

Evaluation of the Xpert MTB/RIF Assay at a Tertiary Care Referral Hospital in a Setting Where Tuberculosis and HIV Infection Are Highly Endemic

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Diagnosis of tuberculosis in most countries where tuberculosis has a high endemicity relies heavily on smear microscopy, a century-old technology. Although it is simple and inexpensive, the specificity and sensitivity is poor, particularly in human immunodeficiency virus (HIV)-positive patients, as well as in children. Furthermore, it cannot differentiate between disease caused by drug-sensitive *Mycobacterium tuberculosis* (MTB) and disease caused by drug-resistant *M. tuberculosis* [1]. In sub-Saharan Africa, an average of only 56% of new cases of pulmonary tuberculosis were smear positive in 2010, with Zimbabwe, Swaziland, and Zambia having the lowest sputum smear-positive case detection rates, at 32%, 37%, and 38%, respectively [2], indicating that smear microscopy is suboptimal for use in Africa. Automated liquid culture, the recommended gold standard tuberculosis diagnostic test, is highly specific and sensitive for the detection of tuberculosis and drug-resistant tuberculosis, but it is labor-intensive, time-consuming (2–6 weeks from sample collection to availability of

results), and expensive and requires specialized equipment and a well-serviced biosafety level 3 facility [1]. The worldwide scale of the drug-resistant tuberculosis problem (estimated at 650 000 prevalent cases in 2010 [2]) is most likely underestimated because of poor laboratory facilities for drug-susceptibility testing, poor surveillance mechanisms and reporting procedures, and suboptimal coverage of the infrequent surveys in middle- and low-income countries [3].

In December 2010, the World Health Organization (WHO) endorsed the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) for the rapid diagnosis of tuberculosis and multidrug-resistant tuberculosis (MDR-TB) [4]. The test is recommended for use in individuals suspected of MDR-tuberculosis or HIV-associated tuberculosis [4]. The Xpert MTB/RIF assay is capable of detecting the *M. tuberculosis* complex while simultaneously detecting rifampicin resistance in <2 hours [5]. A recent meta-analysis of 16 studies gave a pooled sensitivity of 90% (95% confidence interval [CI], 89%–91%) and a pooled specificity of 98% (95% CI, 98%–99%) [6]. Seven of these studies reported on the use of the Xpert MTB/RIF assay to detect rifampicin resistance: pooled sensitivity and specificity were 94% (95% CI, 92%–96%) and 97% (95% CI, 96%–98%), respectively [6]. The detection of rifampicin resistance is considered a proxy marker for MDR-TB, as an estimated 90% of rifampicin-resistant *M. tuberculosis* isolates are also resistant to isoniazid [1, 6, 7].

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Zambia had a tuberculosis incidence of 462 cases per 100 000 population in 2010 and a prevalence of HIV infection of 13.5% among adults [2, 8]. Active tuberculosis is significantly more difficult to diagnose in HIV-positive patients [9], in whom infection is often paucibacillary. The high burden of tuberculosis and HIV infection and the low smear-positive case detection rate [10] in Zambia make this country an ideal setting for the implementation and evaluation of the Xpert MTB/RIF assay.

Several studies have evaluated the Xpert MTB/RIF assay [11–15] since its endorsement by WHO [4]. Further evaluation is required in tertiary care referral hospitals with a high burden of tuberculosis and HIV infection, which concentrate tuberculosis cases missed by primary and secondary healthcare facilities. Tertiary care hospitals receive a large number of seriously ill patients with communicable and non-communicable diseases (CDs/NCDs), many of whom harbor undetected active tuberculosis, which remains undiagnosed because attention is given to the main admission symptoms and referral diagnoses. Many cases in which tuberculosis is comorbid with NCDs or other CDs may be missed, and patients with undiagnosed active tuberculosis may not be investigated for tuberculosis because they are asymptomatic. Most Xpert MTB/RIF assay studies performed to date exclusively recruited patients with suspected tuberculosis [12–14, 16–18]. A different study design was used here, in which all inpatients able to produce sputum were enrolled (irrespective of admission diagnosis and clinical suspicion of tuberculosis), to capture the total tuberculosis load among sputum producers at a tertiary care referral hospital and to detect additional cases that would otherwise have been overlooked.

METHODS

Study Design and Recruitment

This was a descriptive, prospective study designed to evaluate the performance of the Xpert MTB/RIF assay for the detection of pulmonary tuberculosis and MDR-TB in HIV-infected and HIV-uninfected adult inpatients at the medical admission wards of the University Teaching Hospital (UTH; Lusaka, Zambia), a tertiary care referral center.

Each morning, adult inpatients (age, >15 years) admitted during the previous 24 hours to the adult inpatient wards were prospectively recruited into the study between September 2010 and November 2011. Patients who were able to produce a sputum sample were recruited irrespective of admission diagnosis, including patients who were already receiving tuberculosis treatment; the latter patients were included to maximize detection of MDR-TB for the evaluation of the rifampicin resistance detection component of the Xpert MTB/RIF assay. Clinical details, including the admission diagnosis or diagnoses that necessitated hospital admission, were recorded.

Sample Collection, Processing, and Analysis

Consenting patients provided up to 3 sputum samples via the spot-morning-spot strategy. Collection of spot sputum was supervised by clinical staff in the wards, as routinely performed for patients with productive cough. Sputum induction was not performed because this is not routinely practiced at the hospital. Fluorescent smear microscopy was performed directly on all sputum samples. A smear-positive sputum specimen was one in which acid-fast bacilli (AFB) were detected, and a smear-negative sputum specimen was one in which AFB were not detected.

For mycobacterial growth indicator tube (MGIT) culture and Xpert MTB/RIF assay analysis, sputum specimens were processed as previously described [19]. If >1 sputum sample was collected from a patient, the most mucoid sample was used for MGIT culture and Xpert MTB/RIF assay analysis.

Sputum culture and phenotypic drug-susceptibility testing were performed as described previously [19]. A specimen was considered to be culture positive if results of MGIT culture and a confirmatory TBcID test were positive. A specimen was considered to be culture negative if results of MGIT culture were negative, or, if positive, results of a confirmatory TBcID test were negative. The culture-based *M. tuberculosis* load was approximated by the time to positivity (TTP) of the MGIT culture.

For the Xpert MTB/RIF assay, concentrated sputum was added to the sample reagent in a ratio of 1:3 (ie, 0.5 mL of patient sample to 1.5 mL of the sample reagent). Two milliliters of this mixture was added to the Xpert MTB/RIF assay cartridge and then run in the machine in accordance with manufacturer's instructions. The Xpert MTB/RIF assay-based *M. tuberculosis* load was classified using the manufacturer's software as very low, low, medium, and high on the basis of the threshold cycle (C_T) values of *M. tuberculosis*-positive samples.

Data Management and Analysis

Clinical and laboratory data were compiled in databases, using double data entry and Epidata software [16]. Selected variables were exported to SPSS, version 18 (IBM, Armonk, NY), for analysis. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Xpert MTB/RIF assay were calculated with 95% CIs. Analysis of the Xpert MTB/RIF assay-determined *M. tuberculosis* load with respect to both HIV infection status and smear status was performed using the Pearson χ^2 analysis. Analysis of the Xpert MTB/RIF assay-determined *M. tuberculosis* load with respect to MGIT culture TTP was performed using the Kruskal-Wallis test.

Ethics Approval

This study was approved by the research ethics review committee of the University of Zambia School of Medicine (Lusaka, Zambia). All study participants gave written

informed consent, and the study was conducted in accordance with ethics committee guidelines.

RESULTS

Demographic Characteristics

A total of 881 of 937 recruited patients produced a sufficient level of sputum for analysis by smear microscopy, MGIT culture, and the Xpert MTB/RIF assay (Figure 1). The median age of the cohort was 35 years (interquartile range, 28–43 years). The cohort was balanced with regard to sex, with males composing 50.6% of patients (446 of 881), and the HIV infection prevalence was 70.9% (595 of 839; HIV status was unavailable for 42 patients); both characteristics were consistent with the population of adult inpatients in the medical ward (data not shown). Overall, 38.1% of the cohort (336 of 881 patients) was currently receiving tuberculosis treatment. A range of different diagnoses were represented in the cohort. Almost half of the cohort (43.1% [380 of 881]) had pulmonary tuberculosis, extrapulmonary tuberculosis, or a respiratory disorder other than tuberculosis. Other diagnosis categories were cardiac disorders (14.0% of patients), cancer (6.4%), gastrointestinal disorders (6.4%), metabolic disorders (4.3%), renal disorders (5.2%), and diabetes (2%); 18.6% had a diagnosis not specified in this list. Culture-confirmed tuberculosis was found in 201 of 881 patients (22.8%). According to the admission diagnosis recorded by the attending physician, 27 of the 201 patients (13.4%) with culture-confirmed tuberculosis did not have suspected tuberculosis, defined as the presence of cough that persisted for ≥ 2 weeks before admission.

Performance of the Xpert MTB/RIF Assay for the Detection of *M. tuberculosis*, by HIV Infection Status

To evaluate the performance of the Xpert MTB/RIF assay, the 238 culture-negative patients who were currently receiving tuberculosis treatment were excluded from analyses [17]. Consequently, 643 patient samples were included in this analysis.

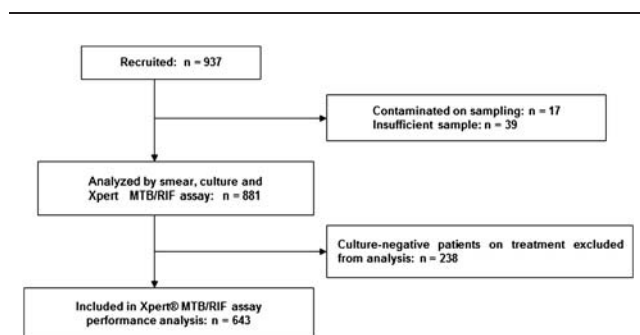


Figure 1. Flow of participants through the study.

The assay performed well, with a specificity of 95.7% (95% CI, 93.3%–97.3%) and a sensitivity of 86.1% (95% CI, 80.3%–90.4%) (Table 1). The PPV and NPV were 89.6% (95% CI, 84.2%–93.4%) and 94.0% (95% CI, 91.4%–95.9%), respectively. Stratification of the analysis by HIV infection status revealed that the sensitivity of the assay was significantly greater for HIV-positive patients (88.2% [95% CI, 81.9%–92.6%] vs 74.3% [95% CI, 56.4%–87.0%]; $P = .033$) (Table 1), although the number of HIV-negative patients in the cohort was low and CIs overlapped. This trend toward reduced sensitivity for HIV-negative individuals was solely due to smear-negative patients, for whom the sensitivity of the assay was 55.6% (95% CI, 31.3%–77.6%), compared with 78.9% (95% CI, 67.8%–87.1%) for HIV-positive patients ($P = .0407$); in smear-positive patients, there was no significant difference in the sensitivity of the assay with respect to HIV infection status (Table 1). The sensitivity and specificity of both the Xpert MTB/RIF assay and smear microscopy did not differ significantly between patients with suspected tuberculosis and those without suspected tuberculosis, although there was a trend of reduced sensitivity of the Xpert MTB/RIF assay for patients without suspected tuberculosis, compared with patients with suspected tuberculosis (Table 1).

Performance of the Xpert MTB/RIF Assay for the Detection of Rifampicin Resistance and MDR-TB

Results of culture to determine drug susceptibility were available for 111 of 202 *M. tuberculosis* culture-positive patients, with 18% of cases (20) resistant to rifampicin and 16.2% (18) MDR. Thirty-three subcultures were contaminated, and 58 were not performed because drug-susceptibility testing was not available at the beginning of the study. A total of 90% of rifampicin-resistant *M. tuberculosis* isolates (18 of 20) were MDR. All 111 samples were analyzed by the Xpert MTB/RIF assay, but findings were negative for MDR in 11.8% of non-MDR-TB cases (11 of 93) and in 22.2% of MDR-TB cases (4 of 18), leaving 96 samples with definitive resistance data from both culture-based drug susceptibility testing and the Xpert MTB/RIF assay. The sensitivity of the Xpert MTB/RIF assay was 81.3% (95% CI, 53.7%–95.0%), and the specificity was 97.5% (95% CI, 90.4%–99.6%). The PPV and NPV were 86.7% (95% CI, 58.4%–97.7%) and 96.2% (95% CI, 88.8%–99.0%), respectively.

Correlation Between *M. tuberculosis* Loads Determined by the Xpert MTB/RIF Assay and Other Markers of Disease Severity

The proportion of smear-positive patients increased as the Xpert MTB/RIF assay-determined *M. tuberculosis* loads increased. However, the Xpert MTB/RIF assay load could not be used to determine smear status because of a mix of smear-positive and smear-negative patients in each Xpert MTB/RIF

Table 1. Sensitivity and Specificity of the Xpert MTB/RIF Assay and Sputum Smear, Using Mycobacterial Growth Indicator Tube Liquid Culture as Gold Standard

Variable	Sensitivity	Specificity	PPV, % [95% CI]	NPV, % [95% CI]	Sensitivity Among Smear-Pos Samples	Sensitivity Among Smear-Neg Samples
<i>M. tuberculosis</i> detection by Xpert MTB/RIF assay						
All patients	173/201 (86.1) [80.3–90.4]	420/442 (95.7) [93.3–97.3]	89.6 [84.2–93.4]	94.0 [91.4–95.9]	102/106 (96.2) [90.1–98.8]	71/95 (74.7) [64.6–82.8]
TB suspected	63/76 (82.9) [72.2–90.2]	189/202 (94.0) [89.6–96.7]	84.0 [73.3–91.1]	93.5 [89.0–96.4]	39/42 (92.8) [79.4–98.1]	24/34 (70.5) [52.3–84.3]
TB not suspected	19/27 (70.3) [49.7–85.5]	231/239 (95.9) [92.2–97.9]	65.5 [45.7–81.4]	96.7 [93.3–98.4]	10/10 (100) [65.5–100]	9/17 (52.9) [28.5–76.1]
HIV positive ^a	142/161 (88.2) [81.9–92.6]	235/247 (95.1) [91.5–97.3]	92.2 [86.5–95.7]	92.5 [88.4–95.3]	82/85 (96.5) [89.3–99.1]	60/76 (78.9) [67.8–87.1]
HIV negative ^a	26/35 (74.3) [56.4–87.0]	155/161 (96.3) [91.7–98.5]	81.3 [63.0–92.1]	94.5 [89.5–97.3]	16/17 (94.1) [69.2–99.7]	10/18 (55.6) [31.3–77.6]
Drug-susceptibility testing						
By Xpert MTB/RIF assay						
All patients	13/16 (81.3) [53.7–95.0]	78/80 (97.5) [90.4–99.6]	86.7 [58.4–97.7]	96.2 [88.8–99.0]	NA	NA
<i>M. tuberculosis</i> detection by smear microscopy						
All patients	106/201 (52.7) [45.6–59.8]	431/442 (97.5) [95.5–98.7]	90.6 [83.4–95.0]	81.9 [78.3–85.1]	NA	NA
TB suspected	42/76 (55.3) [43.5–66.5]	193/201 (96.0) [92.0–98.1]	84.0 [70.3–92.3]	85.0 [79.6–89.3]	NA	NA
TB not suspected	10/27 (52.9) [28.5–76.1]	238/255 (96.2) [92.7–98.1]	50 [26.8–73.2]	96.6 [93.2–98.4]	NA	NA

Data are No. correct/No. tested (%) [95% confidence interval], unless otherwise indicated.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; NA, not applicable; Neg, negative; NPV, negative predictive value; Pos, positive; PPV, positive predictive value; TB, tuberculosis.

^a HIV status was available for 196 culture-positive patients and 408 culture-negative patients.

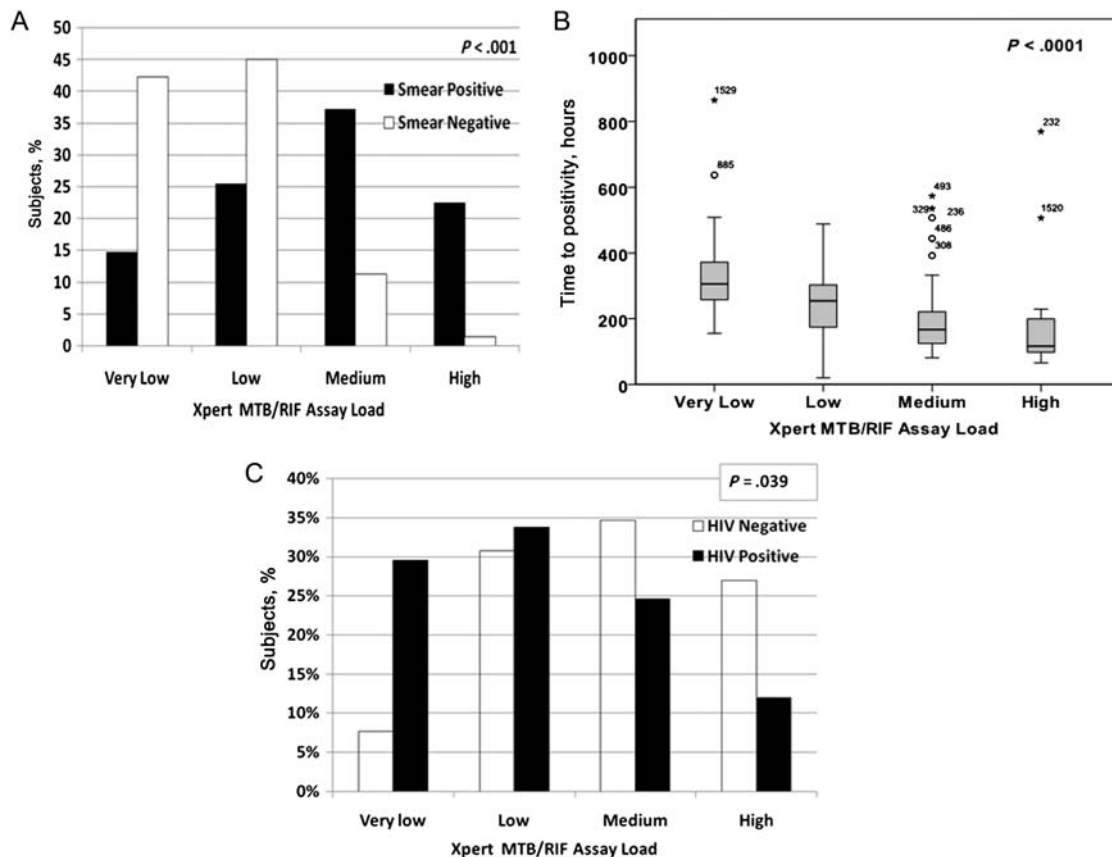


Figure 2. Relationship between Xpert MTB/RIF assay load and smear findings (A), time to positivity (B), and human immunodeficiency virus infection status (C). Abbreviation: HIV, human immunodeficiency virus.

assay load category (Figure 2A). The distribution of MGIT culture TTP results differed significantly by Xpert MTB/RIF assay load ($P < .001$, by the Kruskal-Wallis test) (Figure 2B), and the distribution of Xpert MTB/RIF assay loads differed significantly by HIV infection status ($P = .039$, by the Pearson χ^2 analysis), with medium and high loads more common among HIV-negative patients and very low and low loads more common among HIV-positive patients (Figure 2C).

DISCUSSION

There are several key findings from this study. First, the sensitivity of the Xpert MTB/RIF assay for detecting pulmonary tuberculosis in a hospital setting in a country where tuberculosis and HIV infection are highly endemic was similar to that previously reported in sub-Saharan Africa, whereas the specificity was significantly lower [11–13, 18, 20]. Second, the Xpert MTB/RIF assay detected 71 additional tuberculosis cases that were not detected by smear microscopy, which is a significant improvement over the current smear-microscopy standard being used in most sub-Saharan African countries. Third, the

Xpert MTB/RIF assay had a reduced sensitivity among HIV-negative, smear-negative patients. Fourth, the sensitivity of the Xpert MTB/RIF assay for detecting MDR-TB was significantly lower than previously reported [17, 18]. Fifth, the semiquantitative results of the Xpert MTB/RIF assay correlated well with MGIT culture TTP.

The limitations of our study were as follows. First, only a single sputum sample from each patient was analyzed by culture. Therefore, the number of tuberculosis cases we present here is likely an underestimate of the actual number among participating patients. Second, because sputum specimens were decontaminated, performance may have differed from that for “raw” sputum specimens. Third, because of the nature of the study design, patients who were already receiving tuberculosis treatment were recruited into the study, and there was an overrepresentation of patients with respiratory symptoms, compared with the general adult inpatient population (data not shown). This was inevitable because recruitment was based solely on the ability to produce a sputum sample. Because of this bias, we cannot draw any conclusions about the prevalence of tuberculosis within the broader hospital population.

The Xpert MTB/RIF assay performed well, compared with smear, and was 74.7% sensitive for specimens from smear-negative, culture-positive patients, detecting an additional 71 tuberculosis cases. The overall sensitivity was 86.1%, similar to pooled data from the region (82.4%) [11–13, 18, 20]. Conversely, the specificity of the Xpert MTB/RIF assay was significantly lower than pooled data from the region (95.3% [420 of 442] vs 97.8% [2103 of 2151]; $P = .0101$) [11–13, 18, 20]. Among the 238 culture-negative patients currently receiving treatment who were excluded from our analysis, results of the Xpert MTB/RIF assay were positive in 39 cases (16.4%). Other reports in the literature have suggested that some of the Xpert MTB/RIF assay's "false-positive" results are true positives on the basis of clinical diagnosis [11, 13]. Another possibility is that smear and the Xpert MTB/RIF assay are detecting dead *M. tuberculosis* bacilli, which could confound the use of the assay for use as a biomarker for monitoring response to treatment, cure, and relapse.

The Xpert MTB/RIF assay had a significantly reduced sensitivity among HIV-negative patients. This effect was exclusive to smear-negative, HIV-negative patients, for whom the sensitivity was very low, at 52.9%. Despite the broad CIs, this finding warrants further investigation. Specificity was not significantly affected by HIV infection status. Compared with other data from the region, the sensitivity of the Xpert MTB/RIF assay among HIV-positive patients was significantly higher (88.2% [142 of 161] vs 80.2% [390 of 486]; $P = .02217$). Conversely, the specificity was significantly lower (95.1% [235 of 247] vs 98.2% [560 of 570]; $P = .01684$), as it was overall in our cohort (70.6%), in which the prevalence of HIV infection was high.

Of the 201 patients with culture-confirmed tuberculosis, 27 (13.4%) were not considered to have suspected tuberculosis on admission. The Xpert MTB/RIF assay performed equally well among patients with and patients without suspected tuberculosis. This highlights the fact that, because many tuberculosis cases go undiagnosed in primary and secondary healthcare centers and concentrate in tertiary care health centers, it may be useful to adopt an active case-finding approach that uses the Xpert MTB/RIF assay for routine tuberculosis screening of all inpatients in countries where tuberculosis is highly endemic.

During this study, 18 culture-confirmed cases of MDR-TB were detected. Fourteen cases involved patients with current tuberculosis (who are at greater risk for MDR-tuberculosis, compared with treatment-naive patients); of these, 6 were receiving their first course of first-line tuberculosis treatment and therefore had cases that represented possible acquired MDR-TB or treatment failure, and 4 were about to start relapse therapy. Five patients with current tuberculosis in whom this study detected MDR-TB were not considered by the attending physician to have suspected MDR-TB, which therefore put other patients

and staff at risk. When analyzing the performance of the rifampicin component of the Xpert MTB/RIF assay, culture-determined rifampicin resistance, not MDR-TB, was used as the gold standard. The specificity of the Xpert MTB/RIF assay to detect rifampicin resistance was similar to that previously reported in 2 well-powered studies [17, 18], but the sensitivity was significantly lower (81.3% [13 of 16] vs 95.8% [436 of 455]; $P = .0066$), but our CIs were as broad as those in other studies with relatively small numbers of patients with rifampicin-resistant *M. tuberculosis* [6]. We found that 90% of rifampicin-resistant *M. tuberculosis* isolates were MDR, which is consistent with results from other studies [7]. Two false-positive rifampicin resistance results were produced by the Xpert MTB/RIF assay, representing a significant overcall (15.4% [2 of 13]). *M. tuberculosis* was not detected in 4 of 20 patients with culture-determined rifampicin-resistant *M. tuberculosis* by use of the Xpert MTB/RIF assay, and these samples were excluded when evaluating the assay's ability to detect rifampicin resistance. In clinical practice, these patients would have had undiagnosed rifampicin-resistant tuberculosis. If these samples were included in the analysis, the sensitivity of the Xpert MTB/RIF assay for detecting rifampicin resistance would be significantly lower than reported (65% [13 of 20]). Recent studies have highlighted a problem with false-positive results of tests for rifampicin resistance [18, 21–24], and corrective measures have been instituted in the recent G4 version of the test, including revisions to the diagnostic platform software and redesign of the oligonucleotide probes [25, 26]. WHO recommends further confirmatory tests following detection of rifampicin-resistant *M. tuberculosis* strains [27].

Accurate quantification of the *M. tuberculosis* complex load in patient samples may allow for the evaluation of the patient's infectiousness, the evaluation of the disease severity, and the monitoring of treatment [28]. The semiquantitative *M. tuberculosis* complex load estimates from the Xpert MTB/RIF assay could not be used to determine the smear status of the patients in the patient cohort. Smear grade and Xpert MTB/RIF assay load had a good broad correlation, as has been previously demonstrated [11, 13]. However, because the individual predictive values for each load level were poor, the Xpert MTB/RIF assay loads could not accurately predict smear grade (data not shown). Higher Xpert MTB/RIF assay loads were associated with decreased MGIT culture TTP, consistent with previous data indicating that the Xpert MTB/RIF assay's semiquantitative results could be used to estimate the *M. tuberculosis* load [13, 29]. Finally, low Xpert MTB/RIF assay loads were significantly more common among HIV-positive patients, consistent with previous findings that sputum samples from HIV-positive patients are more often paucibacillary, which makes *M. tuberculosis* more difficult to detect [9].

This study demonstrates that the Xpert MTB/RIF assay performs better than routine smear microscopy in an inpatient

setting at a tertiary care referral center with a high burden of inpatients with tuberculosis and HIV infection. The Xpert MTB/RIF assay detected 74.7% of smear-negative, culture-positive cases and an additional 71 tuberculosis cases, compared with smear microscopy. This study further demonstrates that MDR-TB is present at UTH and that the Xpert MTB/RIF assay may be useful as a dual screening test for tuberculosis and MDR-TB. A recent cost-benefit analysis recommended the use of the Xpert MTB/RIF assay at the community level [22] and for patients initiating antiretroviral therapy [24, 30]. Our study was not designed to evaluate the clinical impact of the assay; rather, it was designed to evaluate the assay at a tertiary care referral center in which the burden of tuberculosis and HIV infection is high among inpatients. Further assessment of the clinical impact of the Xpert MTB/RIF assay in inpatient settings is now required, including evaluation of the outcomes and effect on clinical practice decisions, management outcome, and development of new diagnostic algorithms; the cost-effectiveness and feasibility of implementing the assay; and the usefulness of the Xpert MTB/RIF assay for proactive detection of tuberculosis cases that may be missed by smear and for concomitant screening for MDR-tuberculosis among adult inpatients attending tertiary care referral centers in other countries where the burden of tuberculosis and HIV infection is high.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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