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Comparative performance of urinary lipoarabinomannan assays and Xpert MTB/RIF in HIV-infected individuals with suspected tuberculosis in Uganda

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Abstract

Background—Xpert MTB/RIF (‘Xpert’) and urinary lipoarabinomannan (LAM) assays offer rapid tuberculosis (TB) diagnosis, but have suboptimal sensitivity when used individually in HIV-positive patients. The yield of these tests used in combination for the diagnosis of active TB among HIV-infected TB suspects is unknown.

Design—Study of comparative diagnostic accuracy nested into a prospective study of HIV-infected individuals with signs and/or symptoms of TB in Uganda.

Methods—Xpert testing of archived sputum was conducted for culture-confirmed TB cases and TB suspects in whom a diagnosis of TB was excluded. Additional testing included sputum smear microscopy, sputum culture (solid and liquid media), mycobacterial blood culture, and urinary testing for LAM using a lateral flow test (‘LF-LAM’) and an enzyme-linked immunosorbance assay (‘ELISA-LAM’).

Results—Among 103 participants with culture-confirmed TB, sensitivity of Xpert was 76% (95% confidence interval, CI 0.66–0.84), and was superior to that of LF-LAM (49%, 95% CI 0.39–0.59, $P < 0.001$). Specificity was greater than 97% for both tests among 105 individuals without TB. The combination of smear microscopy and LF-LAM identified 67% (95% CI 0.57–0.76) of culture-confirmed TB cases and approached sensitivity of Xpert testing alone ($P = 0.15$). The sensitivity of the combination of Xpert and LF-LAM was 85% (88/103 95% CI 0.77–0.92), which was superior to either test alone ($P < 0.05$) and approached sensitivity of sputum liquid culture testing (94%, 95% CI 0.88–0.98, $P = 0.17$).

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Conflicts of interest

None declared.

Conclusion—Sputum Xpert and urinary LAM assays were complementary for the diagnosis of active TB in HIV-infected patients, and sensitivity of the combination of these tests was superior to that of either test alone.

Keywords

diagnostics; HIV; lipoarabinomannan; tuberculosis; Xpert MTB/RIF

Introduction

Tuberculosis (TB) is the leading cause of death among HIV-infected persons in many resource-constrained settings [1]. Despite current efforts, global case detection of active TB remains at less than 60% in many endemic settings, with some patients dying before TB diagnosis [1]. A key challenge is the lack of adequate tools for rapid diagnosis of active TB in HIV-infected individuals. Smear microscopy, the most widely utilized TB diagnostic modality worldwide, has poor sensitivity and identifies only a minority of HIV-related TB cases [2]. Sputum culture, the reference standard, is costly, not widely available, and takes weeks to provide results. With advanced immunosuppression, disseminated and extrapulmonary TB become more common and evaluation of sputa alone may be inadequate. Newly emerging TB diagnostic tests may enhance diagnostic algorithms by offering rapid, point-of-care or near-care detection of TB for HIV patients. Among the most promising are the urine tests for lipoarabinomannan (LAM) and the Xpert MTB/RIF test ('Xpert'; Cepheid, Sunnyvale, California, USA).

LAM is a glycolipid component of the mycobacterial outer cell wall and is detectable in urine of HIV-infected individuals with active TB disease [3–6]. In HIV-positive patients, an ELISA for LAM has been shown to have sensitivity of up to 85% in TB patients with low CD4⁺ cell counts; test specificity has varied in range from 88 to 100%, depending on reference standard utilized [3,4,6,7]. The Determine TB LAM test (Alere, Waltham, Massachusetts, USA) is a lateral flow LAM (LF-LAM) assay that requires little equipment or sample processing and yields results in approximately 30 min [6]. In HIV-positive adults, accuracy of the LF-LAM test is similar to that of the ELISA-LAM platform in hospitalized and ambulatory patients [3,6,8]. Both urinary LAM tests offer the benefit of evaluating nonrespiratory samples and previous studies have shown that urine LAM assays have additive value when combined with sputum smear microscopy [3,4,6].

Another strategy for the rapid diagnosis of active TB has been detection of mycobacterial genetic material in sputum after nucleic acid amplification. Xpert is an integrated, automated specimen processing and nucleic acid amplification test for diagnosis of TB and rifampicin susceptibility in as little as 2 h [9]. Xpert sensitivity and specificity for TB have been above 95% in some settings, and the test has been endorsed by the WHO for use in resource-constrained settings [9,10]. However, reliance on sputum Xpert testing alone may be suboptimal for HIV-positive patients [11]. When a single sputum is tested, Xpert identifies only 55–72% of smear-negative pulmonary TB (PTB) cases [9,11]. The increased incidence of smear-negative PTB and extrapulmonary or disseminated TB among HIV-positive

individuals may lessen the yield of Xpert sputum testing, particularly in the most severely immunocompromised patients – the group at highest risk for TB-associated death.

To date, studies among TB suspects have focused on ascertaining diagnostic accuracy of the urinary LAM assays and Xpert individually as stand-alone tests, but these tests may be complementary for the diagnosis of TB in HIV patients. Future TB diagnostic strategies could incorporate use of these tests together. We sought to compare the performance of sputum Xpert with performance of the urinary LAM assays for the diagnosis of active TB among symptomatic adult HIV-positive TB suspects in Uganda, and to evaluate the diagnostic accuracy of using these tests in combination with each other and with conventional diagnostics tools.

Methods

Ethics statement

The study was approved by the institutional review board at the Johns Hopkins University School of Medicine (Baltimore, USA), as well as in Uganda by the scientific review committee of the Infectious Diseases Institute, the Research Ethics Committees of the Ugandan Joint Clinical Research Centre and Mulago National Referral Hospital, the Uganda National Council for Science and Technology, and Boston University Medical Center institutional review board. Witnessed written informed consent was provided by all study participants.

Study site and population

This study was nested in a prospective TB diagnostics study at the Infectious Disease Institute (IDI) and Mulago Hospital in Kampala, Uganda [12]. The parent study enrolled hospitalized and ambulatory HIV-positive adults with at least one sign or symptom of TB (i.e., TB suspects). Participants underwent a panel of protocol-specified TB diagnostic tests and had a study follow-up visit 2 months later conducted in-person or by phone. For the parent study, two fresh spot sputa were tested for acid fast bacilli (AFB) by Ziehl–Neelsen and by fluorescence smear microscopy, and these sputa were cultured using Lowenstein–Jensen and mycobacteria growth indicator tube (MGIT) media; blood was cultured for mycobacteria and a CD4⁺ cell test was performed; one spot urine was tested using LF-LAM and ELISA-LAM tests. Individuals were considered to have culture-confirmed TB if there was at least one positive culture for *Mycobacterium tuberculosis* from sputum or blood; individuals were categorized as ‘Not TB’ if they had no sputum or blood culture positive for *M. tuberculosis*, and they were clinically improved without TB treatment at the 2-month follow-up. For this comparative diagnostics study, 103 consecutively enrolled individuals with culture-confirmed TB and 105 consecutively enrolled individuals categorized as ‘Not TB’ received Xpert testing on the processed frozen pellet derived from the initial sputum specimen collected at the time of enrollment. Sample size was based on resource availability for Xpert testing. Investigators were blinded to LAM results when determining TB status and eligibility for this study. Participants who, in the parent study, did not meet criteria for culture-confirmed or ‘Not TB’ were not eligible for additional Xpert testing as part of this comparative diagnostics study.

Laboratory testing

All tests were conducted by reference laboratories on site in Uganda.

Smear microscopy and mycobacterial cultures—A Ziehl–Neelsen-stained smear of unprocessed sputa was examined by light microscopy and graded according to WHO criteria [13]. Sputa were decontaminated using *N*-acetyl-L-cysteine-NaOH and an additional concentrated Auramine-O smear was prepared, examined using a fluorescent microscope, and graded [13]. A 0.5-ml portion of processed sputum sediment was cultured using the BACTEC MGIT 960 system (Becton and Dickinson, Franklin Lakes, New Jersey, USA); an additional 0.2 ml aliquot was inoculated onto Lowenstein–Jensen media, and the remainder of the sputum sediment was stored frozen at -80°C . Cultures were incubated at 37°C for up to 6 weeks. MYCO/F LYTIC (Becton and Dickinson) tubes were used for mycobacterial blood cultures. Positive cultures were assessed for presence of AFB using Ziehl–Neelsen staining and light microscopy, and for *M. tuberculosis complex* using an anti-MPB64 monoclonal antibody assay (Capilia TB-Neo; TAUNS Laboratories, Numazu, Japan).

Urine lipoarabinomannan testing—Testing using the Determine TB lateral-flow assay was performed and interpreted according to manufacturer recommendations. Sixty microliter of fresh urine was applied to the test strip and incubated at room temperature for 25 min, after which the presence and intensity of bands was graded by a trained laboratorian. A band intensity of ‘grade 2’ was considered positive for primary analysis [3,14]. Analyses were also performed using a ‘grade 1’ intensity band as positive [14]. Urinary LAM testing using the Clearview TB ELISA kit was conducted at the same laboratory and was performed and interpreted according to manufacturer’s directions. Urine specimens were subjected to initial processing and then frozen within 24 h of collection. ELISA testing was performed in batches. Optical density at 450 nm was measured using an ELx800 microplate reader (BioTek Instruments, Winooski, Vermont, USA). Duplicate samples with average optical densities of 0.1 greater than the negative control were considered positive.

Xpert MTB/RIF test—Testing was conducted in batches using frozen sputum sediments. Samples were thawed at room temperature and vortexed for 15 s. A 0.5 ml of resuspended sediment was transferred to a conical screw-capped tube to which 1.5 ml of Sample Reagent was added. The sample was shaken, incubated at room temperature for 15 min, and transferred to the cartridge. The cartridge was loaded into a four-module GeneXpert instrument (Cepheid); an automated readout was generated approximately 2 h later.

Statistical analysis

Student’s *t*-test was used to compare means. Two sample proportions were compared by χ^2 tests. McNemar’s test was used to compare urinary LAM, Xpert, smear, and culture sensitivities and specificities. Sensitivity was defined as the percentage positive by each diagnostic test or combination of tests among those with culture-confirmed TB. When assessing sensitivity of a combination of tests, the combined result was considered positive if any of the individual tests were positive. Specificity was defined as the percentage negative by each diagnostic test or combination of tests among those categorized as ‘Not TB’. When assessing specificity of test combinations, the combined result was considered

negative only if each of the individual tests had a negative result. A P value 0.05 was considered statistically significant and 95% confidence intervals (CIs) were used. Statistical calculations were performed using Stata 10.1 (StataCorp., USA).

Results

Study population

Among 103 HIV-positive patients with culture-confirmed TB disease, median age was 32 (interquartile range, IQR 26–37), 61% (63/103) were women, median CD4⁺ cell count was 63 cells/ μ l (IQR 19–152), and 84% (86/103) were hospitalized at the time of enrollment. Among these 103 patients, 54 (52%) had PTB alone, 43 (42%) had both PTB and *M. tuberculosis* mycobacteremia, and six (6%) had mycobacteremia without PTB. Among these 103 confirmed TB cases, 31 (30%) were smear-positive by Ziehl–Neelsen and 43 (42%) were smear-positive by fluorescence when two sputum samples were tested (Tables 1 and 2).

For 105 HIV-positive TB suspects determined to not have TB, median age was 34 (IQR 27–39, $P=0.19$ vs. the culture-confirmed TB cases), 71 (68%) were women ($P=0.33$), median CD4⁺ cell count was 280 cells/ μ l (IQR 97–486) ($P<0.001$), and only 34% (36/105, $P<0.001$) were hospitalized at time of enrollment.

Comparative performance of Xpert MTB/RIF and urinary lipoarabinomannan assays

Sensitivity and specificity of TB diagnostics evaluated in this study are shown in Table 1. In patients with culture-confirmed TB, sputum Xpert sensitivity was 76% (78/103, 95% CI 0.66–0.84), and did not differ by CD4⁺ cell count (Table 2; $P=0.791$). Xpert sensitivity was significantly lower in patients with smear-negative TB by fluorescence [60% (36/60, 95% CI 0.47–0.72)] compared with those with smear-positive TB [98% (42/43, 95% CI 0.88–1), $P<0.001$]. Xpert specificity among participants without TB was 98% (103/105, 95% CI 0.93–0.99).

Sensitivity of LF-LAM at the grade 2 cut-off (LF-LAM/grade 2) for test positivity was 49% (50/103, 95% CI 0.39–0.59), and specificity was 97% (102/105, 95% CI 0.92–0.99). Sensitivity of LF-LAM/grade 1 assay was 63% (65/103, 95% CI 0.53–0.72, $P=0.001$ vs. LF-LAM/grade 2), but specificity was only 88% [92/105 (95% CI 0.80–0.93), $P=0.002$ vs. LF-LAM/grade 2]. Sensitivity of LF-LAM, using either grade 1 or grade 2 thresholds, was not different between individuals with sputum smear-positive vs. smear-negative results (Table 2, $P>0.05$). Sensitivity of the LF-LAM, using either threshold, differed by CD4⁺ cell count and was highest in those with advanced immunosuppression (Table 2), and was significantly higher among hospitalized patients compared with outpatients (Table 2, $P<0.05$).

Sputum Xpert testing had higher sensitivity (76%) than LF-LAM/grade 1 (63%, $P=0.047$), LF-LAM/grade 2 (49%, $P<0.001$), and the ELISA-LAM (59%, $P=0.01$, Table 1). However, among smear-negative TB cases, there was no difference in sensitivity between Xpert (60%) and LF-LAM/grade 1 (62% sensitivity, $P=0.69$), LF-LAM/grade 2 (43% sensitivity, $P=0.10$), or ELISA-LAM (60% sensitivity, $P=1.0$, Table 2). Xpert sensitivity

was higher than those of all urinary LAM assays among those with CD4⁺ cell counts greater than 100 ($P < 0.001$ for all pairwise comparisons), but Xpert sensitivity was not significantly different from that of the LAM assays among TB cases with CD4⁺ cell counts less than 100 (Table 2). Among hospitalized patients, Xpert MTB/RIF sensitivity was higher than that of LF-LAM/grade 2 (74 vs. 56%, $P = 0.01$, Table 2).

The specificity of Xpert, LF-LAM/grade 2, and ELISA-LAM were all greater than 97% and not significantly different among individuals without TB ($P > 0.05$ for all pairwise comparisons); specificity of LF-LAM at the grade 1 threshold (88%) was significantly lower than Xpert (98%, $P = 0.01$), LF-LAM/grade 2 (97%, $P = 0.002$), or ELISA-LAM (98%, $P = 0.003$).

Performance of combinations of tests

The performance of test combinations was explored and compared to the performance of each test individually (Table 1).

Combination of sputum Xpert with urinary lipoarabinomannan assays—There was incremental yield when Xpert testing was combined with urinary LAM testing, compared with each test individually (Table 1 and Fig. 1). The combination of sputum Xpert and urine LF-LAM/grade 2 had sensitivity of 85% (88/103 95% CI 0.77–0.92), which was superior to the sensitivity of Xpert alone (76%, $P = 0.002$) or LF-LAM/grade 2 alone (49%, $P < 0.001$) (Table 1 and Fig. 1). Moreover, sensitivity of the Xpert and LF-LAM/grade 2 combination was similar to that for two sputa cultured on Lowenstein–Jensen medium (80%, $P = 0.24$) or in MGIT medium (92%, $P = 0.17$). Sensitivity of the Xpert and LF-LAM/grade 2 combination did not differ among inpatient [74/86 (86%)] or outpatient TB cases [14/17 (82%), $P = 0.69$], and was similar across all CD4⁺ cell strata (89% for CD4⁺ cell count < 50 , 88% for CD4⁺ cell count 50–100, 84% for CD4⁺ cell count 100–200, and 76% for CD4⁺ cell count > 200 ; $P = 0.56$). However, among outpatients, the sensitivity of the combination of Xpert and LF-LAM/grade 2 (82%) was not significantly different from Xpert alone (82%, $P = 1.0$), nor was this combination (80%) different from Xpert alone (78%) among those with CD4⁺ cell count more than 100 ($P = 0.317$). Alternatively, this test combination identified significantly more individuals than Xpert alone among hospitalized patients (86 vs. 74%, $P = 0.002$) and among those with CD4⁺ cell count less than 100 (89 vs. 75%, $P = 0.002$).

Among individuals with smear-negative, culture-confirmed TB, addition of LF-LAM/grade 2 increased the diagnostic yield from 60% (36/60) for Xpert alone to 77% (46/60, $P < 0.001$) with the test combination. Among individuals without TB, the specificity of the Xpert and LF-LAM/grade 2 combination was 95% (100/105, 95% CI 0.89–0.98) and was not significantly worse than specificity of Xpert alone (98%, $P = 0.25$) or LF-LAM/grade 2 alone (97%, $P = 0.50$).

Compared with the combination of Xpert and LF-LAM/grade 2, utilizing a lower threshold for LF-LAM positivity, namely, LF-LAM/grade 1, significantly increased the sensitivity of the Xpert and LF-LAM test combination, but resulted in lower specificity. Sensitivity of the combination of Xpert and LF-LAM/grade 1 was 90% (93/103, 95% CI 0.83–0.95) and was

superior to the sensitivity of Xpert alone (76%, $P < 0.001$) or LF-LAM/grade 1 alone (63%, $P < 0.001$), and was not statistically different from testing two sputa by MGIT culture (92%, $P = 0.79$). However, specificity of the Xpert and LF-LAM/grade 1 combination was only 86% (90/105, 95% CI 0.77–0.92) and was significantly lower than that for Xpert alone (98%, $P < 0.001$), but not significantly different from that for LF-LAM/grade 1 alone (88%, $P = 0.16$).

Additional combinations of tests—Figure 1 shows the sensitivity and incremental yield for sputum combinations of Xpert, urinary LAM assays, and conventional diagnostic tests. There was additive yield when sputum smear microscopy (Ziehl–Neelsen or fluorescence) was combined with urine LAM testing (Table 1 and Fig. 1). The combination of two sputa examined by fluorescence and urinary LF-LAM/grade 2 testing identified 69 of 103 culture-confirmed TB cases (67%, 95% CI 0.57–0.76), a sensitivity superior to that for either LF-LAM/grade 2 alone (49%, $P < 0.001$) or fluorescence alone (42%, $P < 0.001$). The combination of fluorescence microscopy and LF-LAM/grade 2 testing identified fewer culture-confirmed TB cases than did Xpert alone (76%), but the difference was not statistically significant ($P = 0.15$). The specificity of Ziehl–Neelsen or fluorescence smear microscopy combined with LF-LAM/grade 2 among individuals without TB was 97% (102/105, 95% CI 0.92–0.99) and was not significantly different from that of either test alone or that of sputum Xpert ($P = 0.65$).

There was further incremental benefit to the performance of sputum Xpert for individuals who were negative by fluorescence microscopy and urine LAM testing (Fig. 1). Among 34 individuals with culture-confirmed TB who were negative by fluorescence and LF-LAM/grade 2, Xpert was positive in 20 of 34 (59%). Addition of sputum Xpert to the combination of sputum microscopy and LF-LAM testing increased overall sensitivity from 67 to 86% (89/103 95% CI 0.78–0.92, $P < 0.001$); specificity of this algorithm among those without TB was 100/105 (95%, 95% CI 0.89–0.98). Addition of MGIT culture for two sputa among those negative by fluorescence smear microscopy, LF-LAM (grade 2), and Xpert MTB/RIF would have identified 101 of 103 (98%, 95% CI 0.93–1) confirmed TB cases.

Discussion

The urinary LAM assays and Xpert are emerging tools with the potential to improve TB diagnostic algorithms, and we found that they are complementary for the diagnosis of HIV-associated TB. As stand-alone tests, Xpert and the urinary LAM assays demonstrated reduced test sensitivity, compared with intensive testing of sputa and blood by mycobacterial culture in HIV-positive individuals with signs/symptoms of TB. However, when utilized together, the combination of testing one sputum with Xpert and testing urine with either the LF-LAM assay or ELISA-LAM assay identified nearly 90% of confirmed TB cases; specificity of this combination was also very high (97%) when using the grade 2 cut-off for LF-LAM positivity. Importantly, combining urine LAM testing with sputum Xpert testing offered improved incremental diagnostic sensitivity compared with either test performed alone, and appeared to be complementary for the detection of HIV-associated TB.

Consistent with previous reports, we found that the urinary LAM assays and Xpert each have higher sensitivity than smear microscopy [4,6,9,15]. A combination of urinary LAM assays with smear microscopy demonstrated additive value and may be an attractive option for initial testing of TB suspects in some settings, especially those with existing capacity for smear microscopy, but not for Xpert. The combination of a lateral flow urine LAM assay and smear microscopy identified nearly 70% of HIV-associated confirmed TB cases, and had an overall sensitivity approaching that of sputum Xpert testing alone. Stepwise algorithms could also be considered. Performance of LF-LAM as a rapid diagnostic in hospitalized patients identified over half of all HIV-associated TB cases, and this approach could limit the number of sputum examinations needed. Sequential testing using Xpert selectively for those individuals who tested negative by sputum smear microscopy and urinary LAM testing has incremental benefit and would rapidly identify over 85% of HIV-associated TB cases; such a strategy may be an attractive option in settings in which Xpert availability is limited.

To date, there is little published literature comparing sputum Xpert performance with that of the urinary LAM assays for the diagnosis of TB in adults with HIV. Among unselected adults initiating outpatient HIV care in Cape Town, Lawn *et al.* [6,8] found that sputum Xpert had higher sensitivity than LF-LAM used as a screening test for active PTB, with little incremental benefit when combining the two tests compared with Xpert used alone. By contrast, our study was conducted in HIV-infected adults suspected of TB disease with at least one sign or symptom of TB; our study population had more advanced immunosuppression than that studied by Lawn *et al.* and the majority of our participants were hospitalized. In this population, we found significant incremental benefit of urinary LAM testing when used in combination with sputum Xpert. Similar to Lawn *et al.*, in subgroup analysis, we found little incremental benefit of urinary LAM testing compared with Xpert alone in individuals with higher CD4⁺ cell counts in the outpatient setting, but our study is limited by relatively few patients in this category.

Our study had several limitations. We included only well categorized patients with cultured-confirmed TB and those in whom active TB was ruled out using a combination of microbiologic and clinical data. As such, sensitivity of the Xpert and urinary LAM assays could not be assessed for individuals in whom a final TB diagnosis could not be culture-confirmed. Nonetheless, the reference standard utilized to identify TB cases was rigorous and included two sputa for Lowenstein–Jensen and MGIT culture and a mycobacterial blood culture. Evaluating test specificity is also challenging, given an imperfect reference standard. To improve accuracy of specificity estimates, we studied a well categorized group of HIV-infected individuals with suspected TB, in whom there was no microbiological or clinical evidence of TB on follow-up at 2 months. Overall, our results regarding individual test sensitivity and specificity are consistent with those reported in the literature for Xpert and urinary LAM assays in HIV patients, respectively [3,4,6,9,15]. Importantly, our study is unique in assessing sensitivity not only in individuals with PTB, but also in those with mycobacteremia in whom sputum examination may be inadequate. TB cases in our study were also largely derived from the hospital setting and consisted of individuals with advanced immunosuppression. Nonetheless, we show that in this sick population with high

morbidity and mortality in whom rapid diagnosis is needed, the addition of urinary LAM testing to either sputum smear microscopy or Xpert is likely to have significant incremental benefit compared with strategies relying on sputum testing alone.

Overall, the LF-LAM represents the first true point-of-care TB diagnostic test, and Xpert offers near-care rapid diagnosis of TB. Our study is unique in evaluating these emerging diagnostics together to provide important covariate data on the sensitivity and specificity of test combinations. Our results show that usage of the urinary LAM assays in combination with a single sputum Xpert offered improved incremental diagnostic sensitivity compared with usage of either test alone; sensitivity of this combination approached that of two sputum Lowenstein–Jensen cultures or two sputum MGIT cultures. Specificity was high for Xpert combined with either LF-LAM/grade 2 or ELISA-LAM. Sequential testing algorithms using combinations of urinary LAM assays and sputum smear microscopy for initial TB assessment with selective subsequent implementation of Xpert testing should also be explored in clinical settings.

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Fig. 1. Sensitivity and incremental sensitivity of combinations of diagnostic tests among HIV-infected adults with culture-confirmed tuberculosis

Sensitivity of individual tests and combinations of tests are shown, along with relative contributions. Error bars represent 95% confidence intervals. Performed individually, FM identified 42% of tuberculosis (TB) cases, urine LF-LAM/grade 2 identified 49% of TB cases, and Xpert identified 76% of TB cases. Addition of urinary LF-LAM/grade 2 testing to FM identified an additional 25% of TB cases, resulting in a sensitivity of 67% for the two-test combination. Addition of one sputum Xpert to the combination of FM and LF-LAM/grade 2 identified another 19% of TB cases, resulting in sensitivity of 86% for the three-test combination. The combination of Xpert and LF-LAM/grade 2 identified 85% of TB cases, a sensitivity similar to that of two sputa cultured in MGIT media ($P=0.17$). ELISA-LAM, urine enzyme-linked immunosorbance assay for LAM; FM, fluorescence microscopy on two sputa; LAM, lipoarabinomannan; LF-LAM, urine lateral flow LAM assay; LJ, Lowenstein–Jensen solid culture on two sputa; MGIT, mycobacteria growth indicator tube liquid culture on two sputa; Xpert, Xpert MTB/RIF test on one sputum; ZN, Ziehl–Neelsen on two sputa.

Table 1

Sensitivity and specificity of tuberculosis diagnostic tests and combinations.

Diagnostic test(s)	Subcategory	Culture-confirmed TB; Sensitivity; <i>N</i> positive/total (%; 95% CI)	Not TB; Specificity; <i>N</i> negative/total (%; 95% CI)
Urine LF-LAM/grade 1		65/103 (63, 0.53–0.72)	92/105 (88, 0.80–0.93)
Urine LF-LAM/grade 2 ^a		50/103 (49, 0.39–0.59)	102/105 (97, 0.92–0.99)
Urine ELISA-LAM		61/103 (59, 0.49–0.69)	103/105 (98, 0.93–0.99)
Sputum Xpert ^b		78/103 (76, 0.66–0.84)	103/105 (98, 0.93–0.99)
ZN smear	1 sputum	25/103 (24, 0.16–0.34)	
	2 sputa	31/103 (30, 0.21–0.40)	105/105 (100, 0.97–1)
FM smear	1 sputum	35/103 (34, 0.25–0.44)	
	2 sputa	43/103 (42, 0.32–0.51)	105/105 (100, 0.97–1)
LJ culture	1 sputum	65/103 (63, 0.53–0.72)	
	2 sputa	82/103 (80, 0.71–0.87)	105/105 (100, 0.97–1)
MGIT culture	1 sputum	79/103 (77, 0.67–0.84)	
	2 sputa	95/103 (92, 0.85–0.97)	105/105 (100, 0.97–1)
Mycobacterial blood culture		49/103 (48, 0.38–0.58)	105/105 (100, 0.97–1)
ZN smear and urine LF-LAM/grade 1		72/103 (70, 0.60–0.79)	92/105 (88, 0.80–0.93)
ZN smear and urine LF-LAM/grade 2		58/103 (57, 0.46–0.66)	102/105 (97, 0.92–0.99)
ZN smear and urine ELISA-LAM		68/103 (66, 0.56–0.75)	103/105 (98, 0.93–0.99)
FM smear and urine LF-LAM/grade 1		82/103 (80, 0.70–0.87)	92/105 (88, 0.80–0.93)
FM smear and urine LF-LAM/grade 2 ^{**}		69/103 (67, 0.57–0.76)	102/105 (97, 0.92–0.99)
FM smear and urine ELISA-LAM		79/103 (77, 0.67–0.84)	103/105 (98, 0.93–0.99)
FM smear and Xpert		79/103 (77, 0.67–0.84)	103/105 (98, 0.93–0.99)
Xpert and urine LF-LAM/grade 1		93/103 (90, 0.83–0.95)	90/105 (86, 0.77–0.92)
Xpert and urine LF-LAM/grade 2 ^c		88/103 (85, 0.77–0.92)	100/105 (95, 0.89–0.98)
Xpert and urine ELISA-LAM		91/103 (88, 0.81–0.93)	101/105 (96, 0.91–0.99)

ELISA-LAM, urine enzyme-linked immunosorbance assay for LAM; FM, fluorescence microscopy; LAM, lipoarabinomannan; LF-LAM, urine lateral flow LAM assay; LJ, Lowenstein–Jensen solid culture; MGIT, mycobacteria growth indicator tube liquid culture; Xpert, Xpert MTB/RIF test; ZN, Ziehl–Neelsen.

^aPairwise comparisons – sensitivity of urine LF-LAM/grade 2 compared with the following: two sputa by ZN ($P=0.001$); two sputa by FM ($P=0.371$); LF-LAM/grade 1 ($P=0.001$), ELISA-LAM ($P<0.001$), Xpert ($P<0.001$), two sputa on LJ medium ($P<0.001$), two sputa in MGIT media ($P<0.001$), mycobacterial blood culture ($P=0.83$). Specificity of LF-LAM/grade 2 did not differ from ZN or FM microscopy, ELISA-LAM, Xpert, or LJ or MGIT culture ($P>0.05$ for all comparisons).

^bPairwise comparisons – sensitivity of Xpert compared with the following: two sputa tested by ZN smear microscopy ($P<0.001$), two sputa tested by FM ($P<0.001$), ELISA-LAM ($P=0.01$), two sputa cultured on LJ medium ($P=0.48$), two sputa cultured in MGIT medium ($P<0.001$), mycobacterial blood culture ($P<0.001$). Specificity of Xpert did not differ from ZN or FM microscopy, LF-LAM/grade 2, ELISA-LAM, or LJ or MGIT culture ($P>0.05$ for all comparisons).

^{**}Pairwise comparisons – sensitivity of LF-LAM/grade 2 and two sputa tested by FM compared to the following: LF-LAM alone/grade 2 ($P<0.001$), two sputa tested by ZN smear microscopy or by FM ($P<0.001$), Xpert alone ($P=0.15$), two sputa cultured on LJ medium ($P=0.02$), two sputa cultured in MGIT medium ($P<0.001$). Specificity of this combination was not significantly different from that of Xpert ($P=0.65$).

^{**}Pairwise comparisons – sensitivity of LF-LAM/grade 2 and two sputa tested by FM compared to the following: LF-LAM/grade 2 alone ($P<0.001$), two sputa tested by microscopy ZN or FM ($P<0.001$), Xpert alone ($P=0.15$), two sputa on LJ medium ($P=0.02$), two sputa in MGIT medium ($P<0.001$). Specificity of this combination was not different from that of Xpert ($P=0.65$).

^cPairwise comparisons – sensitivity of Xpert and LF-LAM/grade 2 was superior to that of the combination of LF-LAM/grade 2 and FM smear ($P < 0.001$), the combination of ELISA-LAM with FM ($P = 0.04$), Xpert alone ($P = 0.002$), LF-LAM/grade 1 alone ($P < 0.001$), LF-LAM/grade 2 alone ($P < 0.001$), and ELISA-LAM alone ($P < 0.001$); Xpert and LF-LAM/grade 2 sensitivity was similar to that for two sputa culture on LJ medium ($P = 0.24$) and for two sputa cultured in MGIT medium ($P = 0.17$). Specificity of the combination was not significantly different from specificity of Xpert alone ($P = 0.25$) or LF-LAM/grade 2 alone ($P = 0.50$).

Table 2

Comparison of sensitivities of urinary lipoarabinomannan assays and Xpert MTB/RIF in culture-confirmed tuberculosis patients.

Category	Total	LF-LAM/grade 1; N (%), 95% CI	LF-LAM/grade 2; N (%), 95% CI	ELISA-LAM; N (%), 95% CI	Xpert; N (%), 95% CI
All confirmed TB	103	65 (63, 0.53–0.72)	50 (49, 0.39–0.59)	61 (59, 0.49–0.69)	78 (76, 0.66–0.84)
FM smear-positive ^a	43	26 (60, 0.44–0.75)	24 (56, 0.4–0.71)	25 (58, 0.42–0.73)	42 (98, 0.88–1)
FM smear-negative ^a	60	39 (62, 0.48–0.74)	26 (43, 0.31–0.57)	36 (60, 0.47–0.72)	36 (60, 0.47–0.72)
PTB alone	54	22 (41, 0.28–0.55)	12 (22, 0.12–0.36)	19 (35, 0.23–0.49)	40 (74, 0.60–0.85)
PTB and mycobacteremia	43	39 (91, 0.78–0.97)	34 (79, 0.64–0.90)	38 (88, 0.75–0.96)	37 (86, 0.72–0.95)
Mycobacteremia alone	6	4 (67, 0.22–0.96)	4 (67, 0.22–0.96)	4 (67, 0.22–0.96)	1 (17, 0.00–0.64)
CD4 ⁺ cell count <50 ^b	46	39 (85, 0.71–0.94)	30 (65, 0.50–0.79)	37 (80, 0.66–0.91)	34 (74, 0.59–0.86)
CD4 ⁺ cell count 51–100 ^b	17	14 (82, 0.56–0.96)	12 (71, 0.44–0.90)	14 (82, 0.57–0.96)	13 (76, 0.50–0.93)
CD4 ⁺ cell count 101–200 ^b	19	7 (37, 0.16–0.62)	4 (21, 0.06–0.46)	6 (32, 0.13–0.56)	16 (84, 0.60–0.97)
CD4 ⁺ cell count >200 ^b	21	5 (24, 0.08–0.47)	4 (19, 0.05–0.42)	4 (19, 0.05–0.42)	15 (71, 0.48–0.89)
Hospitalized ^c	86	58 (68, 0.56–0.77)	48 (56, 0.45–0.67)	55 (64, 0.53–0.74)	64 (74, 0.64–0.83)
Outpatient ^c	17	7 (41, 0.18–0.67)	2 (12, 0.14–0.36)	6 (35, 0.14–0.62)	14 (82, 0.57–0.96)

ELISA-LAM, urine enzyme-linked immunosorbance assay for LAM; FM, fluorescence microscopy on two sputa; LAM, lipoarabinomannan; LF-LAM, urine lateral flow LAM assay; Xpert, Xpert MTB/RIF test on one sputum; ZN, Ziehl–Neelsen on two sputa.

^aTest sensitivity among those who were sputum smear-positive vs. those who were sputum smear-negative: LF-LAM/grade 1, $P=0.638$; LF-LAM/grade 2, $P=0.211$; ELISA-LAM, $P=0.850$; Xpert, $P<0.001$. Among smear-positive patients, sputum Xpert sensitivity was higher than that for each of the LAM assays ($P<0.05$ for all comparisons); among smear-negative patients, Xpert sensitivity was not statistically different from that of any of the LAM tests ($P>0.05$ for all comparisons).

^bTest sensitivity comparing strata of CD4⁺ cell counts: LF-LAM/grade 1, $P<0.001$; LF-LAM/grade 2, $P<0.001$; ELISA-LAM, $P<0.001$; Xpert, $P=0.791$. Xpert sensitivity was higher than that of LF-LAM (grade 1 or 2) ($P<0.001$) and ELISA-LAM ($P<0.001$) for individuals with CD4⁺ cell count >100 ; Xpert sensitivity was not significantly different from LF-LAM/grade-1 ($P=0.24$), LF-LAM/grade 2 ($P=0.40$), or ELISA-LAM ($P=0.48$) for individuals with CD4⁺ cell count <100 .

^cTest sensitivity comparing hospitalized vs. outpatient TB suspects: LF-LAM/grade 1, $P=0.04$; LF-LAM/grade 2, $P=0.001$; ELISA-LAM, $P=0.028$; Xpert, $P=0.486$. Xpert sensitivity was higher than that of urinary LAM assays for outpatients ($P<0.05$ for all comparisons). Among hospitalized TB suspects, Xpert sensitivity was higher than that of LF-LAM/grade 2, $P=0.01$, but not significantly different from that of LF-LAM/grade 1, $P=0.38$ or ELISA-LAM, $P=0.15$.