Diagnostic accuracy of commercial urinary lipoarabinomannan detection in African tuberculosis suspects and patients

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OBJECTIVE: To evaluate a commercially available antigen capture enzyme-linked immunosorbent assay (ELISA) based on detecting lipoarabinomannan (LAM) in urine for the diagnosis of tuberculosis (TB).

DESIGN: Consenting TB suspects and registering TB patients prospectively recruited from three hospitals were asked for two sputum specimens for microscopy and culture, urine for LAM testing and blood for human immunodeficiency virus (HIV) testing, with radiological and clinical follow-up for 2 months.

RESULTS: Of 427 participants, complete data were available from 397 (307 adult and 23 adolescent TB suspects, and 67 registering TB patients). HIV prevalence was 77%. TB was diagnosed in 195 (49%), including 161 culture-positive patients, and confidently excluded in 114 (29%) participants. LAM ELISA sensitivity was 44% (95%CI 36–52) for culture-confirmed TB (52% in smear-positive patients). Specificity was 89% (95%CI 81–94). Sensitivity was significantly higher in HIV-related TB (52%, 95%CI 43–62, \( P < 0.001 \)) compared to HIV-negative TB (21%, 95%CI 9–37). Sensitivity in smear-negative patients was low (28%, 95%CI 13–43) for combined HIV-positive and -negative patients.

CONCLUSION: Our findings confirm greater sensitivity of urine LAM detection for HIV-related TB. However, both sensitivity and specificity were suboptimal, suggesting that this version cannot confirm or exclude TB in either HIV-infected or non-infected patients.

KEY WORDS: tuberculosis; diagnostic; culture; screening; Africa; HIV

EFFECTIVE MANAGEMENT of suspected tuberculosis (TB) and the performance of TB control programmes in resource-poor settings are currently compromised by the lack of a sensitive, specific and timely diagnostic test.1 Consequential delays in diagnosis contribute to both high TB mortality and ongoing TB transmission.2–4

Among the most promising of the new TB diagnostics in development for point-of-care use are antigen-detection assays that are based on lipoarabinomannan (LAM), a carbohydrate cell-wall antigen that is excreted in the urine of TB patients.5,6 Unlike assays that rely on antibody or T-cell responses, antigen-detection assays are less vulnerable to host immune system variables and show the presence of replicating organisms. As such, they have high potential to distinguish active disease from latent TB infection, and will tend to have increased, rather than decreased, sensitivity in the immunosuppressed. Previous studies have shown LAM detection to have moderate sensitivity and good specificity, with the highest sensitivity being in human immunodeficiency virus (HIV) related TB and in patients with smear-positive disease.6–9 A number of different companies and research groups have LAM-based tests under development. One of these was recently launched as a commercial test, Clearview™ (Inverness Medical Innovations, Bedford, UK).

We evaluated the diagnostic performance of the pre-commercial product, a urine-based LAM ELISA, in Harare, Zimbabwe, where adult human immunodeficiency virus (HIV) prevalence is approximately 20%.10,11 We used TB microscopy and culture, and clinical and radiological case definitions for TB, to evaluate test performance in several distinct patient groups, including probable culture-negative TB. The main aims of the study were to estimate sensitivity and specificity according to HIV status and sputum bacteriology.
METHODS

Study design
A prospective cohort of TB suspects and registering TB patients followed up to confirm or exclude TB disease.

Study setting and sample size
The study was conducted at three hospitals in Harare, Zimbabwe, between September 2007 and July 2008. Sample size calculations were based around estimating an assumed test 60% sensitivity in HIV-infected TB patients with precision of 5%, and an estimated HIV prevalence of 85% in TB patients, true TB prevalence of 50% (deliberately oversampled through inclusion of known smear-positive TB patients), and 10% loss to follow-up.12

Study sites and participants
Participants were recruited from a referral hospital, the Beatrice Road Infectious Disease Hospital (BRIDH) in Harare, Zimbabwe: ambulant adult (age ≥18 years) smear-positive TB patients attending for registration (required to obtain a TB treatment card and continue TB treatment), and ambulant primary care TB suspects with cough of ≥3 weeks attending to submit specimens to the BRIDH microscopy laboratory; and two central hospitals in Harare, the Parirenyatwa and Harare Central Hospitals: adolescents (aged 10–18 years) admitted with a febrile or respiratory illness.

Sequential consenting adult TB suspects and registering TB patients (up to a maximum of 15 per day) were recruited from BRIDH. Adolescents were recruited with the consent of their guardians, as previously described.13 TB suspects were excluded if already on TB treatment. Registering TB patients were only considered eligible if they had been taking treatment for ≤24 h.

Investigation, follow-up and case definitions
All participants underwent chest radiography, and were asked to provide four sputum specimens (two spot and two morning), urine and blood for anonymous HIV testing. Adolescent in-patients were recruited with the consent of their guardians, and had TB blood cultures taken in addition to other investigations for the purposes of another study.13

Smear-negative TB suspects were treated with antibiotics (amoxicillin and doxycycline) and had repeat smear and culture (one spot and one morning specimen) before starting TB treatment if they had persistent symptoms and a compatible clinical and/or radiological illness.

Repeat TB investigations (smear, culture, radiography) were carried out in all participants who were not already on TB treatment if symptoms persisted for 2 months, or if there were initial radiological changes.

Patients started on TB treatment were followed up for 2 months to document radiological and clinical response to treatment unless TB was bacteriologically confirmed in the interim (two positive smears or two cultures growing Mycobacterium tuberculosis). Response to TB treatment was assessed on the basis of 1) weight gain, 2) radiological response (complete or partial), 3) resolution of previously documented temperature and 4) reported resolution of previously documented symptoms (cough, haemoptysis, fever, night sweats and chest pain). On the basis of the above symptoms, the attending physician categorised overall treatment response as ‘highly consistent with TB’, ‘TB likely’, or ‘TB unlikely’. All case definitions were reviewed independently by a second person.

TB case definitions were as follows:

Smear-positive TB: at least one positive smear from initial (four specimens) and follow-up (two specimens) microscopy, plus culture-positive (Löwenstein-Jensen [LJ] slope positive or blood culture-positive) for M. tuberculosis.

Smear-negative, culture-positive TB: ≥1 positive culture of M. tuberculosis with all smears negative.

Smear-negative, culture-negative TB: all samples smear- and culture-negative, but clinical and radiological evidence of TB, non-response to broad-spectrum antibiotics, response to 2 months of TB treatment.

Non-TB: All samples smear- and culture-negative, and stable recovery at 2 months without TB treatment.

Indeterminate: not meeting any of the above definitions.

Laboratory methods
Collected urine specimens were kept unprocessed at 2–8°C and run within 3 days of collection. Urine specimens of <10 μl were excluded. Urine was processed by heating for 30 min at 100°C and centrifuged at 10000 rpm for 15 min, and 100 μl of supernatant was used for urine LAM testing following the manufacturer’s instructions on the same day. Automatic washing was used at all stages. The pre-commercial Chemogen (Chemogen, Portland, ME, USA) version of the LAM ELISA was used. All specimens were run in duplicate and optical densities (ODs) read at 450 nm after stopping with 1 molar sulphuric acid. The average OD of the negative control plus 0.1 was considered as the cut-off, with specimens with ODs less than and more than the cut-off taken as negative and positive, respectively. Specimens with one OD value less than the cut-off and one more than the cut-off were recorded as indeterminate. Interpretation of LAM results was independent of any other clinical or laboratory results.

Sputum smears were made from both direct and concentrated decontaminated (4% sodium hydroxide) sputum and read by fluorescence microscopy (auramine O). All positive and 10% of negative slides were...
re-read by a second reader. Auramine positive slides were confirmed with Ziehl-Neelsen (ZN) staining (classified as smear-negative if ZN-negative). Culture used LJ slopes, with the residual concentrate stored at −20°C for re-culture in case of contamination. Species identification used MPB64 antigen detection (Capillia™, Becton Dickinson, Sparks, MD, USA) and visual inspection for cording. Further speciation tests (colony morphology, growth at different temperatures, and growth on para-nitrobenzoic acid-containing LJ slopes) were used for isolates that were ZN-positive but MPB64 antigen-negative. Blood culture used Mycolytic F™ (Becton Dickinson, Johannesburg, South Africa; adolescent participants only).

Confidential HIV serology used Determine™ HIV-1/2 Rapid Test Kit (Inverness Medical Innovations, Waltham, MA, USA), with all positives and 10% of negative specimens confirmed by Uni-Gold™ Test Kit (Trinity Biotech, Dublin, Ireland). For participants not willing to provide serum, oral mucosal transudate was collected and tested using Vironostika® Uniform II Plus O (bioMérieux, Marcy l’Etoile, France).

Data analysis

Data were entered into Epi Info version 3.4.1 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and analysed using STATA 9.0 (STATA Corp, College Station, TX, USA). Between-group differences in patient characteristics and LAM ELISA positivity were tested for statistical significance using the χ² test.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the LAM ELISA were calculated for 1) smear-positive TB, 2) culture-positive TB (regardless of smear results) and 3) all diagnosed TB patients meeting case definitions for TB disease.

Patients with indeterminate TB status and, where relevant, patients with TB who did not meet the bacteriological criteria for which sensitivity and specificity were being calculated, were excluded from the analysis.

Ethical approval

The study was approved by the Zimbabwe Medical Research Council, the Biomedical Research and Training Institute’s Institutional Review Board and the Ethics Committee of the London School of Hygiene & Tropical Medicine. Diagnostic provider-initiated counselling and testing was offered to all participants, according to national guidelines.

RESULTS

Baseline characteristics

Participation was 97% (refusals: 11 registering TB patients and one adolescent). Of 427 participants,

![Figure 1](image.png)

**Figure 1** Participant flow chart. Of 427 recruited TB patients and suspects, 30 were excluded, 397 patients were tested with Chemogen, and 88 patients were TB indeterminate by smear, culture and clinical follow-up. The Chemogen sensitivity and specificity is given for the TB and non-TB patient groups with the corresponding 95%CI. LAM = lipoarabinomannan; TB = tuberculosis; S+ve = sputum smear-positive for acid-fast bacilli; S−ve = sputum smear-negative for acid-fast bacilli; C+ve = sputum culture-positive for *M. tuberculosis*; C−ve = sputum culture-negative for *M. tuberculosis*; CI = confidence interval.
complete data were available for 397 (307 adult TB suspects, 23 adolescents and 67 registering TB patients), as 30 participants with no urine samples or urine sample of <10 ml were excluded (Figure 1).

Participant characteristics are shown in Table 1; overall HIV prevalence was 77%.

TB status
TB was excluded in 114 (29%) and diagnosed in 195 (49%) participants. Disease was culture-confirmed in 161, including 121 (62%) smear- and culture-positive and 40 (21%) smear-negative and culture-positive. Thirty-four (17%) patients had culture-negative TB diagnosis through clinical/radiological criteria (Figure 1). There were no smear-positive, culture-negative patients. TB was neither confirmed nor excluded in 88 (22%) patients, as shown in Figure 1.

Test sensitivity for culture-positive TB
There were major variations in the sensitivity of LAM according to HIV and bacteriological status, as shown in Table 2 and Figure 2. Overall test sensitivity (combined HIV groups), at 44% (95% confidence interval [CI] 36–52), was considerably lower than that of concentrated fluorescent ('sensitive') microscopy (83%, 95% CI 75–89, P < 0.001).

Sensitivity was 50% (95% CI 40–59) in smear- and culture-positive TB, 28% (95% CI 13–43) in smear-negative, culture-positive TB and 27% (95% CI 11–43) in clinically diagnosed smear- and culture-negative TB patients.

As previously reported for LAM tests, sensitivity for culture-confirmed TB was significantly higher in HIV-positive patients (52%, 95% CI 43–62, P < 0.001) compared to HIV-negative TB patients (21%, 95% CI 9–37). Corresponding sensitivities for sensitive smear microscopy were 60% (95% CI 49–70) for

<table>
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<tr>
<th>Group</th>
<th>Prevalence</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tbody>
<tr>
<td>Overall (n = 261)*</td>
<td>58</td>
<td>44</td>
<td>36–52</td>
<td>89</td>
<td>81–94</td>
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<tr>
<td>HIV-positive (n = 182)</td>
<td>58</td>
<td>52</td>
<td>43–62</td>
<td>86</td>
<td>73–93</td>
</tr>
<tr>
<td>HIV-negative (n = 67)</td>
<td>57</td>
<td>21</td>
<td>9–37</td>
<td>93</td>
<td>77–99</td>
</tr>
</tbody>
</table>

* Table results exclude 34 culture-negative TB patients and 14 patients with indeterminate LAM status (12 TB patients and 2 non-TB patients).

1HIV status missing for 12 patients (8 TB patients and 4 non-TB patients).

LAM = lipoarabinomannan; TB = tuberculosis; NPV = negative predictive value; PPV = positive predictive value; HIV = human immunodeficiency virus; CI = confidence interval.
culture-positive, HIV-positive TB and 90% (95% CI 76–97) for culture-positive, HIV-negative TB. The highest LAM sensitivity was in HIV-positive, smear-positive patients (64%, 95% CI 53–76). Test sensitivity in adult TB suspects only (42%, 95% CI 32–53) was similar to overall test sensitivity.

Test specificity

Using patients in whom TB had been confidently excluded, overall LAM specificity was 89% (95% CI 81–94): this was similar in HIV-positive (86%, 95% CI 77–93) and HIV-negative (93%, 95% CI 77–99) TB suspects (Table 2).

Negative and positive predictive values

The overall NPVs and PPVs were respectively 84% (95% CI 74–91) and 54% (95% CI 46–61). NPVs and PPVs by HIV status were similar to the overall predictive values (Table 2).

Test positivity across culture-negative clinical groups

LAM test positivity varied significantly between clinically distinct groups of culture-negative patients (Table 3). Culture-negative patients lost to follow-up because of death (n = 17) or relocation to rural areas (n = 33) had LAM positivity similar to that of patients with culture-confirmed TB, significantly higher (29%, P = 0.035 and 30%, P = 0.014) than LAM positivity prevalence in patients in whom TB had been excluded (Table 3).

DISCUSSION

The main findings from this study are that the LAM ELISA evaluated here had low sensitivity (44%) for culture-positive TB and suboptimal specificity (89%), resulting in low NPVs and PPVs of 84% and 54% in this cohort. There is an urgent need for better TB diagnostics that perform well in detecting HIV-related disease and, in this study, as previously reported, LAM sensitivity was higher in HIV-related than HIV-negative TB. However, the low specificity, and consequently the low PPVs, do not support the use of this test as a first-line diagnostic, even if formatted into a rapid lateral flow test. A recent study in India, using the same version of the test, also concluded that the test is insensitive in detecting active TB in a predominantly HIV-negative cohort of TB suspects.

Unfortunately, the LAM ELISA also performed poorly in detecting smear-negative TB, a patient group where a more effective diagnosis is urgently needed, with sensitivity of 28% in smear-negative, culture-positive patients and 27% in smear- and culture-negative patients who met the case definitions for TB disease through response to TB treatment. Smear microscopy, the first-line investigation of TB suspects in resource-poor settings, has low sensitivity, ranging from 43% to 74% compared to culture in research studies, and perhaps as low as 20% in routine programmes. Mycobacterial culture is more sensitive, although still not 100%, but suffers from a long turnaround time and requirements for expertise and infrastructure that preclude its use in most resource-poor settings. Smear sensitivity is further reduced by concurrent HIV infection, making smears a very suboptimal first-line test in high HIV prevalence settings. The main gaps in the currently available diagnostics are thus rapid point-of-care tests with sensitivity and specificity at least as good as smear, and also diagnostics with good performance in detecting HIV-related smear-negative TB.

Sensitivity in this study is lower than previously reported using other LAM ELISA assays. This may reflect differences in test formats and/or in study design. Studies comparing test results from confirmed TB patients against those of healthy controls typically report higher test accuracies than found in prospective test evaluations in TB suspects. In this study, sensitivity was 64% for HIV-positive, smear-positive TB, whereas in previous reports sensitivity in this group has been as high as 79%. Our use of highly sensitive microscopy (fluorescent and mechanical concentration) may, paradoxically, have contributed to the apparently low performance of LAM ELISA in detecting smear-positive TB in this study.

We also show significant differences in LAM positivity between patients in whom TB was excluded on the basis of negative investigations and complete clinical recovery (12% LAM-positive) and other clinically distinct culture-negative patient groups (clinical TB, died during assessment, lost to follow-up: 27–30% LAM positive and 13% in patients for whom TB was neither confirmed nor confidently excluded by the end of 2 months’ follow-up), suggesting that urine LAM may have potential as an epidemiological tool for investigating the overall performance of TB screening clinical algorithms, even if it is not sufficiently specific to allow individual diagnosis. However, this, too, would require higher sensitivity than demonstrated here. The relatively high LAM positivity in patients

<table>
<thead>
<tr>
<th>Patient group</th>
<th>LAM positivity</th>
<th>Reference group</th>
<th>p value</th>
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<tbody>
<tr>
<td>Non-TB</td>
<td>114</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Clinical TB*</td>
<td>34</td>
<td>27</td>
<td>0.030</td>
</tr>
<tr>
<td>Died during follow-up</td>
<td>17</td>
<td>29</td>
<td>0.044</td>
</tr>
<tr>
<td>Loss to follow-up</td>
<td>33</td>
<td>30</td>
<td>0.009</td>
</tr>
<tr>
<td>Indeterminate†</td>
<td>38</td>
<td>13</td>
<td>0.772</td>
</tr>
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* As described in the Methods, TB was considered in culture-negative patients with symptoms and chest radiographic features consistent with TB with clinical response to TB treatment at 1 month.
† TB neither confidently confirmed (TB treatment started but without clear response) nor confidently excluded (incomplete recovery from presenting condition) in 38 patients by end of 2 months follow-up.

Table 3 The prevalence of Chemogen LAM positivity rates in clinically distinct culture-negative patient groups
lost to follow-up through death, which is similar to that of clinically diagnosed TB, concurs with reports of high rates of death from undiagnosed TB in post mortem studies in Africa.\textsuperscript{10–33}

The study was not able to follow up patients beyond 2 months, resulting in some participants with indeterminate TB status being excluded from the sensitivity and specificity analysis. We also had a small number of adolescents, limiting evaluation of the test in this particular group.

**CONCLUSION**

Our findings confirm the considerably greater sensitivity of LAM urine-based antigen detection tests for HIV-positive TB compared to HIV-negative TB. However, both sensitivity and specificity were suboptimal in this evaluation, and below that of sensitive smear microscopy even in HIV-positive TB patients. The low specificity undermines the potential of this test to be used in a rapid test kit format as a first-line point-of-care test for HIV-related TB, while the very low sensitivity for smear-negative TB patients suggested little added value as a diagnostic in this important subgroup. Overall, the urine-based LAM test cannot be used to either confidently confirm or exclude TB in HIV-positive or HIV-negative patients.

**Acknowledgements**

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**References**

OBJECTIF : Evaluer un test avec immunoadsorbant lié à une enzyme (ELISA) de capture des antigènes commercialement disponible pour la détection du lipoarabinomannan (LAM) dans l’urine, en vue du diagnostic de la tuberculose (TB).

SCHEMA : On a demandé aux suspects de TB et aux patients TB en cours d’enregistrement, consentants, recrutés prospectivement dans trois hôpitaux, deux échantillons de crachats pour l’examen microscopique et la culture, de l’urine pour le test LAM et du sang pour le test pour le virus de l’immunodéficience humaine (VIH) ainsi qu’un suivi radiologique et clinique pendant 2 mois.

RÉSULTATS : Les données complètes ont été disponibles chez 397 des 427 participants (307 adultes et 23 adolescents suspects de TB et 67 patients TB à l’enregistrement). La prévalence du VIH est de 77%. On a diagnostiqué la TB chez 195 participants (49%), parmi lesquels 161 positifs à la culture, et on l’a exclue en toute sécurité chez 114 (29%). La sensibilité du LAM ELISA est de 44% (IC95% 36–52) pour les cas de TB confirmés par la culture et de 52% chez les patients à bacilloscopie positive. La spécificité est de 89% (IC95% 81–94). La sensibilité est significativement plus élevée dans les cas de TB liés au VIH (52% ; IC95% 43–62 ; P < 0,001) par comparaison avec les TB séronégatifs pour le VIH (21% ; IC95% 9–37). La sensibilité est faible chez les patients à bacilloscopie négative (28% ; IC95% 13–43), les patients séropositifs et séronégatifs pour le VIH réunis.

CONCLUSION : Nos observations confirment une sensibilité plus élevée de la détection du LAM dans les urines pour la TB liée au VIH. Toutefois, tant la sensibilité que la spécificité ne sont pas optimales, ce qui suggère que cette version du test ne suffit pas à confirmer ou exclure la TB chez les patients, qu’ils soient infectés ou non par le VIH.