Fujifilm SILVAMP TB-LAM for the diagnosis of tuberculosis in Nigerian adults

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**Abstract:** There is a need for diagnostics for tuberculosis (TB) that are easy to use, able to screen non-sputum samples and to provide rapid results for the management of both immunocompromised and immunocompetent individuals. Fujifilm SILVAMP TB LAM (FujiLAM) assay, a new non-sputum based point of need test for the diagnosis of TB, could potentially address most of these needs. We evaluated the performance of FujiLAM in HIV-positive and HIV-negative patients with presumptive TB attending three district hospitals in Nigeria. Consecutive patients were asked to provide urine samples, on the spot, which were tested with FujiLAM. Results were compared against a positive culture and/or Xpert MTB/RIF, as the reference standard. Forty-five patients had bacteriologically confirmed TB and 159 had negative culture and Xpert MTB/RIF (no TB). FujiLAM test was positive in 23 (sensitivity 65.7%, 95%CI 48-80) HIV-negative and 7 (70%, 95%CI 35-92) HIV-positive patients with bacteriological confirmation of TB. FujiLAM was negative in 97 (specificity 99.0%, 95%CI 94-100) HIV-negative and 56 (93.3%, 95%CI 83-98) HIV-positive patients without TB. The FujiLAM test has good diagnostic accuracy for considering its application in both HIV-positive and HIV-negative patients with TB.

**Keywords:** tuberculosis; diagnosis; lipoarabinomannan; LAM; HIV; urine; point-of-care

1. Introduction

Tuberculosis (TB) continues to cause high morbidity and mortality worldwide [1]. Despite increases in TB notifications in recent decades [2], and a major expansion in the use of the World Health Organization (WHO)-recommended molecular diagnostics (WRDs), 2.9 of the ten million estimated people who develop TB are missed by national TB programs each year.

A major drawback of current WRDs is the poor timeliness of test results, which often return to the clinic several hours or days later, when clinical decisions have been taken and the patients have left the premises [3]. It is recognised that, to be impactful, diagnostic test results need to be available at the time of patient management, to guide treatment initiation and to reduce pre-treatment losses to follow up [4]. Ideally, assays should be conducted at the point of need, using minimal laboratory skills and examining non- or minimally invasive clinical samples [5,6]. Moreover, large proportions of presumptive individuals cannot expectorate sputum, or provide a high quality sample, and therefore non-sputum based tests could be helpful for many populations.

Current non-sputum based, point of need prototypes target serological markers or bacterial components or detritus, including the lipoglycan and virulence factor lipoarabinomannan (LAM). LAM is a heat stable component of the outer cell wall of the bacilli that is released from metabolically active or degenerating bacteria of the genus Mycobacterium. LAM is filtered by the kidney, and can be detected in urine, with test prototypes mentioned in the literature since the 1930s [7,8]. Although current LAM assays have low sensitivity, performing better in individuals with HIV and advanced immunosuppression [9], a recent prototype, Fujifilm SILVAMP TB LAM (FujiLAM, Fujifilm, Tokyo, Japan), is reported to have higher sensitivity in both HIV-infected and uninfected individuals [10,11] thanks to the use of high affinity monoclonal antibodies against Mycobacterium tuberculosis-specific LAM epitopes and a silver-amplification step that increases the visibility of the test lines [11].

We report here a cross sectional study to assess the diagnostic performance of FujiLAM in consecutive adults with presumptive TB attending ambulatory clinics in Nigeria.

2. Materials and Methods

This was a retrospective study of adults with signs and symptoms suggestive of TB attending TB diagnostic clinics at district hospitals of Abuja, Nigeria. Adults above 18 years old with presumptive TB [12] were enrolled consecutively at the time of submitting samples for diagnosis, regardless of their HIV status. Adults who had formerly been diagnosed as having TB or who had received TB treatment in the previous year were excluded.

After obtaining written informed consent, participants were interviewed to obtain clinical and demographic information. Patients were asked to provide sputum, blood samples and one midstream urine sample on-site for routine and study assays. All samples, except urine were processed locally and were used for patient management. Sputum samples were tested with Xpert MTB/RIF (Cepheid, Sunnyvale, CA), and cultured in solid media in duplicate using Löwenstein–Jensen medium. Urine samples were collected in sterile plastic containers and kept in cold boxes until processing for storage the same day. Samples were aliquoted (2 ml) into cryovials and transported frozen to the Institut d’Investigació Germans Trias i Pujol (Badalona, Spain) for LAM testing. One aliquot per participant was thawed at room temperature the day of testing, mixed with a vortex and tested using FujiLAM following the manufacturers' instructions [11]. Briefly, the reagent tube was filled with urine up to the indicator line, mixed without inverting, and incubated for 40 minutes at ambient temperature. During this incubation, the gold (Au)-conjugated primary antibody will capture the 5-methylthio-D-xylofuranose-lipoarabinomannan antigen present in the patient's urine. The tube was then mixed and two drops of the contents of the tube were added to the sample well of the test cartridge. Immediately we pressed the button '2' on the cartridge and waited 10 minutes until the orange mark appeared on the cartridge readout indicating '*go to next step*'. During this incubation, the sandwich immunocomplex will form by binding to the immobilized secondary antibody. At the signal to proceed to the next step, the button "3" was pressed, releasing silver particles (10 um in diameter) which cluster around the gold particles and amplify the intensity of the cartridge reader band. The test results can be read approximately 1 minute later on the test cartridge reader. The test was considered positive if the control and test lines were visible (even if the line was faint) and negative if only the control line appeared. Tests without a control line were considered invalid and were repeated once (See Figure 1). A video describing the test procedures is available at <https://www.youtube.com/watch?v=aK-QtzkLBug>. The test lines were read by two investigators blinded to the patients’ condition and all other test results. In the case of disagreement with the result, the test was repeated once.

HIV status was assessed using two rapid antigen tests and viral loads of patients with HIV were assessed in plasma using the Xpert HIV-1 viral load (VL) assay (Cepheid, Sunnyvale, CA) according to the manufacturer’s instructions. The VL results were interpreted as detected, detected <40 copies/ml, detected >107 copies/ml, undetected, and undetermined. The range of detection of the Xpert HIV-1 VL test is 40 to 107 copies/ml (1.6 to 7.0 log10).

We used Chi-Square and Fisher’s exact tests to test parametric data and Student’s T-tests for continuous variables with normal distributions. Differences were considered statistically significant when the P-value was less than 0.05. Analysis was performed using SPSS (SPSS version 26.0, SPSS Inc, Chicago, IL, USA). Xpert and culture results were used to classify participants as ‘bacteriologically confirmed’ if either the Xpert MTB/RIF or culture results were positive and as ‘non-TB’, if both tests were negative. Sensitivity and specificity of the FujiLAM test were estimated using the combined results of Xpert MTB/RIF and culture as the reference standard (bacteriologically confirmed). We considered invalid FujiLAM results as negative, but annotated these results in separate rows. Written informed consent was obtained from all participants. The study was approved by the Research Ethics Committees of the Liverpool School of Tropical Medicine (protocol 18-176411\_Trop\_942) and the Nigerian National Ethics Committee.

3. Results

Two hundred and four participants with a mean (SD) age of 37 (12.8) years were enrolled, as shown in Table 1. Thirty-seven (18.1%) had positive *M. tuberculosis* culture, 40 (19.6%) were Xpert MTB/RIF positive and 45 (22.1%) culture or Xpert MTB/RIF positive (called ‘bacteriologically confirmed’). Four (10.8%) culture-positive participants were Xpert MTB/RIF-negative and 3 (7.5%) and 4 (10.0%) Xpert MTB/RIF-positive had negative or contaminated culture, respectively. One hundred and fifty-nine (77.9%) participants were culture and Xpert MTB/RIF negative (called ‘not TB’) (Table 1). Overall, 70 (34.3%) participants were HIV-positive, 133 (65.2%) HIV-negative and the HIV status was not known in one. Viral loads among HIV-positive participants were undetectable in 11 (15.7%), <40 copies/ml in 16 (22.9%), and between 40 and 107 copies/ml in 32 (45.7%), with three (4.3%) and 8 (11.4%) participants having indeterminate and missing viral load results, respectively.

**Table 1.** Characteristics of the study participants.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **All**  **(n=204)** | **Not TB**  **(n=159)** | **Bact + TB**  **(n=45)** | **p-value** |
| Age, | mean (SD) | 37.0 (12.8) | 37.65 (13.0) | 34.60 (12.0) | 0.160 |
| Sex | Male | 95 (46.6%) | 69 (43.4%) | 26 (57.8%) | 0.088 |
|  | Female | 109 (53.4%) | 90 (56.6%) | 19 (42.2%) |  |
| HIV | Negative | 133 (65.2%) | 98 (61.6%) | 35 (77.8%) | 0.087 |
|  | Positive | 70 (34.3%) | 60 (37.7%) | 10 (22.2%) |  |
|  | Unknown | 1 (0.5%) | 1 (0.6%) | 0 (0.0%) |  |
| Culture | Negative | 155 (76.0%) | 151 (95.0%) a | 4 (8.9%) a | **<0.01** |
|  | Positive | 37 (18.1%) | 0 (0.0%) a | 37 (82.2%) a |  |
|  | Contaminated | 12 (5.9%) | 8 (5.0%) | 4 (8.9%) |  |
| Xpert | Negative | 164 (80.4%) | 159 (100%) a | 5 (11.1%) a | **<0.01** |
|  | Positive | 40 (19.6%) | 0 (0.0%) a | 40 (88.9%) a |  |

SD: standard deviation. The p-value shows the significant differences observed for each variable between the proportions of bacteriologically confirmed TB patients and non-TB patients. (a) Column proportions that differ significantly with a p-value under 0.05 (in bold).

All 204 participants were tested with FujiLAM. Thirty-six (17.6%) were FujiLAM-positive, 164 (80.4%) FujiLAM-negative, and four (2.0%) had invalid results (Table 2). FujiLAM identified a similar proportion of bacteriologically confirmed individuals by HIV status and was positive in 30 (66.7%) of 45 patients with bacteriologically confirmed TB, including 23 (65.7%) of the 35 HIV-negative and seven (70%) of the ten HIV-positive patients (p = 0.56). Among the 159 participants with no-TB (culture and Xpert MTB/RIF negative), FujiLAM was positive in six (3.8%), invalid in four (2.5%) and negative in 149 (93.7%). Ninety-seven (99%) of 98 HIV-negative and 56 (93.3%) of 60 HIV-positive participants having negative/invalid FujiLAM results. Overall FujiLAM sensitivity and specificity were 66.7% (30/45) (95%CI 51-80) and 96.2% (153/159) (95%CI 92-98) among all participants, varying from 65.7% (95%CI 48-80) and 99.0% (95%CI 94-100) for HIV-negative and 70.0% (95%CI 35-92) and 93.3% (95%CI 83-98) for HIV-positive patients, respectively (Table 3). Positive and negative predictive values are also shown in Table 3 for patients with bacteriologically confirmed TB (Xpert or culture positive) and patients with *No TB* (Xpert and culture negative). The positive predictive value was higher among HIV-negative patients (96%) than HIV-positive (63%) and the negative predictive value was higher among HIV-positive (94%) than HIV-negative patients (89%). However, differences were not statistically significant.

**Table 2.** FujiLAM test results by TB and HIV status.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **All** | | | **HIV-negative** | | **HIV-positive** | | |
| FujiLAM | Negative  n (%) | Positive  n (%) | Invalid  n (%) | Negative  n (%) | Positive  n (%) | Negative  n (%) | Positive  n (%) | Invalid n (%) |
| **Bact + TB** a | 15 (33.3%) | 30 (66.7%) | 0 (0.0%) | 12 (34.3%) | 23 (65.7%) | 3 (30.0%) | 7 (70.0%) | 0 (0.0%) |
| (B+) male | 9 (34.6%) | 17 (65.4%) | 0 (0.0%) | 7 (35.0%) | 13 (65.0%) | 2 (33.3%) | 4 (66.7%) | 0 (0.0%) |
| (B+) female | 6 (31.6%) | 13 (68.4%) | 0 (0.0%) | 5 (33.3%) | 10 (66.7%) | 1 (25.0%) | 3 (75.0%) | 0 (0.0%) |
| Culture pos | 11 (29.7%) | 26 (70.3%) | 0 (0.0%) | 9 (30.0%) | 21 (70.0%) | 2 (28.6%) | 5 (71.4%) | 0 (0.0%) |
| Xpert pos | 11 (27.5%) | 29 (72.5%) | 0 (0.0%) | 11 (32.4%) | 23 (67.6%) | 0 (%) | 6 (100%) | 0 (0.0%) |
| *High* | 3 (23.1%) | 10 (76.9%) | 0 (0.0%) | 3 (27.3%) | 8 (72.7%) | 0 (%) | 2 (100%) | 0 (0.0%) |
| *Medium* | 5 (29.4%) | 12 (70.6%) | 0 (0.0%) | 5 (35.7%) | 9 (64.3%) | 0 (%) | 3 (100%) | 0 (0.0%) |
| *Low* | 0 (%) | 3 (100%) | 0 (0.0%) | 0 (0.0%) | 2 (100%) | 0 (%) | 1 (100%) | 0 (0.0%) |
| *Very Low* | 3 (42.9%) | 4 (57.1%) | 0 (0.0%) | 3 (42.9%) | 4 (57.1%) | 0 (%) | 0 (0.0%) | 0 (0.0%) |
| Culture neg | 143 (92.2%) | 8 (5.2%) | 4 (2.6%) | 92 (97.9%) | 2 (2.1%) | 51 (85.0%) | 5 (8.3%) | 4 (6.7%) |
| Culture cont | 10 (83.3%) | 2 (16.7%) | 0 (0.0%) | 8 (88.9%) | 1 (11.1%) | 2 (66.7%) | 1 (33.3%) | 0 (0.0%) |
| Xpert neg | 153 (93.3%) | 7 (4.3%) | 4 (2.4%) | 98 (99.0%) | 1 (1.0%) | 55 (85.9%) | 5 (7.8%) | 4 (6.3%) |
| **Not TB** b | 149 (93.7%) | 6 (3.8%) | 4 (2.5%) | 97 (99.0%) | 1 (1.0%) | 52 (86.7%) | 4 (6.7%) | 4 (6.7%) |
| Total | 164 (80.4%) | 36 (17.6%) | 4 (2.0%) | 109 (82.0%) | 24 (18.0%) | 55 (78.6%) | 11 (15.7%) | 4 (5.7%) |

a positive culture and/or Xpert. b negative culture and Xpert.

**Table 3.** Sensitivity and specificity of FujiLAM test by HIV status.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **All** | | **HIV-negative** | | **HIV-positive** | |
|  | Sensitivity  [n/N, %, 95%CI] | Specificity  [n/N, %, 95%CI] | Sensitivity  [n/N, %, 95%CI] | Specificity  [n/N, %, 95%CI] | Sensitivity  [n/N, %, 95%CI] | Specificity  [n/N, %, 95%CI] |
| Bact + TBa | 30/45, 66.7%, 51-80 | 153/159, 96.2%, 92-98 | 23/35, 65.7%, 48-80 | 97/98, 99.0%, 94-100 | 7/10, 70.0%, 35-92 | 56/60, 93.3%, 83-98 |
| Culture | 26/37, 70.3%, 53-84 | 147/155, 94.8%, 90-98 | 21/30, 70.0%, 50-85 | 92/94, 97.9%, 92-100 | 5/7, 71%, 31-95 | 55/60, 91.7%, 81-97 |
| Xpert | 29/40, 72.5%, 56-85 | 157/164, 95.7%, 91-98 | 23/34, 67.6%, 49-82 | 98/99, 99.0%, 94-100 | 6/6, 100%, 52-100 | 59/64, 92.2%, 82-97 |
|  | Positive Predictive Value  [n/N, %, 95%CI] | Negative Predictive Value  [n/N, %, 95%CI] | Positive Predictive Value  [n/N, %, 95%CI] | Negative Predictive Value  [n/N, %, 95%CI] | Positive Predictive Value  [n/N, %, 95%CI] | Negative Predictive Value  [n/N, %, 95%CI] |
| Bact + TBa | 30/36, 83%, 67-94 | 153/173, 88%, 83-93 | 23/24, 96%, 79-99 | 97/109, 89%, 82-94 | 7/11, 63%, 31-89 | 56/59, 94%, 86-99 |

apositive culture and/or Xpert. Specificity estimated considering *not TB* cases as not having TB. CI: confidence interval.

FujiLAM results had a similar pattern when analysed using culture or Xpert MTB/RIF results singly, as shown in tables 2 and 3 or when disaggregated by gender. FujiLAM results by Xpert MTB/RIF grades and HIV status are shown in table 2. Although numbers are too small for statistical analysis, the proportion of participants with positive FujiLAM seem to be higher among HIV-negative participants with high Xpert grades (8 (73%) of 11) than among participants with very low Xpert/RIF grades (four (57%) of seven). However, this pattern was not observed among participants with HIV, as all six HIV-positive participants were FujiLAM-positive, independently of their Xpert MTB/RIF grade (Table 3). FujiLAM by HIV viral load are shown in table 4 for bacteriologically confirmed, culture or Xpert MTB/RIF positive participants and Xpert grade. Most of the patients had high HIV viral loads. Sensitivity seemed higher among patients with high viral load, but numbers are too small to conduct a statistical analysis. The presence of clinical signs and symptoms and co-morbidities other than HIV were not associated with FujiLAM results (data not shown).

**Table 4.** Positive FujiLAM test results among HIV positive participants by TB status and HIV RNA viral load.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | HIV viral load | | | | |
|  | Undetected | <40 cps/ml | 40 - 107 cps/ml | Indeterminate | Missing |
| **Bact + TB** a | 1/1 (100%) | 1/2 (50%) | 3/4 (75%) | 0/0 (0%) | 2/3 (67%) |
| Culture pos | 0/0 (0%) | 1/2 (50%) | 3/3 (100%) | 0/0 (0%) | 1/2 (50%) |
| Xpert pos | 1/1 (100%) | 1/1 (100%) | 2/2 (100%) | 0/0 (0%) | 2/2 (100%) |
| *High* | 0/0 (0%) | 0/0 (0%) | 1/1 (100%) | 0/0 (0%) | 1/1 (100%) |
| *Medium* | 1/1 (100%) | 0/0 (0%) | 1/1 (100%) | 0/0 (0%) | 1/1 (100%) |
| *Low* | 0/0 (0%) | 1/1 (100%) | 0/0 (0%) | 0/0 (0%) | 0/0 (0%) |
| *Very Low* | - | - | - | - | - |
| Culture neg | 1/10 (10%) | 0/14 (0%) | 4/28 (14%) | 0/3 (0%) | 0/5 (0%) |
| Culture cont | 0/1 (0%) | 0/0 (0%) | 0/1 (0%) | 0/0 (0%) | 1/1 (100%) |
| Xpert neg | 0/10 (0%) | 0/15 (0%) | 5/30 (17%) | 0/3 (0%) | 0/6 (0%) |
| **Not TB** b | 0/10 (0%) | 0/14 (0%) | 4/28 (14%) | 0/3 (0%) | 0/5 (0%) |
| **Bact + TB** |  |  |  |  |  |
| Sensitivity | 100%, 0.05-1 | 50%, 0.03-1 | 75%, 0.2-1 | 0% | 67%, 0.1-1 |
| **Not TB** |  |  |  |  |  |
| Specificity | 100%, 0.7-1 | 100%, 0.7-1 | 86%, 0.7-1 | 100%, 0.3-1 | 100%, 0.5-1 |

4. Discussion

We have evaluated the diagnostic accuracy of FujiLAM in urine samples of adults with signs and symptoms suggestive of TB attending three district hospitals in Abuja, Nigeria. We found a sensitivity and specificity of 65.7% and 99.0% among HIV-negative patients and 70.0% and 93.3% among HIV-positive patients. These results are higher than reported for the rapid LAM test AlereLAM, which has a reported sensitivity of 42% (95% CI 31-55) among patients with HIV [13]. AlereLAM is recommended by the WHO for the complementary diagnosis of TB in patients with HIV, but not for HIV negative patients due to its low sensitivity in immune-competent individuals [13].

The overall sensitivity of FujiLAM in HIV-positive patients was slightly higher than in HIV-negative participants, but this difference was based on a small number of patients and was not statistically significant. However, the higher sensitivity observed among immunosuppressed individuals with HIV viral loads between 40 and 107 copies/ml (75.0%), was similar to studies of HIV-positive patients in Ghana and South Africa [11,14]. The higher sensitivity of LAM among HIV-positive individuals is said to reflect the increased concentration of LAM in urine due to the haematogenic spread of TB to the kidneys in immunosuppressed individuals [15]. However, LAM detection in urine is not limited to renal involvement [16], and is likely to be associated with the total bacterial burden of *M. tuberculosis* and the severity of disease [17].

The overall sensitivity among HIV-negative patients (65.7%) met the minimum target of 65% for the WHO high priority non-sputum-based TB diagnostic tests [18]. Our results were higher than reported in a multicentre study of HIV-negative patients in Peru and South Africa, which might be explained by the characteristics of the participants. FujiLAM is said to have higher sensitivity in patients with more advanced TB and higher *M. tuberculosis* loads in culture [19], and, as patients in Nigeria were recruited from district hospitals, a high proportion of our participants may have had advanced disease stages.

FujilAM specificity among HIV-negative patients was very high (99%) and similar to other studies among HIV negative patients [19] [18]. Specificity among HIV-positive patients was marginally higher (93.3%) than reported by a study of bio-banked urine samples from HIV-positive patients in three Sub-African countries [14], which reported a 90.8% specificity [11]. Patients with HIV experience multiple opportunistic infections, and positive FujiLAM results may well be false positives. However, it is also possible that these results reflect the difficulty of confirming the diagnosis in patients with disseminated TB disease. Culture and WRDs are imperfect tests, which have a lower performance in patients with HIV, extra-pulmonary and disseminated TB, depend on the quality of the sample and whether the patient is excreting bacilli the day of sampling and it is possible that patients who do not reach microbiological confirmation may have a missed TB diagnosis [20]. Our six patients with positive urine LAM results but negative sputum tests had the same clinical presentation to patients with bacteriologically confirmed TB. As it is likely some patients with TB do not have bacilli in sputum in the absence of cavitations or when the disease is disseminated without communications with the airways (e.g., military TB), we cannot rule out that these patients may have been detected by a test based in urine that does not require expectoration of bacilli and further studies are needed to confirm whether these were true or false positive results. However, it is also true that LAM is a cell wall compound that is present in most Mycobacteria species and is not exclusive in *M. tuberculosis*. Despite this cross reactivity risk, we feel the assay would be useful in the field, as it would allow rapidly identifying about two thirds of patients with TB at the time of the first consultation. The assays would need to be incorporated into diagnostic algorithms, as patients would need to undergo further confirmatory tests, to confirm the presence of MTB and then screen for drug resistance.

Urine is a readily available non-invasive sample, which would be especially useful in patients unable to expectorate, such as children, elderly and adults without productive cough and individuals with disseminated, extra-pulmonary and non-cavitary disease. FujiLAM is simple to use, does not require additional instrumentation and can be used in decentralised laboratories [21]. Therefore, FujiLAM is a promising test for the early detection and treatment of TB in people with signs and symptoms suggestive of TB, with a particular relevance for low resource health centres in low- and middle-income countries with the highest burden of TB.

Our study has several limitations. We only included patients able to provide sputum, which may underestimate the potential of FujiLAM to identify patients who are difficult to diagnose. In addition, the number of bacteriologically confirmed TB cases is small, especially for patients with HIV, and we were unable to follow-up participants, which could have re-classified some individuals with negative culture and Xpert MTB/RIF tests as positive, potentially increasing the specificity.

An important issue for the wider use of FujiLAM is that the test costs are currently too high for its wider implementation outside a research setting. FujiLAM is likely to be most useful in locations with limited resources and tiered pricing mechanisms will be needed to facilitate access to the tests according to need. Moreover, further cost effectiveness studies are needed. A study in South Africa and Malawi examining patients with HIV reported that FujiLAM combined with Xpert MTB/RIF was more cost-effective than using Xpert MTB/RIF alone [22]. However, more studies are needed to assess its cost effectiveness in HIV-negative patients, especially at lower levels of the health system in low resource settings.

Despite the development and scale-up of newer, more sensitive TB diagnostic tests over the last decade, these have not lived up to early promise because they remain too slow, expensive and resource-intensive (liquid culture), have been mostly implemented centrally, or have been found to have high diagnostic accuracy only among a subset of patients (e.g., urine LAM for severe HIV-associated illness). However, there is an emerging pipeline of new TB tests and tools that could allow rapid, accurate point of need diagnosis. Probably these tests are insufficient when used individually, but their performance could be optimized when used in combination as novel diagnostic algorithms. This is the case of the new generation urine LAM rapid test that we have studied: the FUJILAM test. Although the test has limited sensitivity, it would be able to detect two thirds of patients with bacteriologically confirmed TB at the time of first consultation. Future research therefore should evaluate the potential of FujiLAM in immunocompetent adults and children attending primary health care facilities and in patients with extrapulmonary and non-cavitary TB. Further studies are also needed to develop diagnostic algorithms that incorporate drug susceptibility testing for patients identified by FujiLAM at the lower levels of the healthcare system; and whether its combination with other screening tests, such as C Reactive Protein, could be used to develop point of care diagnostic algorithms.

There is also a clear need of new technology, and stronger efforts in the development, validation and market shaping initiatives to expand the use of these devices. FujiLAM manufacturing facilities are currently being expanded, and its manufacturer plans to apply for WHO endorsement in 2022. Other LAM prototype developers are also making strides to develop low-cost lateral flow assays with improved monoclonal antibodies, which are expected to become available as research-use-only prototypes in 2022, while novel LAM concentration methods are being tested in the field with preliminary good performance.

In conclusion, testing urine samples with FujiLAM in HIV-positive and HIV-negative patients with presumptive TB has a higher performance than current urine LAM assays. The use of the FujiLAM test would facilitate the detection and initiation of TB treatment on the same day of consultation in primary health centres.

**Author Contributions:** Conceptualization, LEC, JD; Methodology, PCB, BMM, JB, KK and RA; Validation, LEC, JD; Formal Analysis, PCB, LEC, JD, RA; Investigation, PCB, OO, RA; Resources, JB, JC, JD; Data Curation, PCB, OO, BMM, JB and RA; Writing – original draft, PCB, JD, LEC; Writing – review & editing, All authors; Supervision, JB, LEC, JD; Project administration, JD; Funding acquisition, JC, LEC, JD.

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**Conflicts of Interest:** The authors have no conflicts of interest to declare.

**Appendix A**

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

**Appendix B**

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled starting with “A”—e.g., Figure A1, Figure A2, etc.

References

1. World Health Organization *Global Tuberculsis Report 2019*; Geneva, 2019;
2. Lawn, S.D.; Mwaba, P.; Bates, M.; Piatek, A.; Alexander, H.; Marais, B.J.; Cuevas, L.E.; McHugh, T.D.; Zijenah, L.; Kapata, N.; et al. Advances in tuberculosis diagnostics: The Xpert MTB/RIF assay and future prospects for a point-of-care test. *Lancet Infect. Dis.* **2013**, *13*, 349–361, doi:10.1016/S1473-3099(13)70008-2.
3. Oga-Omenka, C.; Tseja-Akinrin, A.; Sen, P.; Mac-Seing, M.; Agbaje, A.; Menzies, D.; Zarowsky, C. Factors influencing diagnosis and treatment initiation for multidrug-resistant/rifampicin-resistant tuberculosis in six sub-Saharan African countries: A mixed-methods systematic review. *BMJ Glob. Heal.* 2020, *5*, 2280.
4. Dorman, S.E.; Schumacher, S.G.; Alland, D.; Nabeta, P.; Armstrong, D.T.; King, B.; Hall, S.L.; Chakravorty, S.; Cirillo, D.M.; Tukvadze, N.; et al. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. *Lancet Infect. Dis.* **2018**, *18*, 76–84, doi:10.1016/S1473-3099(17)30691-6.
5. Walzl, G.; Mcnerney, R.; Plessis, N.; Bates, M.; Mchugh, T.D.; Chegou, N.N.; Zumla, A. Series Tuberculosis 2 Tuberculosis : advances and challenges in development of new diagnostics and biomarkers. *Lancet Infect. Dis.* **2018**, *3099*, 1–12, doi:10.1016/S1473-3099(18)30111-7.
6. Detjen, A.K.; McKenna, L.; Graham, S.M.; Marais, B.J.; Amanullah, F. The upcoming UN general assembly resolution on tuberculosis must also benefit children. *Lancet Glob. Heal.* 2018, *6*, e485–e486.
7. Sigal, G.B.; Pinter, A.; Lowary, T.L.; Kawasaki, M.; Li, A.; Mathew, A.; Tsionsky, M.; Zheng, R.B.; Plisova, T.; Shen, K.; et al. A novel sensitive immunoassay targeting the 5-methylthio-D- xylofuranose–lipoarabinomannan epitope meets the WHO’s performance target for tuberculosis diagnosis. *J. Clin. Microbiol.* **2018**, *56*, 1–17, doi:10.1128/JCM.01338-18.
8. Marian E. Parker Complement Fixation with Urine in Tuberculosis. *Am. Rev. Tuberc.* **1931**, *23*, 733–738.
9. World Health Organization. Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV. *WHO* **2019**, doi:CC BY-NC-SA 3.0 IGO.
10. Kerkhoff, A.D.; Sossen, B.; Schutz, C.; Reipold, E.I.; Trollip, A.; Moreau, E.; Schumacher, S.G.; Burton, R.; Ward, A.; Nicol, M.P.; et al. Diagnostic sensitivity of SILVAMP TB-LAM (FujiLAM) point-of-care urine assay for extra-pulmonary tuberculosis in people living with HIV. *Eur. Respir. J.* **2020**, *55*, 1901259, doi:10.1183/13993003.01259-2019.
11. Broger, T.; Sossen, B.; du Toit, E.; Kerkhoff, A.D.; Schutz, C.; Ivanova Reipold, E.; Ward, A.; Barr, D.A.; Macé, A.; Trollip, A.; et al. Novel lipoarabinomannan point-of-care tuberculosis test for people with HIV: a diagnostic accuracy study. *Lancet Infect. Dis.* **2019**, *19*, 852–861, doi:10.1016/S1473-3099(19)30001-5.
12. World Health Organization. Who revised definitions and reporting framework for tuberculosis. *WHO* **2013**, *18*, 1–47, doi:10.2807/ese.18.16.20455-en.
13. Bjerrum S, Schiller I, Dendukuri N, Kohli M, Nathavitharana RR, Zwerling AA, Denkinger CM, Steingart KR, S.M. Lateral flow urine lipoarabinomannan assay for detecting active tuberculosis in people living with HIV. *Cochrane Database Syst. Rev.* **2019**, *CD011420*, doi:10.1002/14651858.CD011420.pub3.
14. Bjerrum, S.; Broger, T.; Székely, R.; Mitarai, S.; Opintan, J.A.; Kenu, E.; Lartey, M.; Addo, K.K.; Chikamatsu, K.; Macé, A.; et al. Diagnostic accuracy of a novel and rapid lipoarabinomannan test for diagnosing tuberculosis among people with human immunodeficiency virus. *Open Forum Infect. Dis.* **2020**, *7*, doi:10.1093/ofid/ofz530.
15. Lawn, S.D.; Gupta-Wright, A. Detection of lipoarabinomannan (LAM) in urine is indicative of disseminated TB with renal involvement in patients living with hiv and advanced immunodeficiency: Evidence and implications. *Trans. R. Soc. Trop. Med. Hyg.* **2015**, *110*, 180–185, doi:10.1093/trstmh/trw008.
16. Cox, J.A.; Lukande, R.L.; Kalungi, S.; Van Marck, E.; Van De Vijver, K.; Kambugu, A.; Nelson, A.M.; Colebunders, R.; Manabe, Y.C. Is urinary lipoarabinomannan the result of renal tuberculosis? Assessment of the renal histology in an autopsy cohort of ugandan HIV-infected adults. *PLoS One* **2015**, *10*, doi:10.1371/journal.pone.0123323.
17. Paris, L.; Magni, R.; Zaidi, F.; Araujo, R.; Saini, N.; Harpole, M.; Coronel, J.; Kirwan, D.E.; Steinberg, H.; Gilman, R.H.; et al. Urine lipoarabinomannan glycan in HIV-negative patients with pulmonary tuberculosis correlates with disease severity. *Sci. Transl. Med.* **2017**, *9*, doi:10.1126/scitranslmed.aal2807.
18. World Health Organization. High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. *WHO* **2014**, 1–96.
19. Broger, T.; Nicol, M.P.; Sigal, G.B.; Gotuzzo, E.; Zimmer, A.J.; Surtie, S.; Caceres-Nakiche, T.; Mantsoki, A.; Reipold, E.I.; Székely, R.; et al. Diagnostic accuracy of 3 urine lipoarabinomannan tuberculosis assays in HIV-negative outpatients. *J. Clin. Invest.* **2020**, *130*, 5756–5764, doi:10.1172/JCI140461.
20. Drain, P.K.; Gardiner, J.; Hannah, H.; Broger, T.; Dheda, K.; Fielding, K.; Walzl, G.; Kaforou, M.; Kranzer, K.; Joosten, S.A.; et al. Guidance for Studies Evaluating the Accuracy of Biomarker-Based Nonsputum Tests to Diagnose Tuberculosis. *J. Infect. Dis.* **2019**, *220*, S108–S115, doi:10.1093/infdis/jiz356.
21. FIND DX Pipeline Status Available online: https://www.finddx.org/dx-pipeline-status/ (accessed on Dec 12, 2020).
22. Reddy, K.P.; Denkinger, C.M.; Broger, T.; McCann, N.C.; Gupta-Wright, A.; Kerkhoff, A.D.; Pei, P.P.; Shebl, F.M.; Fielding, K.L.; Nicol, M.P.; et al. Cost-effectiveness of a novel lipoarabinomannan test for tuberculosis in patients with HIV. *Clin. Infect. Dis.* **2020**, doi:10.1093/cid/ciaa1698.