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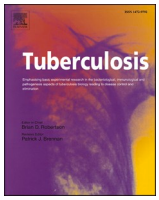

A novel method for detecting Lipoarabinomannan in urine with the promise of meeting the WHO target product profile for the diagnosis of tuberculosis

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ABSTRACT

The diagnosis of tuberculosis largely relies on the detection of *Mycobacterium tuberculosis (M. tuberculosis)* via pathogen-specific DNA or bacterial culture from sputum samples. As the only point-of-care test so far, urinary lipoarabinomannan (LAM) has been endorsed by the World Health Organization for the diagnosis of tuberculosis in people living with HIV.

In this study, the electrochemiluminescence LAM research assay (EclLAM) was used to measure LAM in the urine of HIV-sero-negative individuals with pulmonary tuberculosis and to monitor anti-tuberculosis treatment. Urine samples from 18 patients with microbiologically confirmed tuberculosis were analyzed before and after the initiation of anti-tuberculosis therapy and 17 healthy controls via the S4-20/A194-01 antibody pair.

The assay identified 13/18 (72.2 %) patients with tuberculosis and was negative in 17/17 (100.0 %) controls (AUC 0.88). The results of the reactive urine LAM tests correlated with the detection of *M. tuberculosis* growth in culture ($r = 0.94$, $p < 0.05$).

In conclusion, the LAM-specific antibody assay is promising to fulfill the WHO target product profile for the diagnosis of tuberculosis.

1. Introduction

Tuberculosis (TB) is a leading cause of morbidity and mortality worldwide. Following the Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2) TB has been the second leading cause of death in the field of infectious diseases in the year 2022 and the 13th leading cause of death overall [1].

The most prominent site of infection in humans is the lung, leading to pulmonary TB (PTB). Commonly used tests and WHO-endorsed methods for the diagnosis of active TB are sputum smear microscopy, liquid culture of *Mycobacterium tuberculosis (M. tuberculosis)* with drug susceptibility testing (DST), tuberculin skin test (TST) or interferon-γ release assay (IGRA), LAM lateral flow, and Xpert MTB/RIF, however the gold standard remains growth in culture [2]. In culture, *M. tuberculosis*, a slow-growing bacteria, has a doubling time of 12–24 h [3], making microbiological detection a time-consuming process. While *M. tuberculosis* is cultured from sputum samples, bacterial components can be detected from different sample types, such as bronchoalveolar lavage, stool or urine, with differing degrees of sensitivity and specificity [3].

TB is mostly curable with appropriate treatment [1]. Treatment response monitoring plays a crucial role to reach treatment success in TB. Current gold standards for monitoring TB are sputum smear microscopy and culture-based assays [4]. Limitations of both tests include highly developed infrastructure, trained staff, and the availability of results after several weeks restrict the outcome of these methods [5]. The electrochemiluminescence LAM research assay (EclLAM) with Meso Scale Discovery Inc. (MSD) technology is based on the ELISA-like

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technique and measures potential biomarkers present in *M. tuberculosis* and released during TB drug therapy [6]. The best described pathogen-component used as a TB biomarker is lipoarabinomannan (LAM), which is a component of mycobacterial cell walls and acts as a virulence factor. The lipopolysaccharide is detectable in urine [4,7], but may also be found in sputum [8] and blood [9] as well as in breath condensates [10] of TB patients. Since not all patients can produce sputum, especially children, the preferable test for detecting LAM is in urine or breath, which are non-invasive methods.

LAM is produced by all mycobacteria and accords with their requirements for growth [11]. It has different capping motifs resulting in various structural variants such as ManLAM, which is most abundant in slow growing pathogen species such as *M. tuberculosis* and *M. bovis* [12]. The different epitopes that are unique to *M. tuberculosis* are important for detecting LAM, as different antibodies bind to various epitopes with varying sensitivities towards capping motifs [7]. In particular, the discovered methylthiopentosyl residue attached to the mannosyl caps of LAM, which have an α-xylo configuration, is specific to *M. tuberculosis* and may play an important role in the oxidative protection of the bacterium [13]. Other epitopes consist of polyarabinose Ara4 and Ara6 structures that can be uncapped and capped with mannopyranose (Man*p*) oligosaccharides (Supplement Fig. S1) [14]. The monoclonal antibody (mAb) A194-01 recognizes the uncapped Ara4 and Ara6 structures with the exception of the inositol phosphate-capped Ara4. The S4-20 antibody is uniquely specific for Man*p*-capped structures with an MTX residue. In particular, the modified di- and trimannose – capped structures bind strongly to this antibody [14].

Current diagnostic methods that are used to detect the concentration of LAM in urine are the clinical point of care tests (POCTs) Fujifilm SILVAMP TB lipoarabinomannan (FujiLAM) and Alere Determine TB LAM Ag (AlereLAM). Both tests are lateral flow assays resulting in a semi-quantitative analysis with sensitivities of 10.8 % (AlereLAM) and 53.2 % (FujiLAM) [15]. An alternative method is the laboratory-based EclLAM assay based on MSD technology and reader. This method comprises a quantitative sandwich immunoassay run in a multi-well plate format with a sensitivity of 66.7 % when S4-20 is used as the capture antibody and A194-01 as the detection antibody [15]. The methods mentioned above can be used for people living with HIV (PLHIV) and HIV-sero-negative patients. All characteristics in addition to the WHO target product profiles (TPPs) for a biomarker test [16] are summarized in Supplement Table S2.

In contrast to previously published data, the present study was conducted to ascertain whether the S4-20/A194-01 antibody pair reached the WHO benchmark TTPs for the detection of LAM in urine of TB+/HIV-sero-negative patients and whether the assay is suitable for monitoring the response to anti-TB treatment.

2. Methods

For this retrospective cohort study, the EclLAM assay based on MSD technology (Rockville, Maryland, United States) was used to measure LAM concentrations in urine of TB+/HIV-sero-negative patients.

2.1. Clinical subjects and samples

Patients with PTB confirmed by culture were enrolled. The patients' sputum samples were tested with molecular biological methods beforehand (i.e., GeneXpert (Cepheid Inc., Sunnyvale, California, United States), detection of rifampicin/isoniazid resistance) and *M. tuberculosis* growth was confirmed by culture. Sputum culture was done in triplicates. In addition, healthy individuals were included as controls. The samples used in this study were collected at the "Research Center Borstel, Leibniz Lung Center" between 2015 and 2022 as part of the "Identification of Biomarkers for multidrug-resistant (MDR) tuberculosis therapy response assessment" trial. The inclusion and exclusion criteria are listed in the Supplement Table S3. The participants had not yet started therapy before the collection of samples at the first time point. Before proceeding further, the approval of an independent ethics committee (Ethical Review Board of the University Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany; file number 12–233), regulatory authorities and written informed consent from all participants was obtained. The study was carried out following the Helsinki Declaration contents. In Table 1, the different time points for study visits with sampling of sputum, blood and urine are listed.

Sputum- and *M. tuberculosis* culture conversion as well as the characterization of TB-strains and DST were also obtained.

At least 200 mL of urine was collected from each participant at each time point. The urine was aliquoted in two Falcon tubes (15 mL) with 10 mL each. EDTA (0.5 mL) was added to one aliquot of each sample. In this study, we investigated urine samples with and without the addition of EDTA. The samples with EDTA were used if the samples from the patients without EDTA were missing. After further processing, the samples were stored at −80 °C. The same procedure was done for controls at T0. Since the cohort was enrolled within a longitudinal observation study, patients were not randomized to specific treatment arms. This study aimed to analyze biomarkers to monitor TB therapy independent of treatment.

2.2. Antibodies and control materials

Purified LAM was obtained from BEI Resources, NIAID, NIH (Manassas, Virginia, United States): *Mycobacterium tuberculosis*, Strain H37Rv, Purified Lipoarabinomannan (LAM), NR-14848. This product was used as a control. For immunoassay analysis to measure LAM concentrations, the monoclonal antibodies S4-20 and A194-01 were kindly provided by FIND (Geneva, Switzerland).

2.3. Procedure

Prior to the actual ELISA-like EclLAM assay the capture antibody needed to be conjugated with biotin to bind to streptavidin on the plate. In addition, the detection antibody needed to be conjugated with SULFO-TAG (MSD (Rockville, Maryland, United States), catalogue #R31AA) to emit light due to the chemiluminescence reaction. First, the capture antibody S4-20 was diluted in diluent 100 (MSD (Rockville, Maryland, United States), catalogue #R50AA) until a final concentration of 1.00 µg/mL was reached. The same was done with the detection antibody A194-01 in diluent 3 (MSD (Rockville, Maryland, United States), catalogue #R50AP) until an end concentration of 2.00 µg/mL was reached. The standard samples were generated by adding purified LAM (BEI Resources, Manassas, Virginia, United States) to pooled urine samples from four healthy controls (not included in the EclLAM assay analysis). The starting concentration of 50,000 pg/mL was diluted 1:4 to 0.00 pg/mL.

The MSD GOLD™ Small Spot Streptavidin SECTOR™ Plates (MSD (Rockville, Maryland, United States), catalogue #L45SA) were coated with 25 µL of capture antibody (1.00 µg/mL S4-20) per well and incubated at 23 °C (20 °C 25 °C) for 1 h. The plate was shaken at 750 rpm (VWR Microplate Shaker (Darmstadt, Germany), catalogue # 12620). After incubation, the plate was washed three times with 150 µL/well of PBS +0.05 % Tween-20 (PBS-T). Furthermore, the plates were coated

Table 1
Study visits of participants with sampling of sputum, blood and urine. The table outlines the scheduled time points for collection.

Time point	When?
T0	0–2 days before therapy start
T1	13 days after therapy start ± 3 days
T2	Sputum conversion ± 3 days
T3	8 weeks after culture conversion ± 7 days
T4	6 months after therapy start ± 7 days
T5	After 10 months ± 7 days

with 50 μL/well of standards and samples in duplicate and incubated at 4 °C overnight. The next day, the plates were washed again as previously described. 25 μL of detection antibody (2.00 μg/mL A194-01) was then added to each well and incubated at 23 °C (20 °C 25 °C) for 1 h while shaken at 750 rpm (VWR Microplate Shaker (Darmstadt, Germany), catalogue # 12620). Three washing steps with 150 μL/well of PBS-T followed. In the last step, 150 μL of MSD GOLD Read Buffer B (MSD (Rockville, Maryland, United States), catalogue #R60AM) was added to each well, and the plate was read immediately.

The cut-off for the EclLAM assay was calculated based on our data at 13.35 pg/mL to achieve a sensitivity above 65 %. However, according to other studies with a larger sample size, this cut-off was set at 11.0 pg/mL [7]. Since we worked with a small cohort size and aimed to align our results with the broader population, we decided to adopt the cut-off of 11.0 pg/mL for our study. Below this value, the measurements will be identified as negative for active TB.

2.4. Statistical analysis

The data were statistically analyzed with GraphPad Prism 8.0.1 (Dotmatics, Boston, Massachusetts, United States) and R (version 4.3.2).

Numerical data are reported as the median ($\tilde{x}$) ± 95 % confidence interval (95 % CI). To test whether there was a significant difference in the LAM concentration or fold change between different treatment days, the Wilcoxon-signed rank test was used. The correlation between LAM and the bacterial load was calculated via Spearman's rank coefficient. The TTPs that were measured by the laboratory in Borstel at the time of sample collection were used as surrogate markers for the bacterial load. For the calculation of the diagnostic yield among all those diagnosed with TB the number of people with a positive diagnosis by the test is divided by the total number of people diagnosed with TB.

If indeterminate or missing test results were the case, the test was repeated, or the participant was excluded. If the data was above a coefficient of variation (CV) of 20 % the data was not applicable and excluded for quantitative analysis.

3. Results

3.1. Demographic and clinical characteristics of the study participants

Eighteen randomly chosen participants from a subcohort of the German DZIF TB cohort were included in the study: 10 female and 8 male patients aged between 19 and 57 years (Fig. 1). All patients were tested sputum smear and/or Xpert positive. Seven out of the 18 patients were cigarette smoker. Ten of the patients had confirmed drug-susceptible TB (DS-TB), five out of 18 patients had multidrug-resistant TB (MDR-TB) and three of the patients had extensively drug-resistant TB (XDR-TB) (Table 2). Eleven patients were originally from Europe, and five and two were immigrants from Asia and Africa.

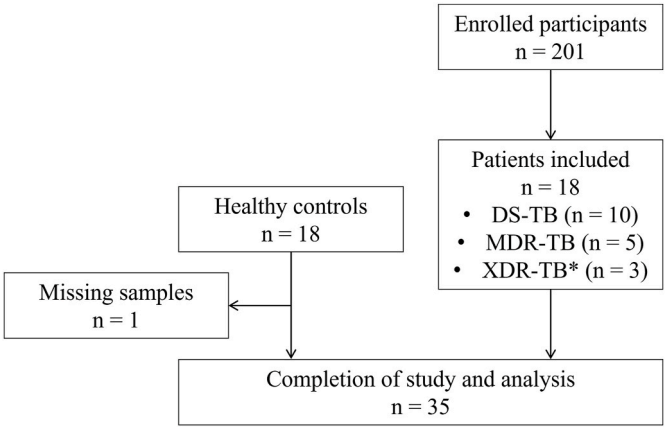

Fig. 1. Flow of participants and healthy controls. *In this study, XDR-TB is defined as resistance to Rifampicin, Isoniazid, any fluorchinolone as well as to one of the WHO II drugs, namely Amikacin, Capreomycin or Kanamycin, according to the WHO definition at the time of cohort recruitment. DS-TB, drug-susceptible tuberculosis; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

Nine healthy male and 8 healthy female participants aged between 21 and 57 years ($\bar{x}$ = 34.8) were included as controls. All were born in Europe. There were no information about the TST/IGRA positivity given but control participants presented to the hospital with no symptoms. Five out of 15 participants were cigarette smokers. No information was given about the cigarette smoking characteristics of two control participants.

3.2. EclLAM assay

To measure LAM concentrations in urine of TB+/HIV-sero-negative patients who received therapy and healthy controls, the EclLAM assay based on MSD technology and reader was used. This assay combines an indirect ELISA-like test with electrochemiluminescence detection by the addition of voltage. Time points 1 to 5 are represented in average days from the start of therapy. The average days and standard deviations (SD) are 13 days (SD: 1.34), 72 days (SD: 40.8), 104 days (SD: 25.2), 189 days (SD: 23.8), and 316 days (SD: 31.8) for time points 1 to 5. Time point 5 comprises 10 months of therapy for DR-TB patients. Time points 4 and 5 take into account the end of therapy for DS-TB patients, which might deviate from the rule. This explains the high standard deviations later on.

3.2.1. LAM concentration measurement

A comparison of the LAM concentrations of the samples obtained prior to the start of therapy ($\tilde{x}$ = 35.0 pg/mL, 13.4–87.5 pg/mL) and day 189 of therapy ($\tilde{x}$ = 5.90 pg/mL, 0.00–157.9 pg/mL) revealed a significant difference ($p < 0.05$, Fig. 2a). This observation was also observed after 13 days of therapy ($\tilde{x}$ = 48.0 pg/mL, 11.4–137.8 pg/mL) where the differences from day 104 ($\tilde{x}$ = 4.34 pg/mL, 0.00–49.0 pg/mL) and day 189 were also statistically significant ($p < 0.05$). When comparing the concentration between baseline and 13 days after the initiation of therapy, an increase in concentration becomes evident, which subsequently decreases again. Total LAM concentrations between 0.00 pg/mL and 857.8 pg/mL were recorded over the course of 316 days of anti-TB therapy (Fig. 2a).

To evaluate the magnitude of the variation in concentration the fold change was calculated. Significant differences in fold change between day 13 ($\tilde{x}$ = 0.91, 0.00 to 1.49) compared to day 189 ($\tilde{x}$ = 0.22, 0.00 to 0.40) were observed ($p < 0.05$, Fig. 2b). Fold changes between 0.00 and 6.67 during 316 days of anti-TB treatment were observed (Fig. 2b).

The most substantial reductions from baseline occurred at days 104 and 316, with the steepest decline observed between day 13 ($\tilde{x}$ = 48.0 pg/mL) and day 72 ($\tilde{x}$ = 14.8 pg/mL). No significant correlations were

Table 2
Demographic data of 18 study participants and 17 healthy controls. *In this study, XDR-TB is defined as resistance to Rifampicin, Isoniazid, any fluorchinolone as well as to one of the WHO II drugs, namely Amikacin, Capreomycin or Kanamycin, according to the WHO definition at the time of cohort recruitment. NA, not available; DS-TB, drug-susceptible tuberculosis; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

Characteristics	TB patients (n = 18)	Healthy controls (n = 17)
Mean age, years	33.1 (19.0–57.0)	34.8 (21.0–57.0)
Sex, M/F	8/10 (44.4 %/55.6 %)	9/8 (52.9 %/47.1 %)
Smoker, yes/no	7/11 (38.9 %/61.1 %)	5/10 (33.3 %/66.7 %)
DS-TB/MDR-TB/XDR-TB*	10/5/3 (55.6 %/27.8 %/16.6 %)	NA

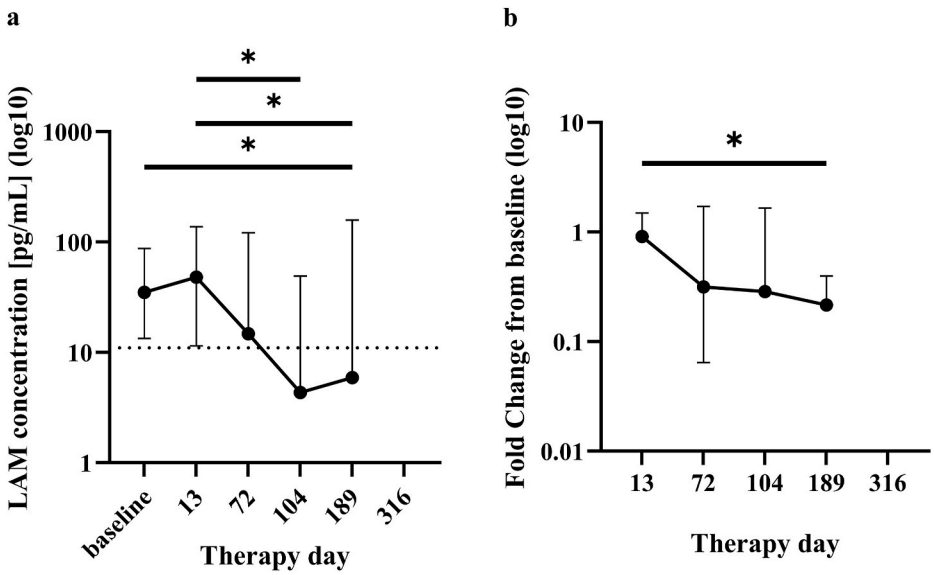

Fig. 2. LAM concentrations in urine of 18 TB+/HIV-sero-negative participants during therapy. The results are shown as the median ($\tilde{x}$) ± 95 % CI. The therapy days represent time points 1 to 5. The time points 3 to 5 are average values of therapy days from 18 participants.
(**a**) The absolute values of the LAM concentrations in urine of 18 participants during TB treatment are shown. The dashed line shows the cut-off value of 11.0 pg/mL. An increase in LAM concentration was detected after 13 days of therapy. A significant decrease in the LAM concentration was measured at day 189 compared with that at baseline. (**b**) The fold change from baseline in LAM concentrations is shown to compare the different relative concentration values from 18 participants. Compared with the baseline measurements, a significant decrease was measured after 189 days. Since the median at 316 days of therapy is 0 for both measurements, the value is not displayed in the diagram. The antibody pair that was used for this measurement was 1 µg/mL S4-20 as the capture antibody and 2 µg/mL A194-01 as the detection antibody. *$p < 0.05$.

found between LAM concentration and culture time to positivity (TTP) from time points 0 to 1 ($r = -0.28$, $p = 0.14$) as well as culture conversion ($r = -0.01$, $p = 0.99$), respectively.

3.2.2. Diagnosis

At the time of diagnosis, the LAM concentration in samples from patients at baseline and healthy controls was compared. The receiver operating characteristic (ROC) curve of the antibody pair for detecting *M. tuberculosis* is shown in Fig. 3. The sensitivity was 72.2 % (95 % CI 46.5 %–90.3 %), with an area under the curve (AUC) of 0.88 (95 % CI 0.76–1.00) with a cut-off value of 11.0 pg/mL (Fig. 3). The same was observed with a cut-off value set to 13.35 pg/mL. The specificity for this antibody pair was 100.0 % (95 % CI 80.5 %–100.0 %). *M. tuberculosis* growth in culture was the reference test (gold-standard) and had 100.0 % sensitivity at baseline measurements.

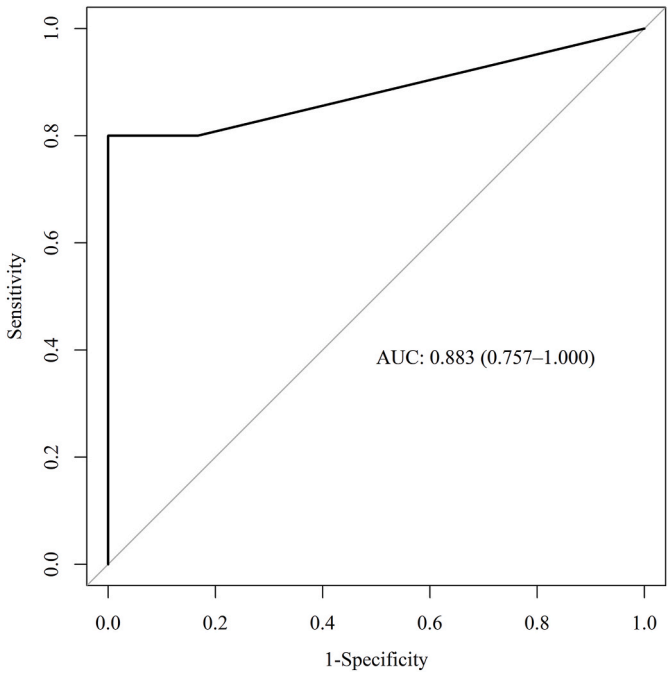

Fig. 3. ROC curve with a cut-off set at 11 or 13.35 pg/mL. The calculated and the already published cut-off values [7] give the same sensitivity and specificity values on the ROC curve. An AUC of 0.88 (95 % CI 0.76–1.00) was measured.

3.2.3. Treatment monitoring

During therapy, the number of positive LAM tests steadily declined. With a cut-off value of 11.0 pg/mL, at baseline, 72.2 % of the patients tested positive for LAM. The diagnostic yield among all those diagnosed was 0.72. This percentage declined to 70.6 % on day 13 to 35.7 % on day 72 of therapy. On day 104, 18.8 % of the samples were positive for LAM, whereas on day 189, 22.2 % were positive. By day 316 of therapy, a total of 12.5 % of the tests were positive for LAM. The same results were observed with a cut-off value of 13.35 pg/mL, except for deviations on days 13 and 189. From day 72 onwards the antibody pair identified more positive tests for LAM than *M. tuberculosis* in culture, staying positive late in treatment after culture turns negative (Table 3). With the cut-off value either set at 11 pg/mL or at 13.35 pg/mL, the number of positive LAM tests significantly correlated with the number of positive tests for *M. tuberculosis* growth in culture ($p < 0.05$; $r = 0.94$, $r = 0.95$, respectively).

4. Discussion

This study evaluated the effectiveness of the EclLAM assay, using the S4-20/A194-01 antibody pair, for detecting LAM in the urine of TB+/HIV-sero-negative patients. LAM levels in urine were also quantified as a surrogate marker for microbiological findings to monitor the response to anti-TB treatment. The antibody pair correctly discriminated HIV-seronegative patients with PTB from healthy controls with a sensitivity and diagnostic yield of 72.2 % and a specificity of 100.0 % (AUC

Table 3
Detection of LAM and *M. tuberculosis* in 18 participants. The TTP was only measured until day 13. In culture, the number of positive tests decreased until day 72 of therapy. After this time point no positive culture tests were identified. The number of positive LAM tests decreased steadily until day 316 of treatment, resulting in 2 positive LAM tests by the end of therapy. TTP, culture time to positivity. IQR, interquartile range. NA, not available. COV, cut-off value.

	COV 11 pg/mL	COV 13.35 pg/mL	Culture	TTP in days (Median, IQR)
Baseline	13/18 (72.2 %)	13/18 (72.2 %)	18/18 (100.0 %)	9.00 (6.25–13.5)
Day 13	12/17 (70.6 %)	11/17 (64.7 %)	16/17 (94.1 %)	25.0 (17.3–37.5)
Day 72	5/14 (35.7 %)	5/14 (35.7 %)	2/14 (14.3 %)	NA
Day 104	3/15 (18.8 %)	3/15 (18.8 %)	0/16 (0.00 %)	NA
Day 189	4/18 (22.2 %)	2/18 (11.1 %)	0/18 (0.00 %)	NA
Day 316	2/15 (12.5 %)	2/15 (12.5 %)	0/16 (0.00 %)	NA

0.88). The diagnostic yield among all those diagnosed is defined as the proportion of people in whom a diagnostic test identifies TB among TB positive people for whom testing is attempted, which is an important metric for the evaluation of the usage of a diagnostic test [17]. This might offer a real-life implementation indicator that enhances conventional accuracy metrics due to the consideration of access, sensitivity, specimen availability, and test completion [17]. The sensitivity and specificity estimates of the test of 72.2 % and 100.0 % were higher than the WHO's TPP recommendations of 65.0 % and 98.0 %, respectively. Setting the cut-off value at 11.0 pg/mL, as previously published by Sigal et al. [7], instead of the calculated 13.35 pg/mL allows for better standardization across a larger population, given the limited size of our cohort. Although a cut-off of 13.35 pg/mL resulted in the same measured sensitivity, it may be less applicable to studies with larger sample sizes, potentially limiting its broader utility. The test cost was $1.00 less than the WHO TPP recommended limit [16]. Thus, the antibody pair met the WHO TPP criteria for sample type, sensitivity, specificity, and cost per test. However, this conclusion should be interpreted cautiously due to the small sample size in the study. A key advantage of the LAM test is its low cost of US$5.00 per test and its ability to be performed in non-sterile environments, making it ideal for use in low-resource settings without the need for specialized laboratories. The primary expense is the plate reader and its maintenance. Additionally, the antibody pair was effective for treatment monitoring, as evidenced by the strong correlations between positive LAM tests and positive culture results.

In other studies using the LAM-ELISA (Chemogen, So. Portland, Maine, United States) as a diagnostic method, the overall sensitivity ranged from 17.8 % to 76.5 % ($\tilde{x} = 54.9$ %) with an overall specificity ranging from 86.9 % to 99.0 % ($\tilde{x} = 88.4$ %) [18–23], generally lower in HIV-sero-negative patients compared to PLHIV [20,21]. In 2009, Mutetwa et al., reported a difference in sensitivity of 31.0 % (HIV-sero-negativity: 21.0 %; PLHIV: 52.0 %) [21]. Reither et al. reported similar results (HIV-sero-negativity: 21.1 %; PLHIV: 62.0 %) [20]. The test specificity in HIV-sero-negative patients was 91.1 % [20] and 93.0 % [21], respectively. Higher specificity (99.0 %) was obtained when unprocessed fresh urine samples were used [19]. The increased sensitivity in PLHIV is likely due to immunosuppression, which higher bacterial burden [24]. Since the WHO Target Product Profile (TPP) criteria were met even with samples from HIV-sero-negative patients, it is possible that results would improve further when using samples from PLHIV.

The initial increase in LAM concentrations during early TB treatment suggests that large quantities of dying mycobacteria are being freely filtered into the urine, leading to higher LAM levels [25]. This observation differs from previous reports [25,26]. The strong correlation ($r = 0.94$) between the number of positive LAM tests and positive culture tests ($p < 0.05$) highlights the relationship between LAM detection and active infection. The most significant decline in both LAM and *M. tuberculosis* culture-positive tests was found between day 13 and day 72 of therapy, which is consistent with findings by Wood et al. in 2012, who observed the greatest reduction in LAM optical density between weeks 2 and 8 of therapy [25]. After week 8, all culture tests were negative, marking the time to culture conversion, defined as the absence of *M. tuberculosis* growth. Ideally, no positive LAM tests should occur after this point. However, the detection of positive LAM tests after culture conversion on day 72 suggests the presence of non-viable bacteria [25]. LAM is secreted into the urine regardless of the infection site and independently of bacilli excretion in sputum [18]. Additionally, in this study, LAM concentrations did not significantly correlate with the TTP within the first two weeks of anti-TB therapy. These factors may explain the occurrence of positive LAM tests even *when M. tuberculosis* culture results are negative or fewer in number.

This study has several limitations: (i) We included only a small number of participants, which might compromise the accuracy and replicability of statistical analyses. More patients would lead to the meaningful statement that this test fulfilled the WHO's TPPs. (ii) The study design was retrospective, which might lead to missing or incomplete data and biases due to existing data. Prospective studies might lead to more control and less confounding. (iii) The data often exhibited a coefficient of variation above 20 %, resulting in the exclusion of these values from qualitative analysis. As a result, only qualitative conclusions could be drawn. For a better quantiative analysis, particularly the concentration measurement, the experiments would need to be repeated. (iv) Not all ethnic groups and ages as well as participants from endemic areas were included, which could lead to biases in drug response and identification of risks and benefits. Especially the inclusion of participants from regions where TB is endemic is needed since lower sensitivities of diagnostic tests were found in high endemic regions [27]. Also, the diversity of TB strains as well as the immune response variability [28] can impact the accuracy of the test. Ensuring that all ethnic groups, ages and individuals from regions with a high disease burden are included in a larger cohort would produce results that are more generalizable to the broader population. (v) The EclLAM assay as well as the antibodies are not commercially available. Users wishing to run the assay must acquire the antibodies independently from FIND and establish the assay in their own laboratory. The accessibility of the antibodies is therefore not given. Commercially available technology and antibodies would thus be more practical for a broader audience.

In conclusion, the EclLAM assay using the S4-20/A194-01 antibody pair demonstrated a sensitivity of 72.2 % and a specificity of 100 % (AUC 0.88), indicating its potential to meet the WHO TPP standards for diagnosing active PTB in HIV-sero-negative individuals. For treatment monitoring, the EclLAM assay showed a strong correlation with *M. tuberculosis* culture results. Overall, these findings underscore the potential of LAM as a biomarker for both diagnosis and treatment monitoring. Further research should prioritize the development of non-invasive, sensitive, specific, and quantitative LAM tests.

CRediT authorship contribution statement

Katharina Budde: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Christoph Lange:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Maja Reimann:** Formal analysis. **Nika Zielinski:** Formal analysis. **Lennard Meiwes:** Visualization. **Niklas Köhler:** Formal analysis. **Patricia Sanchez Carballo:** Writing – review & editing, Visualization, Validation, Supervision, Funding

acquisition, Formal analysis, Conceptualization.

Data availability

All relevant data are within the manuscript and its supporting information files.

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Declaration of competing interest

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Appendix A. Supplementary data

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