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Comparative Performance of Urine Lipoarabinomannan and Urine Xpert MTB/RIF Ultra for Diagnosing Tuberculosis in Adult Inpatients With Human Immunodeficiency Virus in East London, South Africa

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Background. Urine lateral flow lipoarabinomannan (LF-LAM) is a point-of-care tuberculosis (TB) test for patients with human immunodeficiency virus (HIV). Xpert MTB/RIF Ultra (Ultra) has improved sensitivity on sputum compared with the previous generation of Xpert and may improve diagnostic yield for TB on urine-based testing.

Methods. We conducted a diagnostic accuracy study in East London, South Africa. Adults with HIV hospitalized with ≥1 W4SS (World Health Organization–recommended 4-symptom screen) or clinical concern for TB were enrolled; TB cultures were performed on blood, sputum, and urine. Unprocessed urine was tested with LF-LAM and Ultra on the pellet of 15 mL centrifuged urine. The primary outcome was sensitivity of urine Ultra compared with LF-LAM, with microbiological TB (positive TB culture or molecular test, excluding urine Ultra) as the reference. Secondary outcomes included specificity and diagnostic yield.

Results. Two hundred thirty-eight participants were enrolled with a median CD4 count of 76 cells/mm^3. Microbiological TB was diagnosed in 62 (26%). Using microbiological TB as the reference, sensitivity of LF-LAM and urine Ultra was 45% (95% confidence interval, 32–58) and 70% (95% CI, 57–81; McNemar $P = .0013$); specificity was 93% (95% CI, 81–99) and 100% (95% CI, 92–100; McNemar $P = .25$). Diagnostic yields for microbiological TB were 34% for sputum Ultra, 45% for urine LF-LAM, 68 for urine Ultra, and 73% for urine LF-LAM and urine Ultra combined.

Conclusions. Combined urine-based testing (Ultra + LF-LAM) identified nearly three-quarters of medical inpatients with HIV with microbiological TB. Urine Ultra had significantly improved sensitivity compared with LF-LAM.

Keywords. Lipoarabinomannan; urine TB diagnostics; Xpert MTB/RIF Ultra; HIV; hospitalised.

Human immunodeficiency virus–associated tuberculosis causes significant mortality globally, with 161 000 deaths estimated in 2023 [1]. Diagnostic delays lead to delayed treatment initiation, which is associated with increased mortality among patients with HIV and TB [2]. HIV-associated TB is harder to diagnose with sputum-based diagnostics due to paucibacillary sputum, extrapulmonary TB being more common, and fewer patients able to produce sputum (37.0% to 93.7%) [3–5]. The limitations of sputum-based diagnostics have led to investigation of alternative samples, including urine, which is more readily accessible (97.5% to 99.9% of patients) [3–5]. The 2 most studied rapid TB diagnostics on urine are lateral flow urine lipoarabinomannan (LF-LAM) and Xpert MTB/RIF (Xpert).

The LF-LAM assay is a low-cost, point-of-care test with poor overall sensitivity, but it is effective in patients with lower CD4 counts and/or in severely ill inpatients. A recent review found a median pooled sensitivity and specificity in symptomatic people with HIV with CD4 count > 100 cells/mm^3 of 17% and 95%, respectively, and with CD4 count < 100 cells/mm^3 of 54% and 88%, respectively [6]. LF-LAM identifies TB in patients at the highest risk of dying [7]. A review of trials that evaluated urine LF-LAM in the workup for patients with HIV demonstrated a pooled 15% reduction in 8-week mortality [5, 8, 9].

Urine Xpert has been evaluated for the diagnosis of TB in hospitalized adults with HIV. Lawn et al investigated 427 adult

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inpatients with HIV for TB. Diagnostic yields were 64% on urine Xpert, 38% on LF-LAM, and 28% on sputum (Xpert or smear) [4, 10]. The urine Xpert yield was increased by testing the centrifuged pellet compared with an uncentrifuged sample (59.0% versus 42.4%, respectively) [4].

Xpert Ultra (Ultra) is the next-generation Xpert with improved sensitivity but slightly lower specificity on sputum [11]. Sossen et al compared urine Ultra and urine LF-LAM among 1602 inpatients and outpatients with HIV. Against an extended microbiological reference standard, sensitivities of urine Xpert Ultra and AlereLAM were 32.7% and 30.7% and specificities were 98.0% and 90.4%, respectively [3]. Two smaller studies have reported the performance of Ultra on urine. The first measured diagnostic yield of urine Ultra versus LF-LAM among inpatients and outpatients using sputum Xpert or TB culture as the reference. In the people with Human immunodeficiency virus (PWH) subgroup, Ultra was 17% more sensitive than LF-LAM, and Ultra sensitivity was 50% among those with CD4 count < 100 cells/mm^3 [12]. The second study compared the performance of Ultra versus LF-LAM on urine of PWH investigated for meningitis. In definite and probable TB meningitis, 37% were LF-LAM–positive and 41% were Ultra-positive [13].

Early data therefore suggest Ultra performs better than LF-LAM on urine, with the advantage of being MTB-specific and identifying rifampicin susceptibility. In this study, we compared diagnostic performance of urine Ultra and urine LF-LAM in inpatients with HIV and TB symptoms.

METHODS

Population

We conducted a cross-sectional diagnostic performance study with 12-week follow-up. The setting was the medical departments of Frere Hospital and Cecilia Makiwane Hospital, 850-bed tertiary and 600-bed regional referral hospitals in East London, Eastern Cape Province, South Africa. Together, they serve approximately 750 000 people, with a TB incidence of 743 per 100 000 population and 45.7% of TB cases HIV co-infected [12].

Between 10 August 2018 and 22 February 2019, we enrolled adults (aged ≥18 years) with HIV admitted to medical wards and able and willing to consent, with ≥1 W4SS (World Health Organization [WHO]–recommended 4-symptom screen) and/or a clinical suspicion of TB by the treating clinician [13]. Patients were excluded if they were established on TB therapy or had received more than a single dose of TB therapy within the last 60 days. All medical patients admitted during the previous 24 hours were screened for inclusion Monday to Friday.

Data Sources

Case report forms captured demographic data, TB symptoms, details of past TB, HIV history, medications, and the presence of WHO danger signs at study entry (respiratory rate > 30 breaths per minute, temperature > 39°C, heart rate > 120 beats per minute, and unable to walk unaided) [14]. Venous blood was collected for TB blood culture and CD4 count; routine results, including hemoglobin, were recorded from the National Health Laboratory Service (NHLS) database. Additional TB investigations were conducted by treating clinicians directed by clinical presentation, including chest X ray, abdominal ultrasound, lymph peripheral node aspiration, effusion aspiration, and lumbar puncture. All participants attempted expectorated sputum collection for Ultra testing (and TB culture if Ultra was negative) per standard of care. Sputum induction was not performed because no sputum induction facility was available.

A urine sample was collected in a clean, single-use disposable receptacle. Urine was aspirated with a sterile syringe and transferred to 3 sterile plastic containers as follows: 15 mL for Ultra testing, 40 mL for TB culture, and 12 mL for frozen storage. A Determine TB-LAM antigen (Ag) lateral flow strip test (Abbott, Abbott Park, IL) was performed on the remaining urine at the point of care by study staff according to the manufacturer's instructions. Any positive line equal to or greater in intensity than the first band on the reference scale card was read as positive. Ultra testing was performed by a trained technician (see Supplementary Material 1). Trace results were reported as positive according to local protocol. TB culture was performed at a regional NHLS TB laboratory using liquid Mycobacteria growth Indicator Tube (MGIT) TB culture, with MTB typing and rifampicin and isoniazid susceptibility testing by Hain Lifescience polymerase chain reaction. The results of positive urine tests were communicated urgently to the treating clinical team.

The following outcomes were ascertained from a 12-week post-recruitment telephone call, review of hospital and TB clinic notes, and all routine TB laboratory investigations:

subsequent TB diagnosis, receipt and timing of initiation of anti-TB therapy, alternative diagnoses, in-hospital mortality, 12-week mortality, TB culture status, and symptom response to therapy.

TB Reference Standard and Case Definitions

The reference standard for microbiological TB was a positive TB smear, culture, or molecular test on any sample, excluding the urine Ultra result being evaluated [15]. Given the risk for misclassification bias for the microbiological reference standard due to challenges of sampling extrapulmonary compartments, the anticipated significant proportion of sputum-scarce patients, and limitations in performance of a single sputum Ultra assay (and single TB culture if negative), patients were further categorized into the following diagnostic groups for secondary analysis: "probable TB" (not fulfilling criteria for microbiological TB but diagnosed with TB by a clinician and showing response to treatment), "not TB" (no culture or molecular evidence of TB and no

deterioration at 12 weeks without TB therapy), and "unclassifiable TB" (unable to assign to any of the above due to death of unknown cause, ongoing symptoms, or lost to follow-up at 12 weeks; see Supplementary Material 2).

Sample Size Estimation

The primary objective was to compare the sensitivity of urine Ultra with urine LF-LAM using microbiological TB as the reference, with the hypothesis that Ultra would be superior. Assuming sensitivity of 40% for urine LF-LAM, 55% sensitivity for urine Ultra, with 30% of TB cases positive for both tests, 120 patients with microbiological TB would provide 80% power to detect higher sensitivity of urine Ultra at a 2-sided significance level of .05. Thus, assuming a prevalence of microbiological TB of 50%, we planned a sample size of 240.

Analysis

Patient characteristics, including TB diagnostic category and laboratory results, were reported using descriptive statistics. We constructed 2×2 tables to evaluate the diagnostic performance of the 2 urine-based testing methods (LF-LAM and Ultra) for TB. All primary analyses were done using microbiological TB as the reference standard, with "unclassifiable TB" excluded. Urine-based tests were also compared to a composite reference group of microbiological and probable TB combined. The sensitivity, specificity, positive and negative predictive values, and likelihood ratios for each test were calculated. The diagnostic yield was calculated as the proportion with a positive test of all those diagnosed with microbiological TB, with unavailable results (unobtained sample and technical failures) regarded as negative tests. Sensitivity and specificity of urine LF-LAM and Ultra were compared using McNemar's test for correlated proportions. The effects of CD4 count and hemoglobin on absolute and relative diagnostic yields for urine LF-LAM and urine Ultra were estimated and compared using LOESS (locally estimated scatterplot smoothing) regression plots and multinomial logistic regression modeling. Positive urine Ultra rifampicin susceptibility results were compared by visual inspection to susceptibility results from culture-based drug susceptibility tests and Ultra assays performed on nonurine specimens.

Due to a high proportion of unclassifiable TB diagnostic categorization in the per-protocol analysis, modified diagnostic categories were developed by consensus of research clinicians to conduct an additional post hoc analysis. Probable TB was broadened to include patients with at least 1 clinico-radiological feature of TB who were started on TB therapy with no alternative diagnosis. Not TB included cases with no microbiologically confirmed TB and an alternative diagnosis (eg, other infection or malignancy; see Supplementary Material 3). Reclassification was done by a blinded independent TB clinician using the modified criteria.

Ethical Considerations

Informed consent was provided by all participants. The Walter Sisulu University Faculty of Health Sciences Post Graduate Education, Training, Research, and Ethics Committee and the University of Cape Town Human Research Ethics Committee granted ethical permission.

RESULTS

Baseline Characteristics and Outcomes

A total of 238 patients were enrolled; 105 (44%) reported being on antiretroviral therapy currently, and 99 (42%) reported previous TB treatment. Median CD4 count was 76 cells/mm^3 (interquartile range [IQR], 22–203), and 174 (74%) had a CD4 count < 200 cells/mm^3 (Table 1). WHO danger signs were frequent among participants, with 159 (67%) unable to walk unaided. A cough was reported in 168 patients (71%), but only 114 (48%) produced a spontaneously expectorated sputum sample (37% had a sputum Ultra result, 11% had a sputum TB culture only). Urine was collected from all patients; an

Table 1. Baseline Characteristics

Characteristic	Patients (n = 238) N (%)
Age, y	
Median (25th–75th)	39 (32–48)
Sex	
Female	124 (52)
Male	114 (48)
Site	
Cecilia Makiwane Hospital	190 (80)
Frere Hospital	48 (20)
Newly diagnosed human immunodeficiency virus	15 (6)
Current antiretroviral therapy reported	105 (44)
Previously treated tuberculosis	99 (42)
Diabetes mellitus	15 (6)
WHO-recommended 4-symptom screen	
Cough	168 (71)
Reported weight loss	207 (87)
Night sweats	134 (56)
Fever	126 (53)
WHO danger signs	
Respiratory rate >30 breaths pm	139 (58)
Heart rate >120 beats per minute	103 (43)
Temperature >39 °C	50 (21)
Unable to walk unaided	159 (67)
Baseline laboratory results	
CD4 count, median (IQR), [range], cells/mm^3	76 (22–203), [1; 872]
< 50	97 (42)
50–100	36 (15)
101–200	40 (17)
>200	60 (26)
Hemoglobin, median (IQR), [range], g/dL	9.9 (7.7–12.1), [2.4; 16.6]

Abbreviations: IQR, interquartile range; WHO, World Health Organization.

Table 2. Tuberculosis Diagnostic Categories

TB Diagnosis	Per-Protocol Definitions (n = 238) (n, %)	Post Hoc Modified Classifications (n = 238) (n, %)
Microbiological TB	62 (26)	62 (26)
Probable TB	30 (13)	41 (17)
Unclassifiable	102 (43)	34 (14)
Not TB	44 (18)	101 (42)

Post hoc classification modification was undertaken in order to reclassify the large proportion (43%) of "unclassifiable" using the per-protocol definitions. Many of these patients died or were lost to follow-up at 12 weeks and therefore could not meet the "probable" or "not TB" criteria using per-protocol definitions. The modified criteria were developed through consensus by the investigators (see Supplementary Material) and then provided to an independent reviewer (an experienced human immunodeficiency virus and TB clinician and researcher) who reclassified the patients while blind to their lateral flow lipoarabinomannan and urine Ultra results.

Abbreviation: TB, tuberculosis.

LF-LAM result was recorded for all, and 6 of 238 (2.5%) urine Ultra results were "unsuccessful." The minimum number of reference TB tests was 1, the median was 2, and 45% of patients had 3 or more reference tests.

At 12 weeks, 121 (51%) patients were known to be alive, 91 (38%) had died, and 26 (11%) were uncontactable. The 12-week mortality rate for those with a known outcome was 91 of 212 (43%), with 58% of deaths occurring while in the hospital and 42% occurring post-discharge (see the patient flow diagram in Supplementary Material 5). Microbiological TB was diagnosed in 62 of 238 (26%), and probable TB was diagnosed in 30 of 238 (13%); 44 of 238 (18%) were not TB, and 102 of 238 (43%) were unclassifiable using protocol definitions (Table 2; additional details on unclassifiable are provided in Supplementary Material 5). When post hoc revised criteria were applied, the unclassifiable category was reduced to 34 of 238 (14%), with probable TB increasing to 41 of 238 (17%) and not TB increasing to 101 of 238 (42%). The median time to initiate TB therapy after hospital admission (107 patients) was 1.0 day (IQR, 0–4.0; range, 0–22).

Diagnostic Accuracy

Using microbiological TB as the reference, sensitivity for LF-LAM and urine Ultra was 45% (95% confidence interval [CI], 32–58) and 70% (95% CI, 57–81; McNemar $P = .0013$); specificity was 93% (95% CI, 81–99) and 100% (95% CI, 92–100; McNemar $P = .25$); the positive predictive value was 90% (95% CI, 74–98) and 100% (95% CI, 92–100); and the negative predictive value was 55% (95% CI, 43–66) and 70% (95% CI, 57–81), respectively (Table 3). Using a composite reference of microbiological or probable TB, sensitivity for LF-LAM and urine Ultra was 39% and 58%; specificity was 93% and 100%; the positive predictive value was 92% and 100%; and the negative predictive value was 42% and 53%, respectively. The absolute difference in sensitivity between the 2 assays was 25% (95% CI, 12–38) using microbiological TB as the reference and 19%

Table 3. Performance Characteristics

Microbiological TB[a] (Per Protocol)	TB Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
Urine LF-LAM (n = 106)	0.59 (.49–.68)	0.45 (.32–.58)	0.93 (.81–.99)	0.90 (.74–.98)	0.55 (.43–.66)	6.62 (2.15–20.43)	0.59 (.46–.75)
Urine Ultra (n = 103)	0.58 (.48–.68)	0.70 (.57–.81)	1.00 (.92–1.00)	1.00 (.92–1.00)	0.70 (.57–.81)	NA[b]	0.3 (.20–.44)
Composite TB[c] (per protocol)							
Urine LF-LAM (n = 136)	0.68 (.59–.75)	0.39 (.29–.50)	0.93 (.81–.99)	0.92 (.79–.98)	0.42 (.32–.53)	5.74 (1.87–17.62)	0.65 (.54–.78)
Urine Ultra (n = 133)	0.67 (.59–.76)	0.58 (.47–.68)	1.00 (.92–1.00)	1.00 (.93–1.00)	0.53 (.42–.64)	NA[b]	0.42 (.33–.54)
Composite TB (post hoc modified classification)							
Urine LF-LAM	0.51 (.43–.58)	0.39 (.29–.49)	0.95 (.89–.98)	0.89 (.76–.96)	0.60 (.52–.68)	7.84 (3.23–19.07)	0.64 (.55–.76)
Urine Ultra	0.51 (.44–.58)	0.52 (.42–.63)	1.00 (.96–1.00)	1.00 (.93–1.00)	0.67 (.59–.75)	NA[b]	0.48 (.39–.58)

Abbreviations: CI, confidence interval; LF-LAM, lateral flow lipoarabinomannan; NA, not applicable; TB, tuberculosis; Ultra, Xpert Ultra.

[a]Performance characteristics were calculated based on urine LF-LAM results for all definite TB versus not TB patients. The same for urine Ultra less 3 inconclusive results.

[b]Positive likelihood ratios were incalculable due to 100% specificity.

[c]Calculated on all definite or probable TB versus not TB patients with urine LF-LAM results. The same for urine Ultra less 3 inconclusive results.

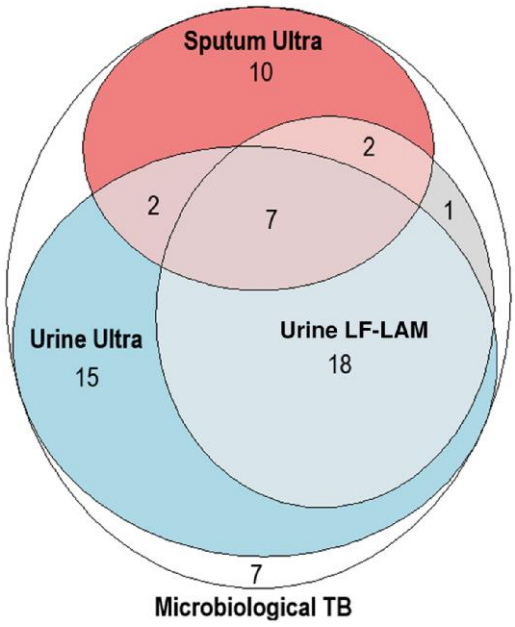

Figure 1. Venn diagram of comparative diagnostic yields for microbiological TB (62 patients). Microbiological TB was defined per protocol. Numbers represent the number of TB cases. The total number of microbiological TB cases was 62. Abbreviations: LF-LAM, lateral flow lipoarabinomannan; TB, tuberculosis; Ultra, Xpert Ultra.

(830) using the composite TB reference. The difference in specificity was 7% (95% CI, −1 to 15) with both references.

Diagnostic Yield

Diagnostic yield for microbiological TB was 34% (95% CI, .22–.47) for sputum Ultra, 45% (95% CI, .32–.58) for urine LF-LAM, 68% (95% CI, .55–.79) for urine Ultra, 73% (95% CI, .60–.83) for urine LF-LAM and urine Ultra combined, and 89% (95% CI, .78–.95) for sputum Ultra, urine LF-LAM, and urine Ultra combined. Incremental yield for a second test after sputum Ultra was 31% for LF-LAM and 53% for urine Ultra. Incremental yield for a second test after LF-LAM was 19% for sputum Ultra and 27% for urine Ultra (Figure 1). The diagnostic yield for composite TB was 23% (95% CI, .15–.33) for sputum Ultra, 39% (95% CI, .29–.50) for urine LF-LAM, 57% (95% CI, .46–.67) for urine Ultra, 64% (95% CI, .53–.74) for urine LF-LAM and urine Ultra combined, and 75% (95% CI, .65–.83) for sputum Ultra, urine LF-LAM, and urine Ultra combined (Table 4). Diagnostic yields for post hoc modified composite TB were 20% for sputum Ultra, 39% for urine LF-LAM, 51% for urine Ultra, 62% for urine LF-LAM and urine Ultra combined, and 72% for sputum Ultra, urine LF-LAM, and urine Ultra combined (Table 4).

Laboratory and Clinico-Radiological Associations With Urine-based TB Detection

Diagnostic yields for the 2 urine tests were strongly related to CD4 count. In patients with microbiological TB, odds of a positive test result were roughly halved for each 1-log increase in CD4 count: urine Ultra odds ratio [OR] = 0.6 (95% CI, .4–.9; $P = .01$) and urine LF-LAM OR = 0.5 (95% CI, .4–.8; $P = .0009$; Supplementary Figure 1). Since both tests were similarly influenced by CD4 count, relative performance was not significantly different across CD4 count range (OR for [urine Ultra + /LF-LAM–] versus [urine Ultra –/LF-LAM +] discordant pairs for 1-log increase in CD4 count = 1.3; 95% CI, .7–2.5; $P = .365$; Supplementary Figure 2). There was no significant association between diagnostic yields for urine Ultra or LF-LAM and hemoglobin (Supplementary Figure 3). Urine Ultra and LF-LAM diagnostic yields were both positively associated with a miliary infiltrate on chest radiograph, positive TB blood culture, and a positive urine TB culture (Supplementary Tables 1 and 2). Urine Ultra positivity was associated with inpatient death ($P = .042$), but neither test result was associated with 12-week mortality.

Urine Rifampicin Resistance Testing

Positive urine Ultra results were 69% (n = 37) rifampicin susceptible, 9% (n = 5) resistant, and 22% (n = 12) indeterminant, with no discordance with rifampicin susceptibility detected on TB culture drug susceptibility testing or sputum Ultra (Table 5). Of the 12 indeterminant rifampicin results, 10 were "MTB trace," 1 was "MTB low," and 1 was unverifiable.

DISCUSSION

This diagnostic performance study compared urine Ultra to urine LF-LAM among adults with advanced HIV admitted to the hospital with at least 1 TB symptom. Urine Ultra demonstrated a significant 25% higher sensitivity and nonsignificant 7% higher specificity for microbiological TB compared with LF-LAM. Compared to composite TB, both tests had modest reductions in sensitivity, but Ultra sensitivity remained 19% higher than sensitivity for LF-LAM. Specificity remained high for both tests. This finding is in keeping with results from an HIV subgroup study by Andama et al who demonstrated a 17.2% higher sensitivity of urine Ultra compared with LF-LAM [12]. However, the multicenter Sossen et al study showed Ultra to have only a 2% higher sensitivity compared with LF-LAM using a microbiological reference and a 9.4% lower sensitivity using a composite reference (95% CIs overlapped for these differences) [3]. This may be due to differences in the enrolled patient population and the immunosuppression and degree of TB dissemination.

There were no false-positive urine Ultra results across all 3 TB reference standards in our study, which is in keeping with reported urine Ultra specificity (98.0% to 99.1%) [3, 12]. Comparative urine diagnostics studies have shown lower specificity for LF-LAM compared with Ultra, which was statistically significant in studies by Sossen et al and others [3, 10, 12]. LF-LAM specificity varies according to the rigor of the gold standard TB

Table 4. Diagnostic Yields

TB Diagnostic Test	Patients With Microbiological TB[a] Per Protocol (n = 62) (95% CI)	Patients With Composite TB[b] Per Protocol (n = 92) (95% CI)	Patients With Composite TB[c] Post Hoc Modified Classification (n = 103) (95% CI)
Urine LF-LAM	0.45 (.32–.58)	0.39 (.29–.50)	0.39 (.29–.49)
Urine Ultra	0.68 (.55–.79)	0.57(.46–.67)	0.51(.41–.61)
Sputum Ultra	0.34 (.22–.47)	0.23 (.15–.33)	0.20 (.13–.29)
LF-LAM + urine Ultra	0.73 (.60–.83)	0.64 (.53–.74)	0.62 (.52–.72)
LF-LAM + sputum Ultra	0.65 (.51–.76)	0.52 (.42–.63)	0.50 (.40–.60)
LF-LAM + sputum Ultra + urine Ultra	0.89 (.78–.95)	0.75 (.65–.83)	0.72 (.62–.80)

Six unsuccessful urine Ultra results were entered as negative for the diagnostic yield analysis.

Per protocol TB reference definitions.

Abbreviations: CI, confidence interval; LF-LAM, lateral flow lipoarabinomannan; TB, tuberculosis; Ultra, Xpert Ultra.

[a]*Microbiological TB:* specimen positive by smear microscopy, culture, or Ultra on sputum or extrapulmonary samples. The urine Ultra and urine LF-LAM were excluded from this reference standard.

[b]*Composite TB:* microbiological and probable TB combined. *Probable TB:* patients did not fulfill the criteria for definite TB but were diagnosed with active TB by a clinician who decided to give the patient a full course of TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation and having good response to anti-TB treatment at 12 weeks follow-up.

[c]For post hoc TB definitions, see Supplementary Material 3.

Table 5. Urine Ultra Rifampicin Susceptibility Agreement With Results From Other Samples

		Rifampicin Susceptibility by Other Xpert Ultra Samples and/or Tuberculosis Culture		
Urine Xpert Ultra Positive Rifampicin Susceptibility Results (n = 54) N (%)		Susceptible	Resistant	Unconfirmed[a]
Indeterminant[b]	12 (22%)	8	2	2
Resistant	5 (9%)	0	4	1
Susceptible	37 (69%)	28	0	9

[a]Unconfirmed rifampicin susceptibility had no other Ultra or culture results with rifampicin susceptibility. Tuberculosis (TB) culture was performed at a regional National Health Laboratory Service TB laboratory using liquid Mycobacterial Growth Indicator Tube (MGIT) TB culture, with mycobacterium tuberculosis typing and rifampicin and isoniazid susceptibility testing by Hain Lifescience polymerase chain reaction.

[b]Ten indeterminant rifampicin susceptibility samples were "MTB trace" results, 1 was "MTB low," and 1 was unable to be verified due to technical error.

investigations. With a large number of baseline TB investigations (including sputum induction) to inform the reference standard, Gupta-Wright et al reported an LF-LAM specificity of 98.9%, which was better than the 93% in our study [7]. Being a point-of-care test, LF-LAM is dependent on accurate interpretation by the operator. Overreporting weakly positive LF-LAM could contribute to false-positive results. Nontuberculous mycobacterial (NTM) infection also results in LF-LAM positivity and reduces LF-LAM specificity [16].

Urine Ultra produced the highest diagnostic yield compared with LF-LAM and sputum Ultra. Combination of the 3 tests resulted in a diagnostic yield of 89%. The composite TB reference resulted in modest reductions in diagnostic yield and sensitivity of the 2 urine diagnostics, but specificities remained similar. The addition of 11 probable TB cases to the post hoc modified composite TB did not change the diagnostic yield of LF-LAM (remained at 39%), but urine Ultra declined from 57% to 51%. Both urine Ultra and LF-LAM had significantly higher yields at lower CD4 counts, which is in keeping with other urine Xpert and LF-LAM studies [4, 17]. The higher yields associated with miliary features on chest X ray and positive urine and blood TB cultures reflect the greatest utility for urine TB testing of severely ill patients with disseminated TB.

Less than half of patients produced a spontaneous sputum sample, which contributed to low sputum Ultra yield. Not offering sputum induction was a limitation of the study (induction can increase absolute TB diagnostic yield by 12%) but reflects reality in most sub-Saharan African hospitals [18]. Despite this, Figure 1 illustrates how sputum Ultra remains an important test. Sputum Ultra was used to diagnose 10 patients with TB who were negative on both urine tests, perhaps representing more localized pulmonary disease.

Another limitation of our study was the higher-than-expected 12-week mortality that contributed to missing data and a large number of unclassifiable categorizations. This was moderated to some degree with the post hoc–modified classification. A second limitation is potential incorporation bias created by sharing positive urine LF-LAM and Ultra results with clinicians. This may have resulted in TB treatment being initiated without other confirmation, thereby resulting in the patient being classified as probable TB. The post hoc probable TB revised criteria required clinico-radiological features of TB as well as a decision to treat, which would have minimized this bias. A third limitation was inclusion of positive smear in the microbiological reference standard that could result in NTM being

misclassified as TB. Smear was performed infrequently; there were only 3 positive results, and all had *Mycobacterium tuberculosis* confirmed by culture. A strength of our study is that it was performed in a real-world resource-constrained setting on fresh urine samples and used existing routine laboratory services, suggesting that urine Ultra introduction is feasible. Including TB blood culture for all patients and having a median of 2 reference TB tests made the microbiological reference standard robust and contributed to reliability of the results.

The way in which urine Ultra is included in algorithms for the workup of inpatients with HIV being investigated for TB, typically with low CD4 counts, depends on access and resources in each setting. The current WHO recommendation is for concurrent sputum low-complexity automated nucleic acid amplification testing and urine LF-LAM for inpatient PWH or patients with a CD4 count < 200 cells/mm^3 [19]. According to our findings, adding urine Ultra would improve yield. The need for urine centrifuging for optimal Ultra performance needs consideration and may require centralization of testing in larger laboratories. A cost-effective strategy could be to reserve urine Ultra for PWH being investigated for TB as inpatients who cannot produce a sputum sample or are negative by the other 2 tests. Larger comparative diagnostic and implementation studies are required to confirm these findings and inform the design of algorithms.

In conclusion, combined urine testing (Ultra and LF-LAM) identified three-quarters of medical inpatients with HIV and definite TB on admission. Urine Ultra had a significantly higher sensitivity than LF-LAM and specificity that was comparable to that of LF-LAM, with the added benefit of providing rapid rifampicin susceptibility results. Xpert Ultra performs well on urine and should be included in TB diagnostic algorithms for inpatients with HIV.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. G. M., D. S., S. W., and A. P. conceptualized the study and wrote the protocol. D. S. directly supervised the study, laboratory interaction, and data entry. E. S. and D. B. performed the data analyses. G. M., D. S., S. W., A. P., E. P., and D. B. wrote and contributed to the final manuscript.

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