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Article

A Novel Chemiluminescent Immunoassay for Urinary LAM Detection Improves Tuberculosis Diagnosis in HIV Cohorts

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Abstract

Aims/Background: Tuberculosis (TB) is a highly prevalent opportunistic infection and a leading cause of mortality worldwide, particularly among those living with human immunodeficiency virus (HIV). Among these patients, the diagnostic accuracy of conventional TB testing is generally limited by atypical clinical manifestations, the frequent occurrence of extrapulmonary TB, challenges in obtaining sputum samples, and consistently low bacterial loads in clinical specimens. Therefore, this study assesses the diagnostic performance of a novel chemiluminescence immunoassay (CLIA) for detecting urinary lipoarabinomannan (LAM) in individuals coinfected with HIV and TB. **Methods**: A prospective cohort study was conducted involving 297 people living with HIV (PLWH) recruited from Beijing Youan Hospital, Capital Medical University, China, between May 2021 and December 2024. By September 2025, when this analysis was finalized, all patients had received at least 6 months of follow-up. Of these, 76 patients had TB (34 definite cases and 42 probable cases), while 221 non-TB patients with confirmed alternative diagnoses were included as a control group. A novel double-antibody sandwich CLIA was specifically designed to detect the *Mycobacterium tuberculosis* LAM antigen in urine specimens. For comparative evaluation, all samples were concurrently analyzed using the commercially available Determine™ TB LAM Ag test (AlereLAM, Abbott Laboratories, USA). Investigators were blinded to the laboratory test results until the completion of enrollment, and laboratory technicians were blinded to patients' diagnoses throughout the study duration. **Results**: Receiver operating characteristic (ROC) analysis showed an area under the curve (AUC) of 0.925 at the optimal cutoff of 1.46. Median CLIA values increased in a graded manner across groups: 0.98 in the non-TB group, 1.96 in the probable TB group, and 4.46 in the definite-TB group ($p < 0.001$). Compared with AlereLAM, CLIA demonstrated significantly higher sensitivity (85.3% vs. 58.8%) but lower specificity (89.6% vs. 98.2%). Neither LAM-CLIA nor AlereLAM exhibited a significant association with patient cluster of differentiation (CD) 4^+ T cell counts ($p > 0.05$). **Conclusion**: CLIA-based urinary LAM detection demonstrates superior sensitivity compared with conventional lateral flow assays, providing a rapid and quantitative approach for detecting HIV-associated TB. This method has significant potential to improve early TB detection in high-risk populations, particularly where culture-based methods are unavailable.

Keywords: electrochemiluminescence; diagnosis; HIV seropositivity; lipoarabinomannan; tuberculosis

1. Introduction

Tuberculosis (TB) is a leading cause of mortality worldwide among individuals living with human immunodeficiency virus (HIV), accounting for approximately one-third of all acquired immunodeficiency syndrome (AIDS)-related deaths. In 2023, an estimated 161,000 HIV-associated TB deaths were reported, corresponding to an approximately 18-fold higher TB risk in this population than among immunocompetent individuals [1].

Among people living with HIV (PLWH), the high burden of extrapulmonary TB (EPTB) and the inability of a significant proportion of individuals (up to 63%) to produce sputum considerably limit the effectiveness of sputum-based diagnostic approaches [2]. In recent years, substantial advances have been made in TB diagnostics using lipoarabinomannan (LAM), a mycobacterial cell wall glycolipid that contributes to the pathogenesis of *Mycobacterium tuberculosis* (MTB) and modulates the host immune response [3,4]. LAM has emerged as a potent diagnostic biomarker in hospitalized PLWH and symptomatic PLWH [5]. Its detection is particularly convenient because it requires only a urine sample and does not rely on sputum, offering a relatively cost-effective and rapid diagnostic option [6].

In 2019, the World Health Organization (WHO) conditionally recommended the AlereLAM lateral flow (LF) assay for use in hospitalized PLWH with advanced immunosuppression [7]. The assay has demonstrated clinical benefits by facilitating early detection of TB in this high-risk cohort [8,9]. However, its sensitivity remains modest (approximately 40–60%) and is primarily effective in patients with cluster of differentiation (CD) 4^+ T-cell counts below 100 cells/µL [10]. Recent advances, including improved urine-concentration techniques and the application of chemiluminescence immunoassay (CLIA), have substantially enhanced the analytical sensitivity of uri-

nary LAM detection [11]. CLIA-based platforms, by providing superior analytical sensitivity and quantitative assessments, offer a promising alternative for the quantification of urinary LAM. Unlike visual-read LF assays, CLIA enables objective quantification of LAM concentrations, which may be advantageous for detecting low-bacillary load and EPTB.

This prospective diagnostic trial was designed to develop and validate novel, highly accurate laboratory methods for the diagnosis of tuberculosis in PLWH. In this study, we evaluated a novel CLIA-based urinary LAM assay in a cohort of 297 PLWH. We hypothesized that automated CLIA-based LAM detection would demonstrate superior diagnostic performance compared with conventional LF-LAM assays in PLWH. The specific objectives were to evaluate the diagnostic accuracy of LAM-CLIA using microbiologically confirmed TB cases as the reference standard and to determine optimal cutoff values for HIV-positive populations.

2. Methods

2.1 Study Design and Recruitment of Study Participants

This prospective study evaluated the diagnostic performance of chemiluminescent urine LAM for detecting TB in PLWH compared with the AlereLAM assay. The reference standards included composite sputum smear microscopy, culture, GeneXpert, and TB-DNA testing. A cohort of 297 PLWH were prospectively enrolled at Beijing Youan Hospital, Capital Medical University, China, between May 2021 and December 2024. Final diagnoses were confirmed by integrating clinical manifestations, biochemical results, histopathological and radiological data, and microbiological and nucleic acid amplification test (NAAT) findings. All participants were monitored for at least 6 months, up to September 2025, to determine diagnostic stability.

Patients were included in the study if they met the following criteria: (1) those provided written informed consent; (2) individuals undergoing clinical evaluation for suspected TB as per national guidelines; and (3) had at least one valid specimen (e.g., sputum, blood, urine) collected both for routine TB diagnostic testing and for the study-specific LAM assay. However, they were excluded if any of the following criteria applied: (1) inability or unwillingness to provide informed consent; (2) those who have already received anti-tuberculosis treatment for more than 72 hours during the current illness episode; and (3) insufficient volume or inadequate quality of the specimen for required testing. All eligible patients meeting the inclusion criteria and none of the exclusion criteria were consecutively enrolled to avoid selection bias.

2.2 Diagnosis of HIV

HIV infection was diagnosed according to national guidelines [12]. Diagnosis required either (1) a positive HIV antibody screening test confirmed by a positive supplemental test (confirmatory antibody assay, qualitative nucleic acid test, or quantitative nucleic acid test with a viral load of $>$1000 copies/mL), or (2) documented HIV seropositivity based on recent epidemiological risk factors or AIDS-defining clinical manifestations together with two positive HIV nucleic acid tests.

2.3 Classification of TB Patients

Study participants were categorized as definite TB, probable TB, and non-TB patients. Definite TB was defined by microbiological or pathological evidence, with any of the following: positive culture (MTB complex isolated from sputum, bronchial lavage fluid, or other clinical specimens); positive molecular test (Xpert MTB/RIF or Xpert Ultra detecting MTB DNA, with *rpoB* gene mutation status if applicable); positive acid-fast staining ($\geq$1 acid-fast bacillus observed in sputum or tissue specimens) combined with clinical imaging consistent with active TB.

Probable TB was defined as those without microbiological confirmation but meeting all of the following criteria: clinical symptoms ($\geq$2) such as cough $>$2 weeks, hemoptysis, night sweats, unexplained weight loss ($>$10%), persistent fever $>$2 weeks; chest computed tomography (CT) or X-ray showing typical TB lesions (e.g., upper lobe cavitation, tree-in-bud sign, multiple nodules with mediastinal lymphadenopathy); and clinical and/or radiological improvement after $\geq$2 months of empirical anti-TB therapy, as assessed by an independent, blinded expert panel.

This standardized classification, while lacking microbiological confirmation, employed objective measures and blinded adjudication to minimize misclassification bias. Sensitivity analyses revealed that reclassification of this group did not alter the primary conclusions regarding LAM-CLIA's superior diagnostic performance compared with AlereLAM.

Furthermore, patients were classified as non-TB if there was no microbiological evidence of TB and either an alternative diagnosis for their symptoms or sustained clinical and radiological improvement over a minimum 6-month follow-up period without anti-TB treatment, consistent with prior diagnostic criteria [13].

All laboratory personnel performing CLIA and AlereLAM assays were blinded to clinical diagnosis, comparator assay results (Xpert/culture), and other laboratory data. Specimens were de-identified with unique barcodes and analyzed in randomly ordered batches.

This study adopted a prospective diagnostic accuracy design, comparing the novel LAM-CLIA assay with the AlereLAM in a paired manner within the same patient cohort. A formal sample size calculation was not performed due to the exploratory nature of this initial clinical evaluation and the challenges in recruiting a large number of microbiologically confirmed TB cases at a single center. Instead, all eligible patients during the study period were

enrolled to maximize the sample size. As a paired diagnostic accuracy study, the sample size was estimated using McNemar's test for correlated proportions. With 34 confirmed TB cases, assuming 20% discordant pairs and α = 0.05, the study has 81% power to detect a significant difference between LAM-CLIA and AlereLAM, supporting the adequacy of the sample for the primary comparison.

All testing personnel completed standardized training, including 8 hours of theoretical instruction on Good Clinical Laboratory Practice, 20 supervised assay runs using blinded quality control samples, and certification requiring >90% concordance with reference results. Standard operating procedures (SOPs) were strictly followed for specimen processing, daily calibration using manufacturer-provided standards, and monitoring of internal quality controls within pre-defined ranges. Automated alerts were generated when the coefficient of variation between duplicates exceeded 15%. TB case classification was validated by an expert panel that was blinded to all LAM assay results.

2.4 Data Collection

Demographic and clinical data, including age, gender, duration of HIV infection, duration of TB-related symptoms, $CD4^+$ T-cell count, and HIV viral load, were systematically extracted from electronic medical records for all enrolled participants. A predefined stratification analysis was performed by categorizing patients into three groups based on $CD4^+$ T-cell count (<100 cells/μL, 100–200 cells/μL, and >200 cells/μL) to evaluate how the degree of immunosuppression affected the diagnostic performance of the LAM assay.

2.5 Urine Specimen Collection

Urine samples were collected under standardized conditions. A required minimum volume of 10 mL was collected for each sample. To ensure accuracy, midstream urine was preferentially obtained, and samples with excessive protein, lipid content, or other visible contaminants were excluded to reduce analytical interference. After collection, samples were stored at room temperature and analyzed within 72 hours. If testing was delayed, samples were stored at 2–8 °C for up to 7 days. However, for longer storage, they were frozen at –15 °C or lower. To prevent degradation, freeze-thaw cycles were minimized to a maximum of three per sample.

2.6 Laboratory TB Diagnostic Methods

2.6.1 Sputum Smears

Acid-fast staining of sputum smears was performed in strict compliance with the manufacturer's instructions and the National Guidelines for Laboratory Detection of Tuberculosis. Microscopic examination was used to detect MTB in sputum samples. Acid-fast staining reagents were provided by BaSO Zhuhai Bioscience Co., Ltd. (lot number: C230802, Zhuhai, China).

2.6.2 Mycobacterium Tuberculosis Culture and Identification

Sputum specimens were transferred to pre-treated screw-capped tubes, mixed with an equal to double volume of 4% NaOH, and vortexed vigorously for 30 s until complete liquefaction. The mixture was then incubated at room temperature for 15 min to facilitate digestion. Using a sterile graduated pipette, 0.1–0.15 mL of the digested sample was aseptically inoculated onto Löwenstein-Jensen medium slopes. Inoculated tubes were incubated horizontally with the medium slopes facing upward and incubated at 36 ± 1 °C for 24 ± 2 hours, then repositioned vertically while maintaining the same temperature. Colony growth was examined on days 3 and 7 post-inoculation and subsequently at weekly intervals of up to 8 weeks. Presumptive mycobacterial colonies were identified using the Bruker MALDI Biotyper system (microflex LT/SH) (Bruker Daltonics GmbH & Co. KG, Bremen, Germany).

2.7 GeneXpert

According to the manufacturer's instructions, the processing solution was mixed with the specimen at a 1:2 volumetric ratio. The mixture was vortexed vigorously for 30 s and incubated at room temperature (20–25 °C) for 15 min. After that, the mixture was vortexed again for 30 s and incubated for an additional 5 min to ensure complete liquefaction. The processed sample was then transferred to a test cartridge and analyzed using the Xpert MTB/RIF assay (GeneXpert® IV system; Cepheid, USA).

2.8 TB-DNA Detection

The specimens were transferred into sterile sputum containers for laboratory testing. MTB DNA was detected using the TB-DNA PCR Fluorescence Detection Kit (DaAn Gene Co., Ltd., Sun Yat-sen University, Guangzhou, China; production lot number: C202407002), following the manufacturer's instructions. Four routine laboratory tests—sputum smear microscopy, MTB culture and identification, GeneXpert MTB/RIF assay, and TB-DNA PCR—were conducted in an ISO 15189-accredited laboratory (CNAS MT0269). All procedures were performed by trained personnel in strict accordance with SOPs for specimen processing, and each assay included both positive and negative quality controls to ensure analytical reliability.

2.9 Urine LAM Detection by Chemiluminescence

This study developed a sandwich CLIA using mouse monoclonal antibodies targeting MTB-LAM for magnetic bead coating and horseradish peroxidase (HRP) (lot number: JB23BA0002, Sangon Biotech, Shanghai, China), enabling quantitative detection of LAM antigens in urine. During the assay, the capture antibody, HRP-labeled antibody, and urine sample were co-incubated at 37 °C for 10 min to promote formation of the immune complex (capture antibody-LAM-HRP-labeled antibody). A four-step

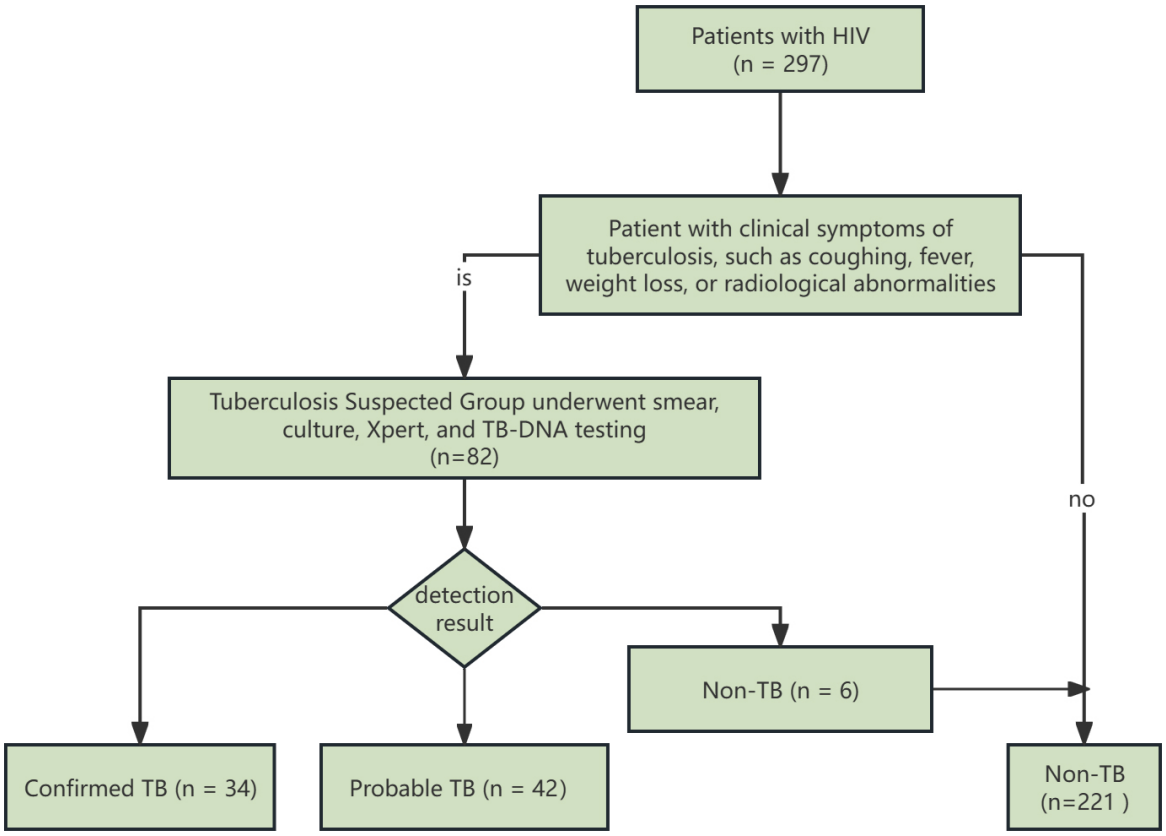

Fig. 1. A flow chart of the study participant selection. TB, tuberculosis; HIV, human immunodeficiency virus; non-TB, non-tuberculosis. The flow chart was created using ProcessOn (https://www.processon.com/, ProcessOn Information Technology Co., Ltd., Beijing, China).

washing procedure was then performed to remove non-specifically bound substances, after which a luminol-based chemiluminescent substrate was added, and photon counts were measured. The capture and labeled antibodies were self-developed by Tianjin YIRUI Biological Technology Co., Ltd. (lot number: LAM250510, Tianjin, China).

The limit of detection (LoD) for the LAM-CLIA assay was determined experimentally. Briefly, a standard curve was generated using a serial dilution of purified LAM antigen in a negative urine matrix. Each concentration was tested in multiple replicates ($n \geq 5$), including zero-concentration (blank) samples. The LoD was defined as the lowest analyte concentration that produced a chemiluminescence signal greater than or equal to the mean signal of the blank replicates plus 3 times their standard deviation (Mean blank + 3SD). The linear range of the assay was established by linear regression analysis of the dose-response data. The concentration interval with a coefficient of determination (R^2) greater than 0.98 was defined as the linear range.

2.10 AlereLAM Test Procedure

Urine samples (60 µL) were applied to the sample pad (white zone marked by an arrow), following the manufacturer's instructions. The test strip was incubated for at least 25 min, and the results were recorded under standard indoor lighting by visual inspection of the patient's result window. For interpretation, the reference scale card (provided with the kit) was placed adjacent to the window. The Alere Determine TB LAM Ag test (Abbott, Chicago, IL, USA) was used according to the manufacturer's instructions, with test kits from multiple lots employed during the 2021–2025 study period.

2.11 Statistical Analysis

Statistical analyses were performed using the SPSS version 20.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed variables were presented as mean ± standard deviation and compared using one-way analysis of variance (ANOVA), followed by a Bonferroni post hoc test for multiple comparisons. Non-normally distributed data were expressed as median and interquartile range (IQR), with the Mann–Whitney U test used for comparisons between two groups and the Kruskal–Wallis test with Dunn-Bonferroni post hoc test for comparisons across three or more groups. Categorical variables were reported as frequencies and percentages and were compared using

Table 1. Baseline characteristics of the study participants based on TB status.

Variable	TB		Non-TB	Statistics
	Definite-TB	Probable TB		
Number of patients	34	42	221	
Age (years, mean ± SD)	36.56 ± 13.09	39.17 ± 10.44	41.78 ± 10.21	F = 4.159, p = 0.017
Gender				
Male	33 (97.06%)	41 (97.62%)	217 (98.19%)	p = 0.481
Female	1 (2.94%)	1 (2.38%)	4 (1.81%)	
HIV duration (years)	1.00 (1.0, 2.0)	1.00 (1.0, 2.0)	3.00 (2.0, 4.0)	H = 75.192, $p < 0.001$
TB symptom duration (weeks)	2.00 (1.0, 4.0)	2.0 (1.0, 3.3)	-	z = –0.238, p = 0.812
Pulmonary TB	31	33	-	
Extra-pulmonary TB	3	9	-	
Lymph nodes	2	2	-	
Meninges	0	1	-	
Pleura	0	3	-	
Intestine	1	1	-	
Muscle	0	2	-	

Comparison of the three groups: definite TB group, probable TB group, and non-TB group. Gender was expressed as a number (percentage). Since the contingency table contained cells with expected counts less than 5, the Fisher-Freeman-Halton exact test was used to compare the gender distribution across groups. HIV duration (years) was compared using non-parametric tests: Kruskal–Wallis test, and the Bonferroni correction method was used for pairwise comparisons. TB symptom duration was compared using non-parametric Mann–Whitney test. Age was compared using analysis of variance (ANOVA) test, and pairwise comparisons were made using the Bonferroni correction.

TB, tuberculosis; HIV, human immunodeficiency virus; SD, standard deviation.

Pearson's chi-squared test or the Fisher-Freeman-Halton exact test, as appropriate.

Predictive performance was evaluated using receiver operating characteristic (ROC) curve analysis to determine optimal cutoff values for continuous predictors and to assess overall diagnostic performance. The DeLong test was used to compare areas under the curves (AUCs), and McNemar's test was employed to compare paired sensitivity and specificity. A two-tailed p-value of <0.05 was considered statistically significant.

3. Results

3.1 Baseline Characteristics Study Participants

A prospective cohort of 297 PLWH was systematically recruited from Beijing Youan Hospital, Beijing, China (Fig. 1). Based on definite diagnostic outcomes and 6-month follow-up, the study population included 34 patients with definite TB, 42 with probable TB, and 221 non-TB controls. Sex distribution did not differ significantly across groups (p = 0.481). Conversely, age, duration of HIV infection, and $CD4^+$ T-cell count differed significantly across groups ($p < 0.05$). Patients in the non-TB group were considerably older than those in the definite TB group (p = 0.017). Both the duration of HIV infection and $CD4^+$ T-cell levels were also significantly higher in the non-TB group than those in the definite and probable TB groups ($p < 0.05$) (Tables 1,2).

3.2 ROC Curve Analysis of Urine LAM (CLIA) Detection

Because the probable TB group lacked pathogenic evidence, there was an inherent risk of misdiagnosis. Therefore, the probable TB group was temporarily excluded to calculate a robust, minimally biased LAM-CLIA diagnostic cutoff, using a high-confidence reference standard composed of microbiologically confirmed definite TB cases and clearly defined non-TB controls.

The ROC curve analysis was performed using the definite TB group as the positive reference and the non-TB group as the negative reference to evaluate the discriminatory performance of urine LAM levels detected by CLIA. The analytical sensitivity and linearity of the LAM-CLIA assay were rigorously evaluated. The limit of detection (LoD) was determined at 50 pg/mL, and the assay demonstrated a broad dynamic range, with excellent linearity from 50 pg/mL to 50,000 pg/mL. The ROC analysis yielded the following findings: the optimal diagnostic threshold (based on the Youden index) was 1.460, with a sensitivity of 85.3% and a specificity of 89.6%. The AUC for distinguishing definite TB from non-TB cases was 0.925 (95% confidence interval [CI]: 0.871–0.980). At the optimal cutoff of 1.460, the Youden index reached 0.749 (Fig. 2).

Table 2. The results of pairwise comparisons among the three groups for CD4.

	Group	Median (Q1, Q3)	H	*p*-value	Note
$CD4^+$ T-cell counts (cells/μL)	Non-TB	598 (413.5, 768.0)	140.953	<0.001	p 1 < 0.001
	Probable TB	146 (43.5, 251.3)			p 2 = 0.386
	Definite TB	64.5 (21.0, 115.8)			p 3 < 0.001

Bonferroni post hoc multiple comparison results for cluster of differentiation (CD) 4 count across the three groups. p 1 = Non-TB vs. Probable TB; p 2 = Probable TB vs. Definite TB; p 3 = Non-TB vs. Definite.

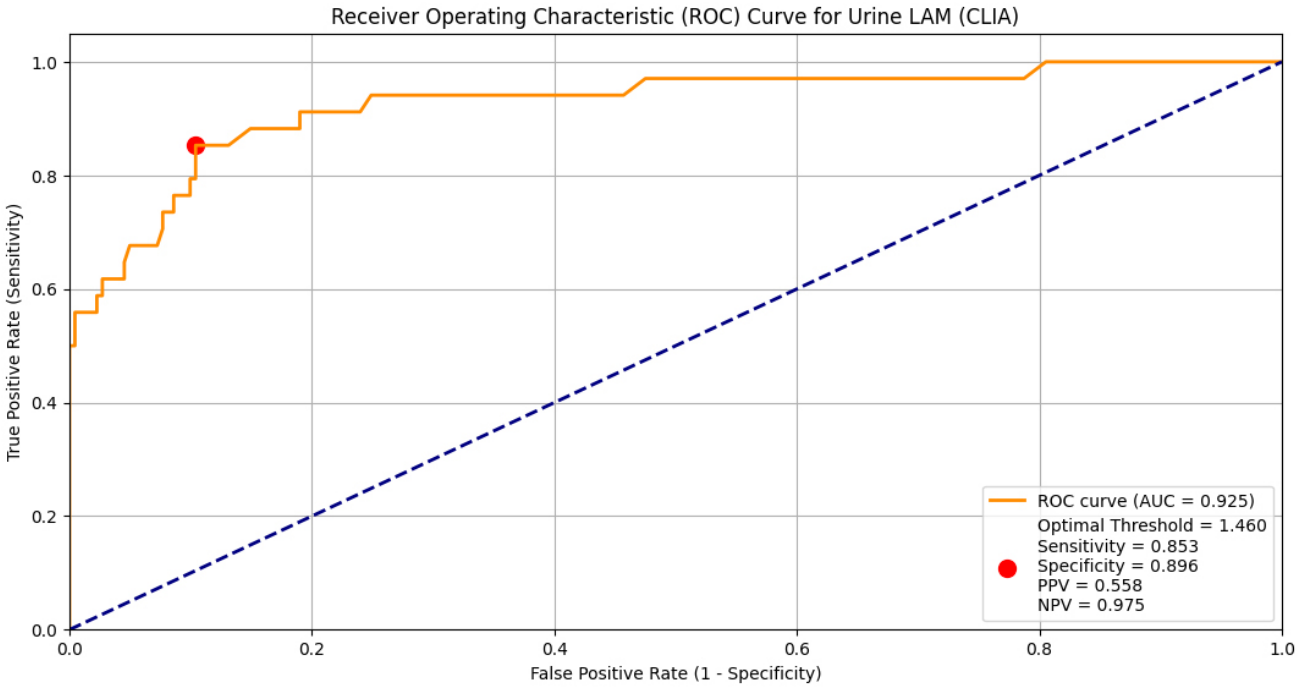

Fig. 2. Receiver operating characteristic (ROC) curve analysis results of urine LAM (CLIA) detection. PPV, positive predictive value; NPV, negative predictive value; CLIA, chemiluminescence immunoassay; LAM, lipoarabinomannan; AUC, area under the curve. The Youden index is an important metric for evaluating the performance of binary classification models. It identifies the optimal decision threshold that balances sensitivity and specificity. Its value ranges from 0 to 1, with higher values indicating stronger discrimination performance.

3.3 Comparative Performance of LAM-CLIA and AlereLAM Positive Rates Across $CD4^+$ Strata in HIV-Associated TB Diagnosis

In the CD4-stratified analysis, the LAM-CLIA and AlereLAM findings for HIV/TB coinfection (definite and probable TB groups) are shown in Table 3. The positivity rates for both LAM-CLIA and AlereLAM exhibited a clear inverse relationship with $CD4^+$ T-cell counts, declining progressively as $CD4^+$ counts increased. Although this inverse trend was suggestive for both assays, it did not reach formal statistical significance in this cohort (AlereLAM: $\chi^2 = 5.254$, $p = 0.072$; LAM-CLIA: $\chi^2 = 4.81$, $p = 0.09$). Notably, across all $CD4^+$ strata, the positivity rate of LAM-CLIA consistently exceeded that of AlereLAM, indicating superior sensitivity, particularly among patients with severe immunosuppression.

3.4 Stratified Analysis of Urine LAM-CLIA Levels Across Diagnostic Categories

Urine LAM-CLIA levels were compared among the definite TB, probable TB, and non-TB groups (Table 4). Median LAM-CLIA values increased progressively across the three groups (definite TB > probable TB > non-TB), with overall differences achieving statistical significance ($p < 0.05$). Pairwise comparisons revealed that both the definite TB and probable TB groups had significantly higher LAM-CLIA values than the non-TB group ($p < 0.05$). In contrast, the difference between the definite TB and probable TB groups was not statistically significant ($p > 0.05$).

3.5 Diagnostic Performance of the Urine LAM-CLIA Assay Versus the AlereLAM Assay for TB Detection

ROC curve analysis was conducted to assess the ability of urine LAM-CLIA and AlereLAM assays to differentiate the definite TB group from the non-TB group. The

Table 3. Comparison of LAM-CLIA and AlereLAM positive rates across CD4$^+$ strata in HIV-associated TB diagnosis.

		CD4 (%)			Total	χ^2	p-value
		<100 cells/μL	100–200 cells/μL	>200 cells/μL			
AlereLAM	Negative	12 (32.43)	11 (50.00)	11 (64.71)	34 (44.74)		
	Positive	25 (67.57)	11 (50.00)	6 (35.29)	42 (55.26)	5.254	0.072
Total		37	22	17	76		
LAM-CLIA	Negative	6 (16.22)	8 (36.36)	7 (41.18)	21 (27.63)		
	Positive	31 (83.78)	14 (63.64)	10 (58.82)	55 (72.37)	4.81	0.09
Total		37	22	17	76		

Pearson's chi-square test (cross-analysis) was used to compare intergroup differences; $p < 0.05$ indicates significant differences.

Table 4. Pairwise comparison of LAM-CLIA levels across three groups.

		Median (Q1, Q3)	H	p-value	Note
LAM-CLIA levels	Non-TB	0.98 (0.8, 1.2)	95.577	<0.001	p 1 < 0.001
	Probable TB	1.96 (1.1, 4.1)			p 2 = 0.224
	Definite TB	4.46 (1.5, 92.4)			p 3 < 0.001

LAM-CLIA data were not normally distributed, and non-parametric statistical analysis was performed using the Kruskal–Wallis test. Pairwise comparisons were conducted using the Bonferroni correction method, p 1 = Non-TB vs. Probable TB; p 2 = Probable TB vs. Definite TB; p 3 = Non-TB vs. Definite TB.

DeLong test was used to compare the AUCs of two assays (Table 5). LAM-CLIA achieved an AUC of 0.874, and AlereLAM yielded an AUC of 0.785; the difference was statistically significant ($p = 0.024$). These ROC findings indicate the performance after final binary classification. Minor shifts in continuous AUC values are expected.

LAM-CLIA showed substantially higher sensitivity than AlereLAM (85.3% vs. 58.8%, $p = 0.004$), whereas its specificity was lower (89.6% vs. 98.2%, $p < 0.001$).

Recall rate analysis (Fig. 3) revealed distinct precision-recall dynamics between the two assays. LAM-CLIA demonstrated a lower initial precision of approximately 0.5–0.6, compared with 0.8–0.9 for AlereLAM. However, as the recall rate increased, LAM-CLIA maintained relatively stable precision until the recall rate exceeded 0.8, after which precision gradually declined. In contrast, AlereLAM exhibited a sharp, sustained decline in precision once its recall rate exceeded 0.6. This pattern indicates that LAM-CLIA offers stable diagnostic performance across a wider range of recall rates and is more suited for screening scenarios where higher sensitivity is required.

3.6 Heatmap of Test Results for 34 Patients With Definite TB (Smear Microscopy, MTB Culture, Xpert MTB/RIF, TB-DNA, AlereLAM, and Urine LAM CLIA)

A heatmap was developed by ranking LAM-CLIA values in the definite TB group from lowest to highest (Fig. 4). Urine LAM-CLIA demonstrated the highest sensitivity, detecting TB in 29 of 34 patients (85.3%), followed by AlereLAM, which showed TB in 20 of 34 (58.8%). Traditional approaches showed lower positivity rates: smear microscopy in 11 of 34 (32.4%), culture in 12 of 34 (35.3%), Xpert in 15 of 34 (44.1%), and TB-DNA in 12 of 34 (35.3%).

3.7 Analytical Performance Validation

To evaluate the reproducibility of the LAM-CLIA assay, both intra-assay and inter-assay precision were determined. For intra-assay precision, a urine sample with a low LAM concentration within the linear range was analyzed 10 times in a single run, yielding a coefficient of variation (CV) of 4.10%. For inter-assay precision, the same sample was evaluated across three independent assay runs, demonstrating a CV of 5.09%. Both CV values were below 10%, indicating acceptable assay reproducibility for clinical application.

3.8 The Impact of Other Opportunistic Infections on the Performance of LAM-CLIA Detection

To evaluate whether common opportunistic infections influenced the specificity of LAM-CLIA, a subgroup analysis was conducted with the non-TB cohort. Among 221 'non-TB' control subjects, 47 (21.3%) were diagnosed with at least one opportunistic infection, including cryptococcosis (n = 9), Pneumocystis pneumonia (n = 16), cytomegalovirus viremia (n = 13), and disseminated fungal infections (n = 9). In this subgroup (n = 47 patients), the specificity of LAM-CLIA was 87.2% (41/47), which did not differ substantially from the overall specificity in the entire 'non-TB' control group (89.6%, $p = 0.65$, chi-square test). Notably, among the 9 patients with confirmed cryptococcosis, only 1 had a false-positive LAM-CLIA result.

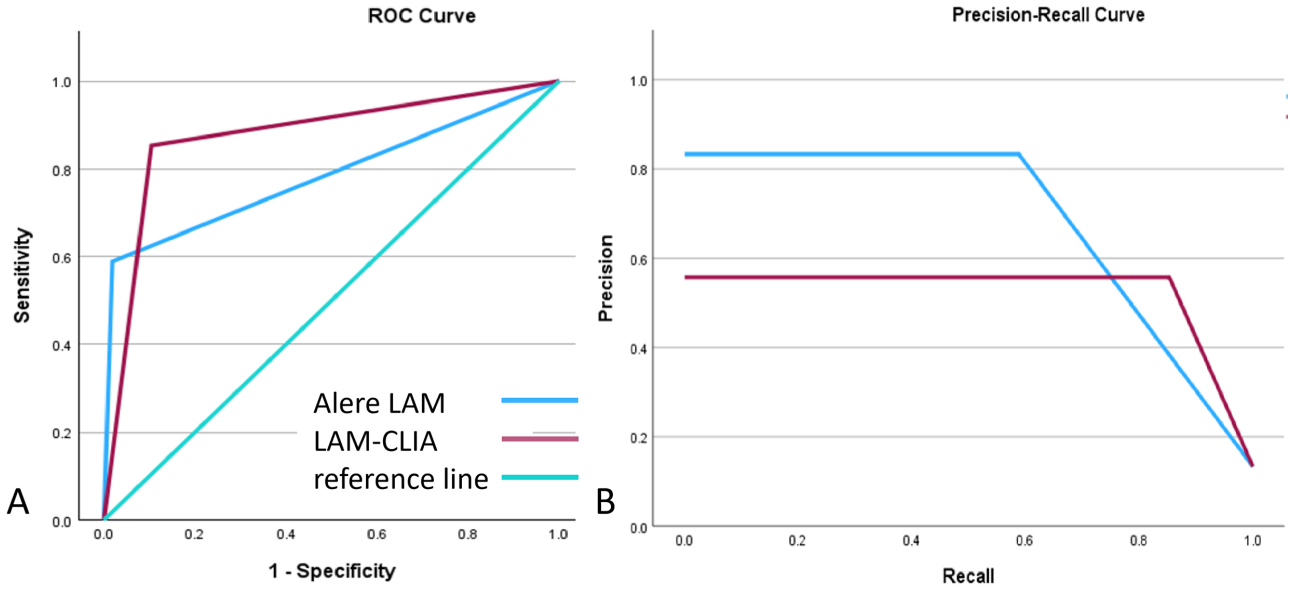

Fig. 3. Comparison of diagnostic performance between LAM-CLIA and AlereLAM assays. (A) ROC curves comparing the overall diagnostic performance of LAM-CLIA and AlereLAM for tuberculosis detection in the study cohort. (B) Precision-recall curves demonstrating the trade-off between positive predictive value (precision) and sensitivity (recall) across different classification thresholds. The model was considered acceptable when the area under the curve exceeded 0.5. A larger area under the precision-recall curve (AUC-PR) indicates better diagnostic performance.

Table 5. ROC curve analysis of LAM-CLIA and AlereLAM.

	AUC	95% CI	Standard error	*p*-value	Sensitivity	Specificity	Delong test
LAM-CLIA	0.874	0.712–0.978	0.053	<0.001	0.853	0.896	$Z = 2.26$, $p = 0.024$
AlereLAM	0.785	0.712–0.858	0.037	<0.001	0.588	0.982	

According to the DeLong test results, combined with the generated Z-value and *p*-value for difference judgment, a *p*-value < 0.05 indicates a significant difference between the two AUCs. In contrast, a *p*-value ≥ 0.05 indicates no significant difference. AUC, area under the curve; CI, confidence interval.

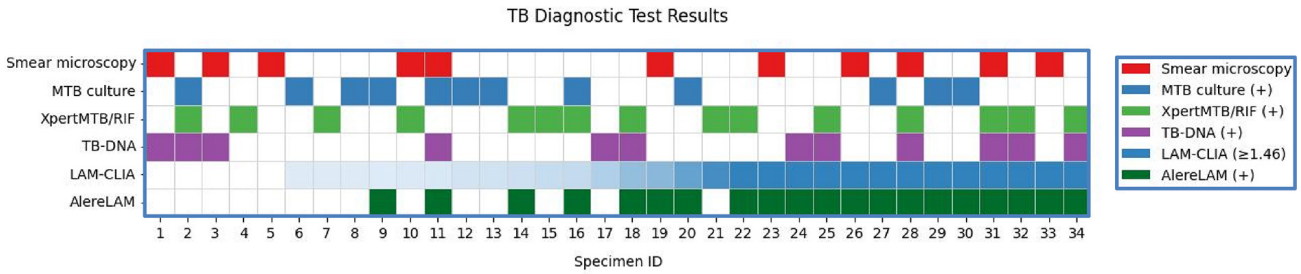

Fig. 4. Heatmap of test results for 34 patients with definite TB (Smear microscopy, MTB culture, Xpert MTB/RIF, TB-DNA, AlereLAM, LAM-CLIA). This figure was created using Python 3.13 (Python Software Foundation, Wilmington, DE, USA). MTB, *Mycobacterium tuberculosis*. White indicates negative results, and the blue gradient of LAM-CLIA represents increasing signal-to-cutoff (S/CO) values from light to dark blue.

4. Discussion

Despite the emergence of new diagnostic technologies, sputum smear microscopy and culture remain the standard approaches for TB diagnosis [5]. However, these traditional methods are limited by delays in diagnosis. In immunocompromised individuals, conventional immunological tests are ineffective, whereas urine LAM testing is considered a promising approach for detecting active TB [14]. The WHO recommends TB screening among high-risk populations, including PLWH, alongside implementing appropriate anti-TB treatment regimens [15]. For patients with advanced HIV, WHO further recommends the use of low-complexity automated NAATs on respiratory samples and LF-LAM testing as part of initial diagnostic strategies [16].

Urine LAM testing for MTB shows higher overall sensitivity in PLWH, particularly among those with CD4 T-cell counts of $\leq$100 cells/µL [17]. Because it enables simple point-of-care testing with minimal biosafety risks, the commercial development of LAM-based assays has accelerated [14]. However, the sensitivity of currently available commercial LAM tests remains suboptimal, which limits their widespread clinical application [18]. For example, an electrochemiluminescence-based LAM assay has been shown to achieve nearly 3-fold higher sensitivity than the Alere LF-LAM test [19], while chemiluminescence LAM testing demonstrated a sensitivity of 50.6%, significantly higher than the TB-DNA assay ($p < 0.05$) [20].

Baseline characteristics in our cohort revealed significant differences in age, HIV duration (in years), and $CD4^+$ cell counts across the three groups (definite TB, probable TB, and non-TB), while sex distribution was comparable. Age, HIV duration, and CD4 counts increased progressively from definite TB to the probable TB group and were highest in the non-TB group. This pattern aligns with the epidemiological trends of HIV/TB coinfection. Typically, individuals with early HIV infection or lower CD4 cell counts are at greater risk of developing active TB. In this study, both urine LAM-CLIA and Alere LAM assays demonstrated a trend toward higher positivity rates with declining $CD4^+$ cell counts among patients with HIV/TB coinfection, consistent with previous evidence that LAM-based assays perform better in advanced immunodeficiency. However, this inverse correlation was not statistically significant in CD4-stratified analysis (definite vs. probable TB groups), which may be due to limited sample size in certain CD4 strata or confounding factors such as TB bacterial load or Antiretroviral Therapy (ART) status. These findings reinforce the utility of LAM testing in immuno-compromised individuals. Further studies with larger cohorts are warranted to clarify the relationship between $CD4^+$ thresholds and LAM assay performance across TB diagnostic categories.

In this study, we developed a LAM-CLIA assay that increases the analytical sensitivity for TB LAM detection from 200 pg/mL [18] to 50 pg/mL, thereby enhancing the ability to detect low-burden infections, such as EPTB and early TB. LAM-CLIA demonstrated strong diagnostic performance in patients with HIV/TB co-infection, with antigen levels in definite and probable TB cases being 2–3 fold higher compared with non-TB controls. The optimal cutoff of 1.46 (AUC = 0.925) suggests high discriminatory power and supports its potential as a rapid, non-invasive diagnostic tool for TB in high-risk populations. While further multicenter clinical studies are needed to validate specificity and minimize cross-reactivity, these findings suggest significant clinical utility for more precise TB diagnosis.

Comparative analysis demonstrated that LAM-CLIA provided better overall diagnostic accuracy than AlereLAM (AUC: 0.874 vs. 0.785) and higher sensitivity (0.853 vs. 0.588), making it preferable for screening where minimizing false negatives is critical. Although AlereLAM showed higher specificity (0.982 vs. 0.896) and superior initial precision (0.8–0.9), its precision declined sharply once recall exceeded 0.6, limiting its utility as a stand-alone approach when higher case detection is needed. By contrast, the precision of LAM-CLIA began to decline more noticeably when the recall rate exceeded 0.6, yet it still provided a usable level of detection at high recall. This supports its potential role as an initial screening tool, particularly in resource-limited settings where identifying most cases is prioritized over minimizing false positives. Thus, LAM-CLIA may be best suited for primary screening, whereas AlereLAM may be more appropriate as a confirmation assay. Future studies should explore optimized thresholds or combined testing strategies to leverage the complementary strengths of both assays. These findings are consistent with the WHO's classification of AlereLAM as a highly specific confirmatory tool.

Traditional diagnostic methods in this study showed relatively lower positivity rates for TB: smear microscopy (32.4%), culture (35.3%), Xpert (44.1%), and TB-DNA (35.3%). These results are consistent with previous studies indicating limited sensitivity of conventional tests for EPTB, with positivity rates ranging from 17.3% to 33.2% [21]. From a diagnostic integration perspective, prioritization of Xpert MTB/RIF and culture on respiratory specimens should be prioritized, with LAM-CLIA as an alternative when these approaches are unavailable. In immunocompromised patients, a positive LAM-CLIA result may warrant consideration of empirical anti-TB treatment. Furthermore, combining LAM-CLIA with molecular assays has the potential to substantially improve diagnostic sensitivity, particularly in cases of EPTB and HIV coinfections [22].

This study provides valuable insights into diagnostic performance, clinical utility, and implementation aspects of LAM-CLIA for diagnosing active TB in PLWH. Use of an automated CLIA platform reduces operator dependence and enhances assay reliability and consistency. Compared with existing diagnostic approaches, LAM-CLIA offers significantly greater sensitivity, highlighting its significance for TB diagnosis in PLWH, especially in resource-limited settings. Its non-invasive nature and automated workflow further enhance its feasibility for routine clinical use.

This study has several limitations that should be considered before interpreting these results. First, the relatively small number of microbiologically confirmed TB cases (n = 34) may affect the precision of diagnostic estimates and highlight the need for validation in larger cohorts. Second, excluding clinically diagnosed TB cases from the primary ROC analysis—though methodologically necessary for establishing a definitive cutoff using confirmed reference standards—limits direct assessment of assay performance in this clinically complex subgroup. Third, although

no incident TB cases were identified during the 6-month follow-up in the non-TB control group, this may be insufficient to fully exclude latent infection or late-onset disease in immunocompromised individuals. Fourth, the generalizability of these findings is constrained by the lack of data from immunocompetent TB patients and other immunosuppressed populations, such as individuals with diabetes. Finally, despite supportive sensitivity analysis, the inherent subjectivity is unavoidable in defining "clinically diagnosed TB", which relies on treatment response. Future multicenter studies with expanded sample sizes and longer follow-up are warranted to validate and refine these findings.

5. Conclusion

The newly developed LAM-CLIA assay demonstrates significantly greater sensitivity than AlereLAM for diagnosing HIV-associated TB, particularly in both definite and probable cases, while maintaining acceptable specificity. Its ability to stratify TB risk based on antigen levels, combined with its high diagnostic accuracy (AUC = 0.925), underscores its potential as a rapid, non-invasive tool for early TB detection in HIV-positive populations.

Key Points

• The urine LAM-CLIA test provides a non-invasive and biosafe alternative with higher sensitivity for patients with HIV, which is consistent with World Health Organization recommendations for screening high-risk populations.

• The urine LAM-CLIA assay offers improved sensitivity (85.3% vs. 58.8%) than AlereLAM, underscoring its clinical significance in HIV-positive populations.

• LAM-CLIA is more appropriate for screening with stable high recall, whereas AlereLAM is better suited for confirmatory testing due to its high initial specificity.

• LAM-CLIA represents an automated, non-invasive tool with high accuracy (AUC = 0.925) and the ability to stratify TB risk, helping address critical diagnostic gaps for TB in people living with HIV, particularly in resource-limited settings.

Availability of Data and Materials

All data included in this study are available from the corresponding authors upon reasonable request.

Author Contributions

Conceptualization, YL and JLL; methodology, YL; data acquisition and analysis, HJW; validation, YL, YWH and YHY; formal analysis, YL; investigation, YWH; resources, YHY; data curation, LMS; writing—original draft preparation, YL; visualization, YWH; supervision, YWH; project administration, YHY. All authors contributed to revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study design strictly adhered to the ethical standards outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Beijing Youan Hospital, Capital Medical University (ethical approval number: LL-2021-053-K). Written informed consent was obtained from all participants before urine collection, confirming that each patient correctly understood the study objectives and agreed to participate voluntarily.

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Conflict of Interest

The authors declare no conflict of interest.

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