Venn diagram displayed 40 participants with TB detected by each diagnostic test and the overlap between tests. (**C**) SCMLP-TB signals of samples from TB and non-TB patients. Two-sided *p* values between these two groups were obtained by the Mann-Whitney *U* tests. Dashed line denoted the threshold values ascertained by constructing ROC curve (Figure S1). (**D**) Correlation of FL intensity and the TTP of culture-positive samples (n=25). Data points represented individual patient values. Solid lines represented the linear regression line and shaded areas represented the 95% CIs. FL intensity was presented in a.u.. Pearson correlation was used to assess the statistical significance of the relationship between FL intensity and TTP. SCMLP-TB, single cell metabolic labeling probe for tuberculosis; TB, tuberculosis; CT, confirmed tuberculosis; CDxT, clinically diagnosed tuberculosis; ROC, receiver operating characteristic; FL, fluorescence; TTP, time to positivity; a.u., arbitrary units; Culture, MTB culture; Xpert, Xpert MTB/RIF; AFB, acid-fast bacilli; IGRA, interferon-gamma release assay; CIs, confidence intervals; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Table 2. Diagnostic performance of culture, Xpert, and SCMLP-TB for tuberculosis compared to final clinical diagnosis

	Sensitivity (95% CI; n/N)	Specificity (95% CI; n/N)	PPV (95% CI; n/N)	NPV (95% CI; n/N)
Culture	62.5% (45.8-76.8; 25/40)	100.0% (82.8-100.0; 24/24)	100.0% (83.4-100.0; 25/25)	61.5% (44.7-76.2; 24/39)
Xpert	72.5% (55.9-84.9; 29/40)	100.0% (83.4-100.0; 25/25)	100.0% (85.4-100.0; 29/29)	69.4% (51.7-83.1; 25/36)
SCMLP-TB	97.5% (85.3-99.9; 39/40)	96.7% (80.9-99.8; 29/30)	97.5% (85.3-99.9; 39/40)	96.7% (80.9-99.8; 29/30)

PPV, positive predictive value; NPV, negative predictive value. Xpert, Xpert MTB/RIF. SCMLP-TB, single cell metabolic labeling probe for tuberculosis.

culture, AFB, and mNGS are used for TB diagnosis. These methods exhibited varying levels of sensitivity, with Xpert demonstrating high sensitivity (about 67-89%), culture showing limited sensitivity (50-70%), AFB exhibiting lower sensitivity (30-70%), and mNGS providing moderate sensitivity (*24-27*). These methods were still limited by the requirement for a relatively high bacterial load. Fluorescence-based metabolic labeling techniques had great potential for diagnosis and therapeutic evaluation owing to their high sensitivity (*28*). However, the chemical interaction of Auramine O with MTB was constrained by incomplete dye binding, resulting in low accuracy of only about 80% (*29*). Quantitative PCR faced challenges in differentiating live from dead bacteria and was limited by accessibility and cost in resource-limited settings (*4*). Our study designed a metabolic labeling probe (SCMLP-TB) to engage in MTB cell wall synthesis *via* antigen-85 (Ag85), achieving rapid, sensitive and accurate tuberculosis diagnosis and demonstrated great promise for point-of-care testing diagnostic applications (*11)*.

This study retrospectively evaluated the diagnostic accuracy of SCMLP-TB for the rapid detection of pulmonary TB in a real-world setting for the first time. Our data demonstrated that SCMLP-TB was an effective diagnostic tool for detecting pulmonary TB in clinical settings with 97.5% sensitivity and 96.7% specificity, significantly superior to culture and Xpert. Remarkably, the diagnostic accuracy for patients with negative culture and Xpert tests was 100%, suggesting that SCMLP-TB had excellent performance in detecting samples with low bacterial loads, such as individuals co-infected with HIV (*24*).

In this study, the sensitivities for culture and Xpert respectively were 62.5% and 72.5% compared to final clinical diagnosis by the diagnosis criteria for pulmonary tuberculosis (WS 288-2017), while both exhibited specificities of 100.0%. Indeed, our research data was essentially consistent with the reported results (*24*). Patients with subclinical TB were prone to being overlooked during screening due to the absence of obvious symptoms (*30*), which heightened the urgency to develop novel detection methods capable of identifying such patients. Previous data indicated that subclinical tuberculosis represented a median of 50.4% of all TB cases, with approximately one-third of cases in China classified as subclinical (*31,32*). Employing a combination of chest imaging and SCMLP-TB technology for asymptomatic screening is expected to enhance the detection rate of subclinical TB and is of great significance in controlling the spread of the disease. In addition, individuals co-infected with HIV are more susceptible to a variety of infections owing to their impaired immune systems, with TB being one of the most frequent opportunistic infections (*33*). A meta-analysis revealed that the sensitivity of Xpert was relatively inferior in patients with HIV-associated tuberculosis (*24*). In this study, among a total of 5 TB-HIV co-infected participants, Xpert managed to correctly diagnose merely three cases; in contrast, the SCMLP-TB test presented positive results for all five patients. Although this subgroup was too small to estimate assay performance, SCMLP-TB demonstrated a potential advantage in diagnosing TB co-infected with HIV.

While the current study illustrated the potential of SCMLP-TB in accurate TB diagnosis, it was a preliminary investigation with several limitations and thus future large-scale, multicenter prospective studies are warranted for further validation. Firstly, our study had an insufficient sample size and all the samples were from a single center. Secondly, the study failed to take children into account, thereby overlooking a substantial clinical necessity for enhanced TB diagnostic methods within pediatric populations. Thirdly, the sample size of immunosuppressed patients was limited, including only 13 individuals co-infected HIV infection and 11 with diabetes. The scarcity of participants with these specific conditions made it difficult to draw definitive conclusions about the effectiveness of the test in these sub-populations. Despite the small sample size in these subgroups, SCMLP-TB managed to diagnose all TB patients co-infected with HIV.

In conclusion, we were the first to report the application of the SCMLP-TB test on sputum or BALF specimens and showed its excellent diagnostic accuracy for pulmonary TB, particularly in patients with paucibacillary TB. *Via* one-step labeling and centrifugation, SCMLP-TB realized simple and high-throughput MTB detection by microplate reader, representing a clinically accessible and low-cost assay (~10 cents per test) for TB diagnosis. Future development will focus on an automated sample processing, fluorescence imaging, and AI-assisted analysis for efficient sample analysis.

Considering the current burden of TB and the shortcomings of existing diagnostic technologies, the implementation of the user-friendly SCMLP-TB was likely to enhance the diagnosis and treatment monitoring of TB infections that presented with a variety of symptoms and low bacterial loads.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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[§]These authors contributed equally to this work.

**Address correspondence to:*
Hongzhou Lu and Pengfei Zhao, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital, Southern University of Science and Technology, Shenzhen, 518112, China.
E-mail: luhongzhou@fudan.edu.cn (HL); pengfeizhao@aliyun.com (PZ)

Yang Zhou, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital, Southern University of Science and Technology, Shenzhen, 518112, China; Molecular Biology Research Center & Center for Medical Genetics, School of Life Sciences, Central South University, Changsha, 410078, China.
E-mail: yangzhou@szsy.sustech.edu.cn

Mingbin Zheng, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital, Southern University of Science and Technology, Shenzhen, 518112, China; The Affiliated Dongguan Songshan Lake Central Hospital, Guangdong Medical University, Dongguan, 523808, China.
E-mail: mingbinzheng@126.com

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