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# Diagnostic Yield of urine Xpert MTB/RIF ultra to detect *Mycobacterium tuberculosis* among severely immunosuppressed inpatients with HIV: A prospective cohort study in Ghana

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ABSTRACT

*Objectives:* Due to the higher prevalence of sputum scarce pulmonary tuberculosis (TB) and extrapulmonary TB among people with HIV compared to HIV-negative people, diagnosis of HIV-associated TB cannot rely solely on sputum-based methods. We estimated the TB diagnostic yield of urine Xpert MTB/RIF Ultra (Ultra) and its prognostic significance among inpatients with HIV.

*Methods:* Adults admitted with HIV in Ghana between October 2019 and November 2021 were enrolled if they had a positive WHO 4-symptom screen, advanced HIV, or severe illness according to WHO criteria for people with HIV. Urine samples were frozen and later analyzed using Ultra. Two TB reference standards were used: an extended microbiological reference (eMRS) including culture, Xpert/Ultra on non-urine samples, or urine LF-LAM; and a composite reference (CRS) including eMRS or TB diagnosis by microscopy, recommended TB treatment, or TB at death. Patients were followed for 8 weeks.

*Results:* Of 141 participants, 118 (83.7%) provided urine samples for storage. TB prevalence was 25.0% by eMRS and 37.3% by CRS. Urine Ultra was positive in 34/118 (28.8%) patients. Ultra-positive patients had lower median CD4 count compared to Ultra-negative patients (31 vs 78 cells/mm$^3$, $P = 0.025$). If urine Ultra had been used to guide TB diagnosis, it would have identified an additional 20 patients not detected by CRS. No difference in 8-week mortality was observed between Ultra-positive and negative patients.

*Conclusion:* Urine Ultra shows promise as a supplementary point-of-care TB test among inpatients with HIV.

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## Introduction

To help close the tuberculosis (TB) diagnostic gap and improve patient outcomes, there is growing attention on the diagnostic yield of rapid tests [1], as well as on the value of concurrent testing with multiple point-of-care diagnostics across sputum and non-sputum specimen types [2,3]. This approach is particularly important in people with HIV evaluated for TB, in whom sputum scarcity is common with up to 32% unable to provide sputum samples [4]. Immunosuppression further increases the risk of disseminated or extrapulmonary disease, and low mycobacterial load in sputum due to impaired granuloma formation, supporting the need to add non-sputum testing for TB [5,6].

The Xpert MTB/RIF Ultra ("Ultra", Cepheid, USA) is a low-complexity automated nucleic acid amplification test (LC-aNAAT) developed to detect *Mycobacterium tuberculosis complex* (MTBC) and rifampicin resistance in sputum [7].

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Ultra offers improved sensitivity to diagnose TB compared to the earlier-generation Xpert MTB/RIF cartridge ("Xpert") with a lower limit of detection of 16 vs 114 bacterial colony forming units per millilitre [8,9]. The increase in sensitivity with use of Ultra versus Xpert comes with an increased risk of false-positive results, particularly for "Trace" positives that in clinical practice are advised to be considered as bacteriological confirmed TB among people with HIV [10].

LC-aNAATs like Xpert and Ultra have traditionally been evaluated using specimens from suspected TB sites, such as sputum for pulmonary TB or cerebrospinal fluid for TB meningitis, demonstrating high sensitivity and specificity [11]. Few studies have evaluated Ultra testing of urine as a test to detect all forms of HIV-associated TB not limited to genitourinary TB [12–15]. These studies suggest that diagnostic accuracy was best among sub-populations with low CD4 cell count, with sensitivities ranging from 50% to 70% and specificities approaching 100% [12,14,15].

Sossen et al. [14] reported a promising diagnostic yield for HIV-associated TB by combining urine Ultra, sputum Ultra, and urine Determine TB-LAM (Abbott, USA) in patients with CD4 counts below 200 cells/mm$^3$. Similarly, Stead et al. [15] recently found that urine Ultra alone identified 68% of microbiologically confirmed TB cases among severely immunosuppressed inpatients with HIV in South Africa. As suggested by the World Health Organization (WHO), more data on the performance of LC-aNAATs on urine are needed to determine whether it can be recommended as an initial test for TB [10].

In a cohort of severely immunosuppressed inpatients with HIV in Ghana, our primary aim was to estimate the diagnostic yield [1] of urine Ultra in addition to routine TB tests and clinical criteria.

## Methods

### *Design and Participants*

This is a prospective single-center cohort study based on patients enrolled and biobanked samples collected between 14 October 2019 and 21 November 2021. The study is a sub-study to a larger multicenter LF-LAM intervention trial (Clinicaltrials.gov ID: NCT04122404) with a temporary pause from March to October 2020 due to the COVID-19 pandemic as previously reported [16]. Participants received oral information in their local language and written information in English, and consented via signature or thumbprint. Enrolment occurred in Korle-Bu Teaching Hospital's medical wards within 72 hours of admission for HIV-positive adults meeting at least one of the following criteria: a positive WHO 4-symptom screen (any cough, night sweat, weight loss or fever), advanced HIV (CD4 count $<$200 cells/mm$^3$ or WHO HIV stage 3 or 4), or being seriously ill (respiratory rate $>$30/minute, temperature $>$39°C, heart rate $>$120/minute, or unable to walk unaided) [17]. Enrolment also required no intake of TB treatment in the past 60 days.

Data was collected on sociodemographic characteristics, medical history, symptoms, physical examination, routine test results, TB investigations, HIV status, and treatment status. Follow-up occurred at 8 weeks ($\pm$14 days), with physical consultations and review of medical records, or via phone interviews and review of mortality records.

### *Procedures*

Urine was collected upon enrolment, cryopreserved the same day at $-$20°C, and stored at the Department of Medical Microbiology, University of Ghana, Korle-Bu. In October 2024, urine samples were cold-chain transferred for testing at the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. Thawed samples with a median volume of 9 ml (IQR 8.5-12.0) were centrifuged at 3000 $\times$ g for 15 minutes. After removal of the supernatant, 2 ml of the remaining concentrated urine including the pellet was mixed with sample reagent in a 1:1 ratio for Ultra analysis according to the manufacturer's instructions [7]. The laboratory team was blinded to all patient clinical data, including previous reference test results. Urine Ultra results were not shared with the clinicians as they were performed after follow-up.

Sputum Xpert or Ultra was routinely available, while urine LF-LAM was offered in the study's second half at the physician's discretion or as retrospective testing of cryopreserved urine. Sputum microscopy and TB liquid culture (MGIT) were provided through the National Tuberculosis Control Programme (NTP).

The diagnostic yield was defined as the proportion of patients in whom urine Ultra identified TB among all patients tested positive for TB by an extended microbiological reference standard (eMRS) or a composite reference standard (CRS). A positive eMRS included any of the following positive tests: sputum culture (MGIT), Xpert or Ultra on non-urine samples, or urine LF-LAM. A positive CRS included a positive eMRS and/or a positive sputum smear microscopy, clinician referral for TB treatment, or TB-related death according to medical records. Patients without a valid eMRS TB test were unclassifiable and excluded from eMRS analyses.

### *Outcomes*

The primary outcome was the diagnostic yield of urine Xpert Ultra among patients classified by a valid eMRS or CRS result [1]. Secondary analyses characterized the urine Ultra-positive population, urine Ultra diagnostic accuracy against eMRS and CRS, and 8-week outcomes by urine Ultra results.

### *Statistical Analysis*

Descriptive analyses were used to characterize the study population. Group differences were assessed using the Wilcoxon rank-sum test for continuous variables and chi-square or Fisher's exact tests for proportions. Diagnostic yield was described as proportion and percentages, and visualized using Venn diagrams against eMRS and CRS. Agreement between urine Ultra and LF-LAM was assessed with kappa statistics. We calculated prevalence, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals (CIs). Survival by urine Ultra result was analyzed using Kaplan–Meier curves and the log-rank test, while a Cox proportional hazards model quantified the association of urine Ultra-positivity with 8-week mortality adjusted for sex, CD4 cell count, known comorbidities, being on ART, and TB treatment initiation. Analyses were conducted in Stata (version 17). The STARD guidelines for reporting diagnostic studies were applied [18].

### *Sample Size*

The number of participants with urine available for retrospective Xpert Ultra testing was determined by the available study population during the LF-LAM intervention study period [16] rather than by a formal sample size calculation.

## Results

### *Cohort and TB Reference Standard*

Of 245 patients screened for eligibility, 141 (57.6%) were enrolled from the medical wards. After routine care sampling for TB diagnosis at the discretion of the treating physician with the samples obtained listed in Table S1, 118 (83.7%) patients provided a

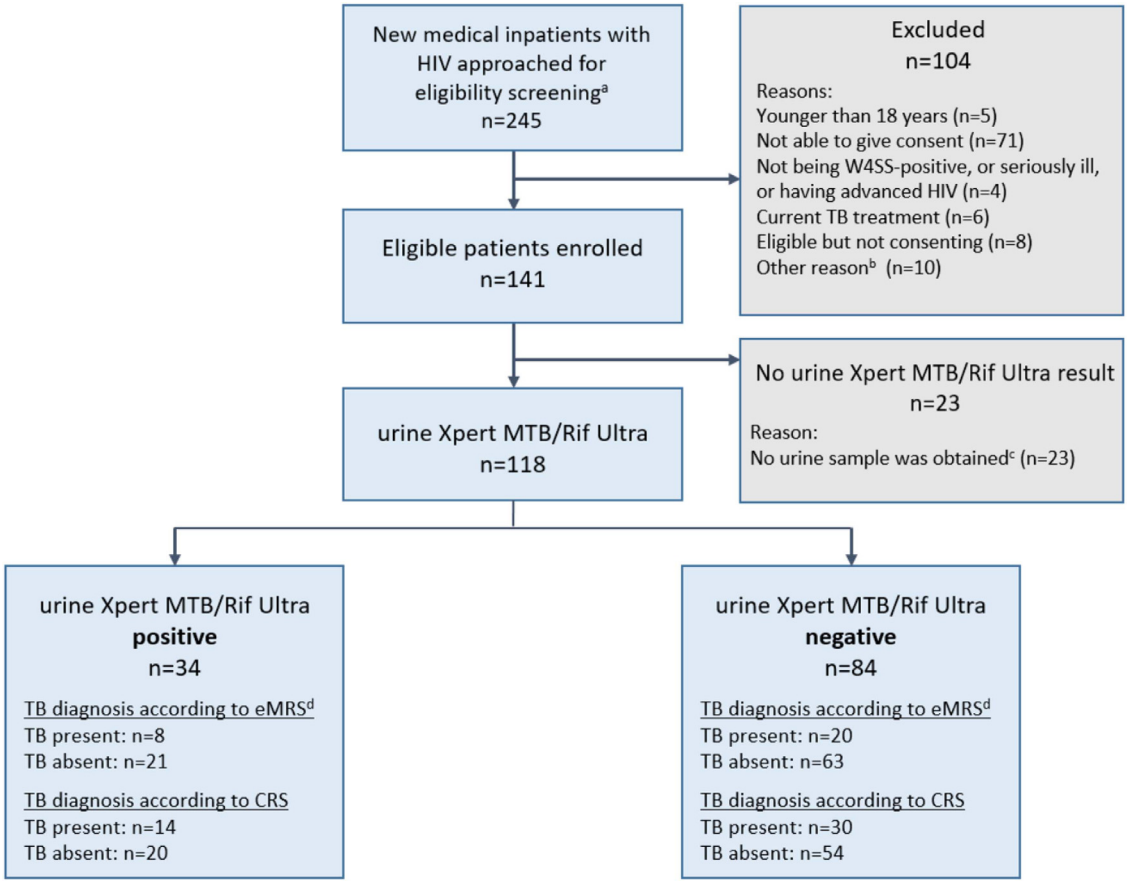


**Figure 1.** Study flow diagram. Abbreviations: TB = tuberculosis; aMRS = extended microbiological reference standard; CRS = composite TB reference.
[a]Patients were not screened for eligibility due to death (n = 123), discharge (n = 59), prior enrollment (n = 12), or HIV-negative/unknown status (n = 3217).
[b]In some cases, HIV status was undisclosed to the patient, and factors like the presence of unaware relatives prevented informed consent.
[c]Among those who did not provide a urine sample for index testing, 7/23 (30.4%) met the composite TB reference standard. Of the total, 16/23 (69.6%) died during follow-up, with 8/16 (50%) dying within the first five days post-enrollment. Among survivors, 3/7 (42.9%) underwent urine Ultra testing at eight weeks, all of which were negative.
[d]Six patients with urine Ultra results lacked valid results for sputum culture, or urine LF-LAM, or any specimen Xpert/Ultra (excluding urine), and could not be classified for TB status, leading to their exclusion from eMRS-based analyses.

urine sample for cryopreservation and future testing and formed part of the primary analysis (Figure 1). Four of 118 (3.4%) urine Ultra tests yielded errors but were successfully repeated once, and the valid results were included in the analysis. Of 118 patients with a valid urine Ultra result, 72 (61.0%) had a routine sputum Xpert/Ultra, the median CD4 count was 68 cells/mm³ (IQR 25-149), 70 (59.3%) patients were female, and 37 (31.4%) patients were on ART upon admission.

Among patients classified according to eMRS, 28/112 patients (25.0%, 95% CI 17.3-34.1) had TB. Using the CRS, 44/118 patients (37.3%, 95% CI 28.6-46.7) were identified with TB.

For an overview of patients with valid TB test results and test positivity rates, see Table S1.

### *Diagnostic Yield*

The urine Ultra was positive in 34/118 patients classified by the CRS (28.8%, 95% CI 20.8-37.9) with 25/34 (73.5%) "Trace" positive, 6/34 (17.7%) "Very low" positive, and 3/34 (8.8%) "Low" positive (Table S2). Among nine patients with "Very low" or "Low" positive results, rifampicin resistance was detected in 1/9 (11.1%, 95% CI 0.3-48.2).

Urine Ultra identified 8/28 (28.6%) of patients with TB according to eMRS and 14/44 (31.8%) according to CRS. The concordance between urine Ultra and urine LF-LAM was low at 68.0%, $\kappa = 0.103$, $P = 0.155$, with 7/22 (31.8%) urine Ultra-positive patients also having a positive urine LF-LAM (Figure 2A).

If urine Ultra had guided TB diagnosis, an additional 21 patients missed by eMRS, or 20 patients missed by CRS, would have been detected based on a positive urine Ultra alone (Figure 2 B-C).

Of 20 urine Ultra-positive patients missed by the CRS, 85.5% (17/20) had "Trace" results, and the median CD4 cell count was low at 21 cells/mm³ (IQR 8-131). Half (10/20) had a routinely performed sputum Xpert or Ultra test result (Table S3).

In sensitivity analyses among all 141 patients enrolled, whether or not a urine sample was available for retrospective Ultra testing, a missing urine Ultra result for any reason was imputed as negative, showing that urine Ultra was positive in 34/141 patients (24.1%, 95% CI 17.3-32.0) (Figure S1 in the supplementary files).

### *Patient Characteristics by Urine Ultra Result*

In exploratory analysis, urine Ultra-positive vs Ultra-negative patients had lower median CD4 of 31 (IQR 11-103) vs 78 (IQR 28-169) cells/mm$^3$, $P = 0.025$; lower median blood sodium, 134 (IQR 131-139) vs 138 (IQR 134-141) mmol/l, $P = 0.023$, and higher rate of subjective and objective respiratory distress (Table 1). Conversely, urine Ultra-negative patients compared to positive patients were more often newly diagnosed with HIV, 49 (58.3%) vs 13 (38.2%), $P = 0.048$, and had a higher rate of renal dysfunction, 34 (40.5%) vs 6 (17.7%), $P = 0.018$.

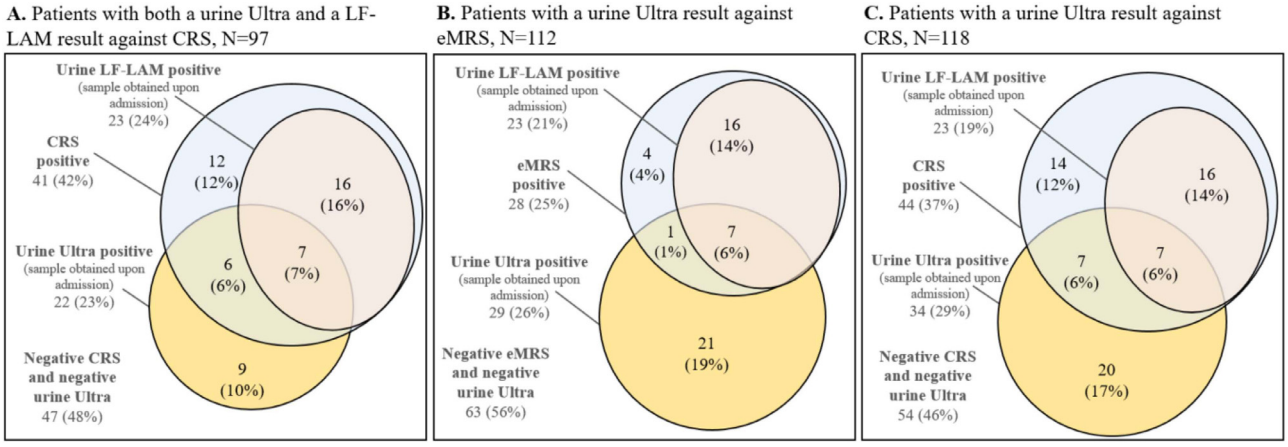


**Figure 2.** Venn diagram showing urine ultra diagnostic yield.

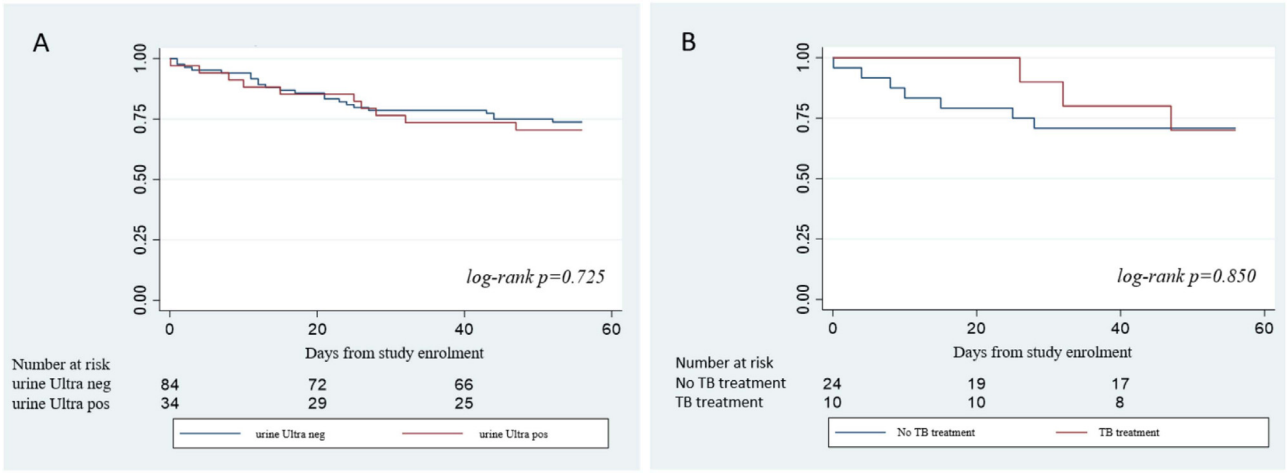


**Figure 3.** Eight weeks survival probability among (A) 118 patients stratified by urine Ultra result, and (B) 34 urine Ultra-positive patients stratified by TB treatment initiation.

*Diagnostic Accuracy*

The sensitivity, specificity, PPV, and NPV of urine Ultra against the eMRS for TB were 28.6%, 75.0%, 27.6%, and 75.9%, respectively (Table S4). Against the CRS for TB, the sensitivity, specificity, PPV, and NPV of urine Ultra were 31.8%, 73.0%, 41.2%, and 64.3%.

*The Prognostic Significance of Urine Ultra-positivity*

We had 8-week outcome data on all patients enrolled. All-cause mortality among urine Ultra-positive patients was similar to that of urine Ultra-negative patients, with rates of 10/34 (29.4%) vs 22/84 (26.2%), log-rank $P = 0.725$ (Figure 3A). Among mortality cases, median survival was 20 days (IQR 8-28) for Ultra-positive vs 16 days (IQR 11-25) for Ultra-negative, $P = 0.849$. The HR for 8-week all-cause mortality for urine Ultra-positive patients compared to negative patients was 1.14 (0.54-2.41), $P = 0.726$, in the univariate analysis, and 1.30 (0.53-3.16), $P = 0.562$, in the multivariate analysis.

In a post-hoc analysis of urine Ultra-positive patients, mortality rates were similar between those who initiated TB treatment (3/10, 30.0%) and those who did not (7/24, 29.2%), log-rank $P = 0.850$ (Figure 3B).

## Discussion

In this cohort of hospitalized patients with advanced HIV, more than one in four had a positive urine Ultra test for TB. Urine Ultra-positive patients were characterized by severe immunosuppression and respiratory distress. Urine Ultra demonstrated substantial added value by identifying a significant number of TB cases that were missed by traditional tests and clinical criteria.

The urine Ultra-positivity rate in our study was higher than reported in the large multi-country study by Sossen et al. [14] of 10.8% (65/600) among inpatients with HIV, and 16.7% (72/432) among those with CD4 count <200 cells/mm$^3$ and as reported by Falci et al. [19] among inpatients with advanced HIV in Brazil of 11.3% (15/133). This difference may reflect the local TB incidences and the severe immunosuppression in our study, which increases the risk of TB dissemination [20] and bloodstream infection [21], potentially leading to shedding of MTBC DNA into urine.

We did not have mycobacterial blood culture in our study, but a high rate of respiratory distress and low blood sodium level among urine Ultra-positive patients may reflect the severity of TB disease as hyponatremia previously has been associated with miliary TB [22] and TB meningitis [23].

We found a high proportion of “Trace” results compared to other urine Ultra studies that reported “Trace” proportions rang-

**Table 1**
Patient characteristics stratified by urine ultra result, n = 118.

| | | Urine ultra-positive n = 34 | Urine ultra-negative n = 84 | *P*-value |
|---|---|---|---|---|
| Demographics | Age (years) | 43 (32-49) | 39 (33-52) | 0.865 |
| | Female sex | 20 (58.8) | 50 (59.5) | 0.944 |
| | Alcohol consumption on a daily or weekly basis | 4 (12.2) | 6 (7.1) | 0.465 |
| | Current or past smoking of tobacco products | 3 (8.8) | 6 (7.1) | 0.716 |
| HIV status | CD4 count (cells/mm$^3$) | 31 (11-103) | 78 (28-169) | **0.025** |
| | Newly diagnosed with HIV (diagnosed <3 months prior current admission) | 13 (38.2) | 49 (58.3) | **0.048** |
| | ART coverage | 14 (41.2) | 23 (27.4) | 0.143 |
| Co-morbidity | Previously treated for TB | 2 (6.1) | 3 (3.6) | 0.620 |
| | Diabetes (any type) | 3 (8.8) | 9 (10.7) | 1.000 |
| | Hypertension | 6 (17.7) | 13 (15.5) | 0.771 |
| | Renal dysfunction (acute and chronic) | 6 (17.7) | 34 (40.5) | **0.018** |
| Symptoms | Cough | 25 (73.5) | 54 (64.3) | 0.334 |
| | Fever | 24 (70.6) | 54 (64.3) | 0.512 |
| | Night sweat | 16 (47.1) | 39 (46.4) | 0.950 |
| | Weight loss | 32 (94.1) | 82 (97.6) | 0.341 |
| | Dyspnoea | 17 (50.0) | 18 (21.4) | **0.002** |
| | Hemoptysis | 3 (8.8) | 4 (4.8) | 0.409 |
| Vital signs | Respiratory rate >30 breaths/min | 11 (32.6) | 4 (4.8) | **<0.001** |
| | Peripheral oxygen saturation <96% or receiving oxygen supply | 12 (35.3) | 15 (17.9) | **0.041** |
| | Heart rate >100/min | 13 (38.2) | 34 (40.5) | 0.822 |
| | Mean arterial pressure <70 mmHg | 7 (20.6) | 11 (13.1) | 0.305 |
| | Axillary temperature >37.5°C | 7 (20.6) | 10 (11.9) | 0.224 |
| Physical examination | Positive finding on lung auscultation | 19 (55.9) | 34 (40.5) | 0.128 |
| | Abnormal abdominal examination | 11 (32.4) | 31 (36.9) | 0.640 |
| | Palpable enlarged lymph nodes | 8 (24.2) | 17 (20.7) | 0.680 |
| | Skin or mucosal manifestation | 15 (44.1) | 45 (53.6) | 0.352 |
| | Unable to walk unaided | 12 (35.3) | 26 (31.0) | 0.648 |
| | Middle upper arm circumference <230 mm | 14 (41.2) | 38 (45.2) | 0.687 |
| Blood test results | Hemoglobin (g/dl) | 8.8 (7.6-10.0) | 8.4 (7.0-10.7) | 0.535 |
| | Platelets (x10^9 cells/l) | 254 (191-371) | 214 (167-311) | 0.352 |
| | Leukocytes (x10^9 cells/l) | 5.8 (4.1-7.9) | 5.8 (4.2-9.6) | 0.396 |
| | Lymphocytes (x10^9 cells/l) | 1.1 (0.6-1.5) | 1.2 (0.7-1.8) | 0.300 |
| | Neutrophils (x10^9 cells/l) | 4.3 (2.4-6.3) | 4.1 (2.0-7.6) | 0.803 |
| | Creatinine (mg/dl) | 0.8 (0.7-1.3) | 1.2 (0.8-6.1) | **0.016** |
| | Urea (mmol/l) | 4.3 (2.7-8.8) | 6.8 (3.9-17.2) | **0.041** |
| | Albumin (g/dl) | 2.8 (2.4-3.2) | 2.9 (2.4-3.5) | 0.359 |
| | Potassium (mmol/l) | 4.1 (3.5-4.5) | 4.2 (3.6-4.9) | 0.413 |
| | Sodium (mmol/l) | 134 (131-139) | 138 (134-141) | **0.023** |
| | ALT (iu/l) | 21 (15-38) | 31 (18-55) | 0.244 |
| Imaging | Any pathology on chest X-ray | 20/24 (83.3) | 43/59 (72.9) | 0.313 |

Abbreviations: ALT = alanine aminotransferase; ART = anti-retroviral therapy; WHO danger sign = respiratory rate >30 breaths/min, heart rate >120 beats/min, temp >39°C, or unable to walk unaided); WHO TB symptom = cough, fever, night sweat, or weight loss.
Missing variables: CD4 count (1), palpable enlarged lymph nodes (3), hemoglobin (6), platelets (6), leukocytes (5), lymphocytes (4), neutrophils (4), creatinine (9), urea (12), albumin (22), potassium (8), sodium (9), ALT (25), chest X-ray (35).
Statistical analysis: Data are median (IQR) for non-normal distributions, mean (SD) for normal distributions, or n (%). Distributions were compared using Chi-squared or Fisherś test for categorical data and Mann–Whitney test for continuous data. Two-sided *P*-values are presented. P-values < 0.05 are shown in bold.

ing from 18.0% to 29.7% [14,19,24]. The high proportion of "Trace" results in our study may be a result of the relatively small pre-centrifugation urine volume used or other factors related to the sample pre-treatment method. Previous studies have used slightly different pre-treatment protocols than ours, including larger pre-centrifugation volumes reaching 30 ml [13,14], resuspension of the pellet with urine [13] or phosphate-buffered saline [14,19] and loaded into the Ultra cartridge without buffer [13], or after addition of Ultra sample reagent in a 1:2 sample-to-reagent ratio [14].

The analysis showed that urine Ultra and LF-LAM detected TB in mostly different patient populations, with only limited overlap, consistent with findings from other studies [12–15,25]. It remains unclear if discordant cases represent different patient characteristic phenotypes or different forms of HIV-associated TB. Notably, in the study from Sossen et al. [14], a positive urine Ultra result, unlike LF-LAM, was often confirmed by another positive molecular or culture-based TB test. In the same study, urine Ultra identified 60/135 (44.4%) of inpatients with TB and 68/133 (51.1%) of TB among patients with CD4 count below 200 cells/mm$^3$. In the severely immunosuppressed inpatient cohort by Stead et al. [15] urine Ultra identified 42/62 (68%) of TB cases. The higher TB yield by urine Ultra in the studies by Sossen et al. and Stead et al. is likely explained by a more rigorous reference testing enabling identification of bloodstream TB.

Furthermore, in the two South African inpatient cohorts well investigated for disseminated TB with mycobacterial blood culture, Boloko et al. [26] showed strong agreement between urine Ultra and mycobacterial blood culture ($\kappa$ = 0.71) and Mntonintshi et al. [25] demonstrated higher diagnostic yield for urine Ultra compared to LF-LAM or sputum Ultra in subgroups with CD4 count <200 cells/mm$^3$, hemoglobin <8 dl/l, and bloodstream TB.

Our findings may be applicable among patients with HIV admitted to hospital in a low- to middle-income setting with moderate to high TB prevalence. The addition of urine Ultra to routine TB investigation with minimal invasive sampling and clinical judgment identified a substantial number of additional patients with possible TB that otherwise would have gone undetected. Ours and others' yield analyses highlight that a single TB test from one specimen cannot detect all forms of HIV-associated TB, emphasizing the need for parallel test strategies (testing several specimens with two or more tests) as evaluated recently using LF-LAM and respiratory LC-aNAATs [2] and recommended by WHO to improve case detection among people with HIV [27].

It is also important to note that urine Ultra may have less value in other settings. For example, Reeve et al. [24] studied ART-naïve outpatients with higher CD4-cell counts in South Africa and found that urine Ultra had a very low incremental TB diagnostic yield compared to LF-LAM.

The suboptimal diagnostic accuracy of urine Ultra against eMRS in our study likely reflects a suboptimal TB reference standard, which lacked blood and extrapulmonary TB cultures. Expanding the reference to CRS, including positive microscopy and clinical TB cases, only slightly improved accuracy.

We included LF-LAM in the reference standard to capture all forms of HIV-associated TB, but some LF-LAM positive patients may have been misclassified as TB although not having TB and some may have been the result of cross-reactivity to non-tuberculous mycobacterial antigens [28,29]. The study by Falci et al. [19] reported a lower urine Ultra sensitivity of 20.7%, while other studies without LF-LAM in the reference but including TB blood cultures, found higher sensitivities at 52.3% [14] and 70% [15]. The latter two studies, also found higher specificity for urine Ultra (98.7% and 100%) [14,15], suggesting its potential as a complementary test for TB in severely immunosuppressed patients with HIV. The limited extrapulmonary testing in our study and especially the lack of mycobacterial blood cultures, likely explains our suboptimal specificity.

Urine Ultra-positive cases missed by CRS were extremely immunosuppressed, clinically unstable, and had a lower rate of routine TB testing. This aligns with our previous findings that severely ill patients such as those unable to walk unaided, were less likely to undergo routine TB testing [30], increasing their risk of underdiagnoses. In contrast, five patients who were urine Ultra-positive but reference standard negative in the study by Mntonintshi et al. [25] had moderate CD4 counts and were negative on comprehensive TB testing, including mycobacterial blood culture, suggesting these were urine Ultra true false-positive cases.

Contrary to Sossen et al. [14] and for inpatient mortality by Stead et al. [15], we found no association between urine Ultra-positivity and mortality. This may be due to our small sample size and highly immunosuppressed inpatient population, where Ultra-negative patients had high rates of comorbidities competing with TB mortality.

A key strength of this study is its focus on estimating TB burden using urine Ultra in a vulnerable, hard-to-reach population of severely immunosuppressed inpatients with HIV. However, two key limitations are the small sample size, which reduces precision and results in wide CIs; and an incomplete microbiological reference for extrapulmonary and disseminated TB. This together with the inclusion of LF-LAM in the reference standard, may have introduced misclassification bias, potentially underestimating urine Ultra's diagnostic accuracy for HIV-associated TB.

A significant proportion of severely immunosuppressed HIV-infected inpatients in Ghana tested positive for urine Ultra, many of whom were undiagnosed by routine tests. Given urine Ultra's high specificity in studies with robust reference standards and its incremental diagnostic yield in ours and similar studies, it shows promise as a supplementary point-of-care TB test alongside sputum-based LC-aNAAT and urine LF-LAM, particularly in severely immunosuppressed inpatients with HIV, where sputum collection is challenging and the risk of extrapulmonary or disseminated TB is high. Further research is needed to optimize sample processing, clarify the mechanism of MTBC DNA detection in urine, and evaluate the feasibility, cost-effectiveness, and patient-centered outcomes through clinical trials.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Ethical Approval

The study was approved by the Korle-Bu Teaching Hospital Scientific and Technical Committee and the Institutional Review Committee on September 9, 2019 (KBTH-IRB/00052/2019), with amendments for COVID-19 safety measures approved on August 7, 2020.

## Authors Contributions

JÅ, SB, ML, and ISJ conceptualized the overall research project and oversaw the project activities and communication. JÅ, SB, ML, DYM, JAO, and ISJ designed the methodology for the Urine Ultra study. JÅ collected and managed the data and performed the formal analysis. JÅ, SB, and ISJ validated the results for accuracy. PA, SOW, TA, and DYM conducted the Urine Ultra analyses in the laboratory. JÅ, FS, YAP, JAO and ISJ provided resources or funding for the study. JÅ wrote the initial manuscript draft, and all authors reviewed and edited the final manuscript.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.108254.

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