Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirations in children in sub-Saharan Africa: a prospective descriptive study

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Summary

Background Rapid and accurate diagnosis of pulmonary tuberculosis in children remains challenging because of difficulties in obtaining sputum samples and the paucibacillary nature of the disease. The Xpert MTB/RIF assay is useful for rapid diagnosis of childhood tuberculosis with sputum and nasopharyngeal samples. We assessed this assay for the detection of tuberculosis and multidrug resistant (MDR) tuberculosis with gastric lavage aspirate (GLA) samples in children admitted to hospital.

Methods We did a prospective study to assess the sensitivity and specificity of the Xpert MTB/RIF assay with GLA samples for the detection of pulmonary tuberculosis and MDR tuberculosis in new paediatric inpatient admissions at the University Teaching Hospital, Lusaka, Zambia. Children aged 15 years or younger were recruited between June, 2011, and May, 2012. GLA and sputum were analysed by standard smear-microscopy, mycobacterial growth indicator tube (MGIT) culture, MGIT drug-susceptibility testing, and the Xpert MTB/RIF assay. Sensitivity of the Xpert MTB/RIF assay was assessed with the Pearson χ² or Fishers exact test.

Findings Of 930 children, 142 produced sputum and GLA was obtained from 788 non-sputum producers. Culture-positive tuberculosis was identified in 58 (6·2%) of 930 children: ten from sputum producers and 48 from GLA of non-sputum producers. The sensitivity and specificity of the Xpert MTB/RIF assay were similar: sensitivity was 68·8% (95% CI 53·6–80·9) for GLA versus 90·0% (54·1–99·5; p=0·1649) for sputum samples; specificity was 99·3% (98·3–99·8) for GLA and 98·5% (94·1–99·7; p=0·2871) for sputum samples. The Xpert MTB/RIF assay detected an extra 28 tuberculosis cases compared with smear microscopy and was significantly more sensitive than smear microscopy for both sputum (90·0% [54·1–99·5] vs 30·0% [8·1–64·6]; p=0·01) and GLA (68·8% [53·6–80·9] vs 25·0% [14·1–40·0]; p=0·0001). The assay load did not differ significantly by sample type (p=0·791). 22 children were infected with HIV and tuberculosis and significant differences in assay performance could not be detected when stratifying by HIV status for either sample type. The Xpert MTB/RIF assay detected rifampicin resistance in three GLA samples: two confirmed as MDR tuberculosis and one false positive.

Interpretation Analyses of GLA samples with the Xpert MTB/RIF assay is a sensitive and specific method for rapid diagnosis of pulmonary tuberculosis in children who cannot produce sputum. The single site nature of our study invites caution.

Introduction WHO estimates the global burden of tuberculosis at 9 million new cases a year, with up to 15% of the burden in children.1 In sub-Saharan Africa, an estimated 20% of all cases of active pulmonary tuberculosis are in children.2–4 Currently available diagnostic techniques are insufficient for the rapid and accurate diagnosis of tuberculosis and multidrug resistant (MDR) tuberculosis (ie, tuberculosis caused by strains of Mycobacterium tuberculosis resistant to at least isoniazid and rifampicin) in children in countries highly endemic for tuberculosis and HIV.5–8 In such settings sputum microscopy is often the only test available. This test performs poorly in children for several reasons: the inability of young children to expectorate sputum, the paucibacillary nature of sputum from those co-infected with HIV, and inability to identify MDR tuberculosis.2 Where optimum culture facilities are available, confirmation is delayed and the combination of sputum smear and culture tests still miss many cases of childhood tuberculosis.9

Tertiary referral hospitals in sub-Saharan Africa have a high inpatient load of children admitted to paediatric wards with respiratory illnesses, many of whom die of undiagnosed pulmonary tuberculosis.10–11 A large burden of tuberculosis in children is evident at all points of the health-care system in sub-Saharan Africa, emphasising an urgent need to develop, assess, and introduce more rapid and accurate diagnostics and diagnostic
algorithms for childhood tuberculosis. In December, 2010, WHO endorsed the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) for rapid diagnosis of tuberculosis and MDR tuberculosis with sputum in regions highly endemic for tuberculosis and HIV. Several studies have assessed the Xpert MTB/RIF assay in adults with pulmonary tuberculosis in sub-Saharan Africa and a meta-analysis of 16 studies gave a pooled sensitivity of 90% and a pooled specificity of 98%. In an inpatient hospital setting, the assay can identify the M tuberculosis complex and simultaneously detect rifampicin resistance in sputum samples from adult patients operationally within 24 h.

Recent studies investigating the Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in children using sputum samples in Cape Town, South Africa, and Mbeya, Tanzania, have suggested that the assay is better than sputum-smear microscopy. A major hindrance in the assay’s application for diagnosis of childhood tuberculosis is the inability of a large proportion of children with pulmonary tuberculosis to expectorate sputum. In these children, the Xpert MTB/RIF assay has proven useful with induced sputum. A recent study by Zar and colleagues showed that testing two nasopharyngeal aspirates in children with suspected pulmonary tuberculosis with the Xpert MTB/RIF assay can be useful, especially in settings where facilities for inducing sputum and culture are not available. Further assessment of the assay in children from settings with high burdens of tuberculosis and HIV, with specimens other than sputum, are warranted.

Zambia has a high incidence of tuberculosis (462 cases per 100 000 population) and a high HIV prevalence (13-5%). Only 6% of reported cases of childhood tuberculosis are sputum-smear positive, with the remaining cases diagnosed on the basis of clinical criteria. Furthermore, no data exist on the prevalence of MDR tuberculosis in children in Zambia. Tertiary care hospitals in sub-Saharan Africa receive many seriously ill children with communicable and non-communicable diseases with HIV and M tuberculosis co-infection. Many of these children harbour active tuberculosis but remain undiagnosed.

We sought to assess the Xpert MTB/RIF assay with gastric lavage aspirate (GLA) samples for the rapid diagnosis of active pulmonary tuberculosis and MDR tuberculosis in children.

Methods
Study design and patients
We did a prospective study to assess the performance of the Xpert MTB/RIF assay with GLA samples for the detection of pulmonary tuberculosis and MDR tuberculosis in new paediatric inpatient admissions at the Department of Paediatric and Child Health, University Teaching Hospital, Lusaka, Zambia—a tertiary referral centre serving an urban population of about 1.2 million people from Lusaka province of whom about 40% are children. The hospital admits patients who present acutely to the accident and emergency units, are referred from primary health clinics in Lusaka province, and are sent from health-care centres from other parts of the country for further investigation and specialist management. Respiratory disorders, diarrhoeal diseases, and malnutrition are three of the most common reasons for admission.

Any new child inpatients, aged 15 years or younger, with a primary or secondary admission diagnosis of suspected tuberculosis were eligible for enrolment in the study. A patient with suspected tuberculosis was defined as having tuberculosis on the basis of a symptom-and-risk-factor screen (one or more of five factors: cough for more than 2 weeks, weight loss, malnutrition, HIV, or tuberculosis contact) according to the Zambia National TB Programme and WHO guidelines. This screen included children currently on treatment for tuberculosis for whom failure of treatment or presence of drug resistant disease necessitated diagnosis of active tuberculosis. Patients were excluded if they were deemed to have a poor prognosis or if parents or guardians refused consent.

Between June, 2011, and May, 2012, parents or guardians of new admissions with a primary or secondary admission diagnosis of suspected tuberculosis, were approached by the attending physician to have their children enrolled into the study. Informed consent was obtained. The admission diagnoses, as ascertained by the admitting paediatrician, necessitating hospital admission, were recorded. Because of the high prevalence of HIV co-infection in patients with tuberculosis in Zambia, HIV screening is done as routine on all tuberculosis suspects after appropriate counselling and consent. Sputum samples were collected from children who could expectorate. GLAs were obtained from children unable to produce a sputum sample. This study was approved by the research ethics review committee of the University of Zambia School of Medicine, Lusaka, Zambia. The guardians of all study participants provided written informed consent.

Procedures
Fluorescent smear microscopy was done directly on all samples as described previously. Samples (sputum or GLA) were then homogenised and digested in N-acetyl-L-cysteine–NaOH (1·5% final concentration) and vortexed for 30 s at 5 min intervals for 15 min, with subsequent concentration at 4000 g for 15 min. The supernatant was removed and the sediment was resuspended in 2 mL phosphate buffer (pH 6-8), irrespective of the original sample volume. The resulting suspension was used for Mycobacterial Growth Indicator Tube (MGIT; BD, Franklin Lakes, NJ, USA) culture and Xpert MTB/RIF assay analysis.

One MGIT tube was inoculated with 0·5 mL concentrated sample (sputum or GLA) and incubated in the
BACTEC 960 system (BD, Franklin Lakes, NJ, USA). Cultures were classed as negative when no growth was evident after 42 days incubation. Positive MGIT cultures were confirmed as containing *M tuberculosis* complex with no growth on blood agar plates and a positive TBcID (BD, Franklin Lakes, NJ, USA) culture confirmation test. Contaminated samples were retreated and recultured, and excluded if still contaminated. Phenotypic drug-susceptibility testing was done on *M tuberculosis* positive cultures with the BACTEC MGIT 960 SIRE kit (BD, Franklin Lakes, NJ, USA) in accordance with the manufacturer’s instructions.

The concentrated sample (sputum or GLA) was added to the Xpert MTB/RIF sample reagent in a 1:3 ratio (0.5 mL of sediment to 1.5 mL of the sample reagent). 2 mL of this mixture was added to the Xpert MTB/RIF cartridge and run in the machine in accordance with manufacturer’s instructions.

Statistical analysis
All clinical and laboratory data were compiled in databases with double data entry and Epidata software. Selected variables were exported to SPSS (version 18) for analysis. Comparisons of Xpert MTB/RIF assay sensitivity between different groups of patients, and comparisons between HIV status or sample type and Xpert MTB/RIF assay load were done with Pearson $\chi^2$ or Fishers exact test. Median age and time to positivity (TTP) were compared with sample type and Xpert MTB/RIF assay load by Mann Whitney U and Kruskal Wallis tests, respectively.

Role of the funding source
The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
1037 children were recruited and had sputum or GLA obtained for analysis (figure 1). About half (516; 49.8%) of children enrolled were younger than 2 years (median 24 months; IQR 12–74) and did not differ by HIV prevalence, which was 30.5%.

Some children were excluded from our assessment of the performance of the Xpert MTB/RIF assay (figure 1). We were able to obtain GLAs from all children who could not produce sputum. 431 children (46.3%) had suspected tuberculosis on admission, 488 (52.5%) did not have tuberculosis as a primary diagnosis, and 11 were already on tuberculosis treatment before admission. The proportions of sputum and GLA collected did not differ significantly with respect to the patients’ tuberculosis status (table 1; Pearson $\chi^2$, $p=0.879$). Common primary admission clinical diagnoses were respiratory disorders (322 [34.6%]), malnutrition (210 [22.6%]), gastrointestinal disorders (166 [17.9%]), and tuberculosis (76 [8.2%]).

Culture-positive tuberculosis was detected in 58 (6.2%) of 930 children: ten (7%) of 142 in sputum and 48 (6.1%) of 788 in GLA samples. 19 (32.8%) samples were from children younger than 2 years of age. 18 (31.0%) of 58 were aged between 2 and 4 years, six (10.3%) were aged between 5 and 9 years, and the remaining 15 (25.9%) were aged between 10 and 15 years.

Smear positive tuberculosis was detected only in 15 of 58 culture-positive children: smear microscopy performed poorly in both sputum and GLA (table 2). There were 30 smear-positive, culture-negative cases, two of which were Xpert MTB/RIF assay positive, and might represent false negatives with respect to the gold standard. 12 GLA and five sputum specimens produced an indeterminate Xpert MTB/RIF assay result and were repeated. The performance characteristics of the Xpert MTB/RIF assay compared with culture and smear are described in table 2. No significant difference was identified in the sensitivity, specificity, positive predictive value, or negative predictive value of the Xpert MTB/RIF assay between sputum and GLA samples (table 2). Confounded by low numbers, no significant differences in assay performance were noted between HIV-positive and HIV-negative patients for either sample type, even

![Figure 1: Study profile](http://dx.doi.org/10.1016/S1473-3099(12)70245-1)
Within the GLA group, which contained most culture-confirmed cases of tuberculosis.

The Xpert MTB/RIF assay detected an extra 28 cases of tuberculosis (22 GLA and six sputum) compared with smear microscopy. The assay was significantly more sensitive than smear microscopy for both sputum (p=0.01) and GLA (p<0.0001; table 2).

52 of 58 MGIT culture positive samples were analysed with the MGIT drug-susceptibility test (six were contaminated and were excluded), and two of 52 were MDR tuberculosis. The Xpert MTB/RIF assay detected rifampicin resistance in three cases, of which two were culture-confirmed MDR tuberculosis, and the third was culture negative. The assay was 100% (95% CI 19.8–100) sensitive and 97.4% (84.9–99.9) specific for the detection of rifampicin resistance compared with culture (table 2).

M. tuberculosis load in the Xpert MTB/RIF assay was analysed according to age and MGIT culture TTP (a putative marker of bacillary load). Older children seem more likely to have a higher M. tuberculosis load (figure 2).

The median age of children who produced sputum was 119 months (IQR 84–144), which was significantly higher than the age of children from whom GLA was obtained (20 months, 11–40; p<0.0001). Although sample numbers were low, median age did not differ significantly by Xpert MTB/RIF assay load, either overall (p=0.261) or within GLA (p=0.118; data not shown). Assay load did not differ significantly by sample type (p=0.791; data not shown).

The median TTP for cultures was 13.5 days (IQR 11.0–15.5) and it did not differ significantly between sputum and GLA samples (p=0.866). Reduced TTP was associated with higher Xpert MTB/RIF assay load (p=0.071; figure 3).

![Figure 2: Effect of sample type and age on Xpert MTB/RIF assay load](image-url)

Table 2: Specificity and sensitivity of the Xpert MTB/RIF assay and smear microscopy versus culture in sputum and GLA samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sensitivity (smear positive)</th>
<th>Specificity (smear negative)</th>
<th>Sensitivity (culture positive)</th>
<th>Specificity (culture negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>81.8% (95.2–100.0)</td>
<td>99.2% (95.2–100.0)</td>
<td>3/3 (100%)</td>
<td>6/7 (85.7% 42.0–99.2)</td>
</tr>
<tr>
<td>HIV positive</td>
<td>100% (95.2–100.0)</td>
<td>100% (95.2–100.0)</td>
<td>2/2 (100%)</td>
<td>4/4 (100% 39.6–100.0)</td>
</tr>
<tr>
<td>HIV negative</td>
<td>98.8% (92.7–100.0)</td>
<td>98.8% (92.7–100.0)</td>
<td>1/1 (100%)</td>
<td>2/3 (66.7% 12.5–98.2)</td>
</tr>
<tr>
<td>Gastric lavage aspirate</td>
<td>86.8% (71.1–95.1)</td>
<td>98.0% (96.6–99.8)</td>
<td>12/12 (91.7% 97.9–99.6)</td>
<td>22/26 (61.1% 43.5–76.4)</td>
</tr>
<tr>
<td>HIV positive</td>
<td>83.3% (50.9–97.1)</td>
<td>97.3% (94.0–98.9)</td>
<td>5/5 (100%)</td>
<td>5/11 (45.5% 18.1–75.4)</td>
</tr>
<tr>
<td>HIV negative</td>
<td>88.5% (68.7–97.0)</td>
<td>98.3% (96.6–99.2)</td>
<td>6/7 (85.7% 42.0–99.2)</td>
<td>12/24 (70.8% 48.7–86.6)</td>
</tr>
<tr>
<td>Gastric lavage aspirate and sputum combined</td>
<td>92.3% (62.1–99.6)</td>
<td>98.4% (96.7–99.3)</td>
<td>2/2 (100%)</td>
<td>10/17 (58.8% 33.5–80.6)</td>
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<tr>
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<tr>
<td>HIV negative</td>
<td>3/3 (100%)</td>
<td>95.2% (93.4–96.6)</td>
<td>NA</td>
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<td>Smear vs culture</td>
<td>3/10 (30.0% 81.6–84.6)</td>
<td>73/74 (99.3% 98.3–99.8)</td>
<td>NA</td>
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<td>Gastric lavage aspirate</td>
<td>12/18 (66.7% 41.1–85.6)</td>
<td>182/183 (99.4% 96.5–100.0)</td>
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<td>HIV positive</td>
<td>115/118 (97.5% 92.2–99.3)</td>
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<td>HIV negative</td>
<td>152/155 (100% 74.7–100)</td>
<td>121/123 (98.4% 93.7–99.7)</td>
<td>92.3% (62.3–97.9)</td>
<td>96.8% (92.9–98.7)</td>
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<td>Xpert MTB/RIF assay vs culture drug-susceptibility test</td>
<td>All patients</td>
<td>100% (95.2–100)</td>
<td>94.7% (89.0–97.7)</td>
<td>NA</td>
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Data n/N (%; 95% CI) or % (95% CI); *p=0.1649, Fisher’s exact; †p=0.0001, Pearson χ²; ‡p=0.0468, Fisher’s exact.
Discussion

Our study prospectively assesses the Xpert MTB/RIF assay for the rapid diagnosis of childhood tuberculosis using GLA samples in a high tuberculosis and HIV endemic setting. The study has four key findings: the Xpert MTB/RIF assay performs well in GLA samples for rapid and accurate diagnosis of childhood tuberculosis; no significant difference was evident in the accuracy of the assay between sputum and GLA samples, although a larger study would be needed to confirm this finding—ideally with paired sputum and GLA samples; the assay is more sensitive than smear microscopy for the diagnosis of childhood tuberculosis with both sputum and GLA samples; and the assay detected an extra 28 cases of tuberculosis compared with smear microscopy. Our study was done in a single, tertiary referral centre with high tuberculosis and HIV burden; therefore, it cannot be assumed that the accuracy of the Xpert MTB/RIF assay for the detection of childhood pulmonary tuberculosis will be the same in other patient cohorts or settings.

In sub-Saharan Africa and other resource-poor countries, lack of rapid accurate diagnostic tests for childhood tuberculosis and continued reliance on sputum-smear microscopy as a rapid screening test leaves a large proportion of active tuberculosis cases undiagnosed. In resource-rich countries, induced sputum, nasopharyngeal aspirates, GLA, bronchoalveolar lavage fluid, and fine needle aspirate of the lung or thoracic lymph nodes are routinely used for pulmonary tuberculosis screening in children who cannot expectorate sputum to increase diagnostic yield. In some European countries, the Xpert MTB/RIF assay is also being used in clinical practice on an ad-hoc basis for rapid diagnosis of childhood tuberculosis with sputum, GLA, and other biological samples. The assay was specifically developed, optimised, and validated for use on sputum samples from adult patients with pulmonary tuberculosis, and WHO endorsement of the assay in 2010 recommended its use for rapid diagnosis of pulmonary disease with sputum in countries highly endemic for tuberculosis and HIV. Many studies have assessed the Xpert MTB/RIF assay in adults from various geographical regions. So far, three studies from sub-Saharan Africa have assessed the Xpert MTB/RIF assay for the diagnosis of childhood tuberculosis with sputum samples: in South Africa, Nicol and colleagues used induced sputum samples and Zar and colleagues used induced sputum and nasopharyngeal aspirates; in Tanzania, Rachow and colleagues used frozen sputum and induced sputum (panel). Sputum induction increases diagnostic yield but is not practised widely across sub-Saharan Africa because it needs specific training, equipment, consumables, staffing, and infection control with major resource implications. At the University Teaching Hospital in Lusaka, routine screening for pulmonary tuberculosis in children is done via sputum-smear microscopy and culture, and if sputum is not available, a GLA is obtained. We had ethical approval for obtaining only one GLA sample in patients with suspected tuberculosis who were unable to produce a sputum sample. The convention for use of gastric lavage in children is to obtain at least two GLA samples. Thus, in our study, the GLA yield could have been even higher if multiple specimens were obtained from each patient. The use of multiple GLA samples would increase the cost significantly and would be impractical for resource-poor settings.

The proportion of children with culture-positive tuberculosis (58 [6.2%] of 930) confirmed the large tuberculosis load that presents to the University Teaching Hospital in Lusaka. Culture is not infallible—it has sensitivity limitations and takes time to yield a clinically useful result. However, this method remains WHO’s recommended gold standard for diagnosis of active tuberculosis, and performance assessment of any new diagnostic test is universally measured against this standard. Although the Xpert MTB/RIF assay for assessment of GLA and sputum samples compares well with gold standard culture, many children in our study who were negative by all methods received tuberculosis therapy on the basis of a probable or possible tuberculosis diagnosis based on clinical diagnostic algorithms. Autopsy studies by our group have shown that many children with tuberculosis are misdiagnosed or missed when assessed with clinical diagnostic algorithms and gold standard diagnostic tests. The unanswered issues of true positives missed by gold standard culture, accuracy of clinical diagnostic algorithms, and
Panel: Research in context

**Systematic review**
We searched PubMed for articles published up to July 25, 2012, with search terms “Xpert” or “MTB/RIF” and “tuberculosis” and “children”. No language restrictions were used. The search returned three studies in which the Xpert MTB/RIF assay had been assessed in children, all of which were from sub-Saharan Africa (one Tanzanian and two South African).16–18 Two of these studies have been included in a systematic review. The studies were combined with studies of adults in a meta-analysis of accuracy of the Xpert MTB/RIF assay.14

**Interpretation**
All three studies of the Xpert MTB/RIF assay for childhood tuberculosis used induced sputum.16–18 The Tanzanian study made comparisons with non-induced sputum, finding significantly more cases detected with non-induced sputum, but the study did not state sensitivity.18 One of the South African studies showed improved sensitivity with induced sputum (73·6% [64 of 87]) versus nasopharyngeal aspirates (56·3% [49 of 87]; p=0·0171).17 The routine specimen type in our setting for the microbiological diagnosis of childhood tuberculosis, if the child could not expectorate, was gastric lavage aspirate. We showed that analyses of gastric lavage aspirate samples with the Xpert MTB/RIF assay is a sensitive and specific method for rapid diagnosis of pulmonary tuberculosis in children who cannot produce sputum.

Assessment of new tests, which might be better than the gold standard, need to be resolved by incorporating tissue biopsy, autopsy, and large cohort studies.12

The sensitivity of the Xpert MTB/RIF assay did not differ significantly between GLA and sputum samples, although the number of culture-positive sputum samples was low compared with GLA. Older children were more likely to provide a sputum sample but sample type had no significant effect on TTP or Xpert MTB/RIF assay loads, suggesting that bacillary load in both sample types was similar. Reduced TTP seemed to be linked to higher Xpert MTB/RIF assay load, as observed in previous studies;15–18 suggesting that semiquantitative Xpert MTB/RIF assay load could be used as a marker of disease severity, similar to MGIT TTP. HIV-positive children had significantly lower assay load but MGIT-culture TTP was not significantly affected by HIV status.

Combining the data from the three previous assessments of the Xpert MTB/RIF assay for diagnosis of childhood tuberculosis in South Africa17 and Tanzania27 results in a pooled sensitivity of 74·1% (137 of 185) in sputum and induced sputum. In our study, the expectorated sputum samples produced similar results (90·0% vs 74·1%; p=0.2337) as did GLA samples (68·8% vs 74·1%; p=0.4610), although the yield from GLA versus sputum could not be assessed. In this small sample size, with wide confidence limits, we detected no significant difference in the sensitivity of the assay in GLA samples between HIV-positive and HIV-negative children, as previously reported in induced and non-induced sputum from children in Tanzania.28 This finding was contrary to those from South African studies with induced sputum19 and nasopharyngeal aspirate,27 which showed significantly better performance in HIV-positive children. These discrepancies could relate to differences in the clinical characteristics of the study groups. Our recent assessment of the Xpert MTB/RIF assay in adult inpatients at the University Teaching Hospital in Zambia,11 with a very high HIV prevalence (70·9%), showed higher assay sensitivity in HIV-positive patients.

The detection of MDR tuberculosis in Zambian children is consistent with other childhood tuberculosis Xpert MTB/RIF assay assessments from sub-Saharan Africa, which reported prevalence of rifampicin resistance between 0–5%.26–28 MDR tuberculosis was detected in two (3·8%) of 52 samples tested with MGIT culture drug-susceptibility testing, both from GLA samples. The Xpert MTB/RIF assay detected rifampicin resistance in both MDR tuberculosis cases, but also in a third child who was culture negative, representing a false positive result. One of the two confirmed cases of MDR tuberculosis in our study was an HIV-positive 13-year-old child on relapse tuberculosis treatment with negative smear. The case of a rifampicin-resistant false-positive Xpert MTB/RIF assay was an HIV-negative infant with no history of tuberculosis contact. False positive results for rifampicin resistance are well documented29 and the manufacturers of the assay have attempted to resolve this with the latest version (G4).30 Further studies of the use of the Xpert MTB/RIF assay for the rapid diagnosis of MDR tuberculosis are needed because false-positive results could expose children to unnecessary second-line treatment with toxic drugs. Additional confirmatory tests are recommended by WHO after detection of rifampicin resistance with the Xpert MTB/RIF assay.11

Our study shows that testing GLA samples with the Xpert MTB/RIF assay is a useful alternative to sputum, in children who cannot expectorate, for the rapid, sensitive, and accurate diagnosis of tuberculosis in a paediatric inpatient setting. The Xpert MTB/RIF assay is significantly better than smear microscopy and could be used on sputum and GLA. Additional studies are needed to assess the effect of this assay on: the starting of tuberculosis therapy, moving away from empirical treatment; outcome of clinical management; and inpatient duration of stay. Further studies of the cost-effectiveness of the Xpert MTB/RIF assay for the diagnosis of active tuberculosis in children in resource-poor settings are needed before recommendations for implementation can be made. Other assessments should be extended to various respiratory and non-respiratory clinical samples from children with extrapulmonary tuberculosis.29–31 In addition to developing and assessing new tests for
tuberculosis, more accurate, rapid diagnostic platforms for simultaneous detection of many bacterial, viral, and fungal respiratory pathogens are urgently needed for practical and improved management of childhood respiratory infections.

Contributors
AZ, MH, MMa, and Pmw designed and wrote the study protocols. Pmw, MB, JO’G, and AZ coordinated the study. LC, CC, JM, PMu, MC, LM, MMu, JT, and RK recruited patients, undertook laboratory diagnostics, and managed the data. AZ, JO’G, and MB wrote the first and final drafts. All authors contributed to writing of the report.

Conflicts of interest
We declare that we have no conflicts of interest.

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