# Rapid diagnosis of pulmonary tuberculosis in African children in a primary care setting by use of Xpert MTB/RIF on respiratory specimens: a prospective study

*Heather J Zar, Lesley Workman, Washiefa Isaacs, Keertan Dheda, Widaad Zemanay, Mark P Nicol*

## Summary

**Background** In children admitted to hospital, rapid, accurate diagnosis of pulmonary tuberculosis with the Xpert MTB/RIF assay is possible, but no paediatric studies have been done in the primary care setting, where most children are given care, and where microbiological diagnosis is rarely available. We assessed the diagnostic accuracy of Xpert MTB/RIF in children in primary care.

**Methods** For this prospective study, we obtained repeat induced sputum and nasopharyngeal aspirate specimens from children (<15 years) with suspected pulmonary tuberculosis at a clinic in Khayeliwtsha, Cape Town, South Africa. We compared the diagnostic accuracy of Xpert MTB/RIF with a reference standard of culture and smear microscopy on induced sputum specimens. For the main analysis, specificity of Xpert MTB/RIF versus liquid culture, we included only children with two interpretable Xpert MTB/RIF and induced sputum culture results.

**Findings** Between Aug 1, 2010, and July 30, 2012, we enrolled 384 children (median age 38·3 months, IQR 21·2–56·5) who had one paired induced sputum and nasopharyngeal specimen, 309 (81%) of whom had two paired specimens. Five children (1%) tested positive for tuberculosis by smear microscopy, 26 (7%) tested positive by Xpert MTB/RIF, and 30 (8%) tested positive by culture. Xpert MTB/RIF on two induced sputum specimens detected 16 of 28 culture-confirmed cases (sensitivity of 57·1%, 95% CI 39·1–73·5) and on two nasopharyngeal aspirates detected 11 of 28 culture-confirmed cases (sensitivity of 39·3, 23·6–57·6; p=0·18). The specificity of Xpert MTB/RIF on induced sputum was 98·9% (95% CI 96·9–99·6) and on nasopharyngeal aspirates was 99·3% (97·4–99·8).

**Interpretation** Our findings suggest that Xpert MTB/RIF on respiratory secretions is a useful test for rapid diagnosis of paediatric pulmonary tuberculosis in primary care.

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**Department of Paediatrics and Child Health** (Prof H J Zar PhD, L Workman MPH, W Isaacs MSc), **Department of Medicine** (Prof K Dheda PhD), **and Division of Medical Microbiology, Institute for Infectious Diseases, and Molecular Medicine** (W Zemanay PhD, Prof M P Nicol PhD), **University of Cape Town, Cape Town, South Africa; Red Cross War Memorial Children's Hospital, Cape Town, South Africa** Prof H J Zar, L Workman, W Isaacs); **and the National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa** (W Zemanay, Prof M P Nicol)

Correspondence to:
Prof Heather J Zar, Department of Paediatrics and Child Health, 5th Floor, ICH Building, Red Cross War Memorial Children's Hospital Cape Town, South Africa
**heather.zar@uct.ac.za**

## Introduction

Diagnosis of pulmonary tuberculosis in children can be challenging because of non-specific symptoms, signs, and radiological features.[1] Diagnostic uncertainty is compounded by HIV co-infection because tuberculosis can be difficult to distinguish from other HIV-associated diseases. Missed diagnosis of paediatric pulmonary tuberculosis is an important problem, with up to 40% of children admitted to hospital with culture-confirmed tuberculosis being discharged without appropriate treatment because culture confirmation can take several weeks.[2–4] In the community setting, the proportion of missed cases can be even higher because many patients have less severe disease presentation. Rapid microbiological confirmation of tuberculosis and identification of drug resistance is therefore desirable because it enables definitive diagnosis and initiation of appropriate treatment.[5]

The Xpert MTB/RIF assay (Xpert; Cepheid, CA, USA) has enabled rapid diagnosis of pulmonary tuberculosis and detection of resistance to rifampicin in children admitted to hospital. We previously reported that Xpert MTB/RIF on two induced sputum specimens correctly identified 44 (76%) of 58 children in hospital with culture-confirmed disease.[6] Similar results have been reported in hospital-based studies of children with suspected tuberculosis in Uganda and Tanzania using sputum or induced sputum specimens.[7,8] Findings from a Zambian study showed that Xpert MTB/RIF done on gastric lavage specimens in children admitted to hospital had a sensitivity of 65%, whereas in older children the sensitivity on spontaneously produced sputum was 90%.[9] We also reported that Xpert MTB/RIF on two nasopharyngeal aspirates was useful for microbiological confirmation in children in hospital.[10] Although nasopharyngeal aspirates provided a lower culture yield than induced sputum, the sensitivity of two Xpert MTB/RIF tests on nasopharyngeal aspirates was similar to Xpert MTB/RIF on two induced sputum specimens, 71% on induced sputum and 65% on nasopharyngeal aspirate specimens.[10]

Most children with suspected pulmonary tuberculosis present to primary clinics, but microbiological diagnosis

is rarely attempted because of limited capacity and the perception that microbiological diagnosis is neither feasible nor useful in children in primary care. Nevertheless, microbiological investigations might enable definitive diagnosis and improve treatment in primary care, as shown by findings from a South African study in which culture and smear on induced sputum increased diagnostic yield by 22% in a paediatric clinic.[11] Xpert MTB/RIF could offer a feasible option for rapid microbiological diagnosis and detection of rifampicin resistance in children in primary care. Moreover, nasopharyngeal aspirates are an attractive option for use in primary care, where lack of capacity to collect induced sputum specimens has been an obstacle to effective microbiological diagnosis and where gastric lavage can be difficult as an ambulatory procedure. We know of no studies that describe the accuracy of Xpert MTB/RIF in children recruited exclusively from a primary care clinic. We therefore prospectively assessed the accuracy of Xpert MTB/RIF on induced sputum and nasopharyngeal aspirate specimens using culture of induced sputum as a reference standard for diagnosis of pulmonary tuberculosis in children at a primary care clinic.

## Methods

### Study design and participants

For this prospective study, we recruited participants from Nolungile primary care clinic in Khayelitsha, Cape Town, South Africa, an area in which the incidence of tuberculosis is high. We enrolled consecutive children presenting from Aug 1, 2010, to July 30, 2012, with suspected pulmonary tuberculosis. Children younger than 15 years were eligible if they had a cough for more than 14 days and one of the following: a household tuberculosis contact within the previous 3 months, weight loss or failure to gain weight within the previous 3 months, a positive skin test to purified protein derivative (PPD 2TU, PPD RT23; Staten Serum Institute, Copenhagen, Denmark), or a chest radiograph suggestive of pulmonary tuberculosis. A positive skin test was defined as 5 mm or more of transverse induration in children with HIV or 10 mm or more in children without HIV. We excluded children for the following reasons: if they had received tuberculosis treatment or prophylaxis within the previous 72 h, if they could not be followed up, if informed consent was unobtainable, or if an induced sputum and nasopharyngeal aspirate specimen could not be obtained.

A history and physical examination were done at enrolment. Clinical investigations included chest radiography, skin test, and HIV testing in children whose HIV status was unknown (HIV rapid test, followed by a confirmatory PCR for children younger than 18 months or HIV ELISA in older children). HIV-infected children were classified by WHO clinical staging; CD4 count and HIV viral load were recorded and children were classified by the CDC immunological classification. Two independent reviewers, masked to microbiological and other results, reported chest radiographs using a standardised format. Signs suggestive of tuberculosis included airway compression or lymphadenopathy, diffuse miliary pattern, pleural effusion, or cavitary disease.

Tuberculosis treatment was given at the discretion of the treating clinician on the basis of clinical, radiological, and microbiological information. Xpert MTB/RIF results were communicated to the clinic doctor as soon as they were available. Follow-up visits were done at months 1, 3, and 6 for children on tuberculosis treatment and at months 1 and 3 for those untreated. Response to treatment was assessed at follow-up by recording symptoms, signs, weight, and a repeat chest radiograph at completion of treatment.

Written, informed consent was obtained from a parent or legal guardian. The study was approved by the

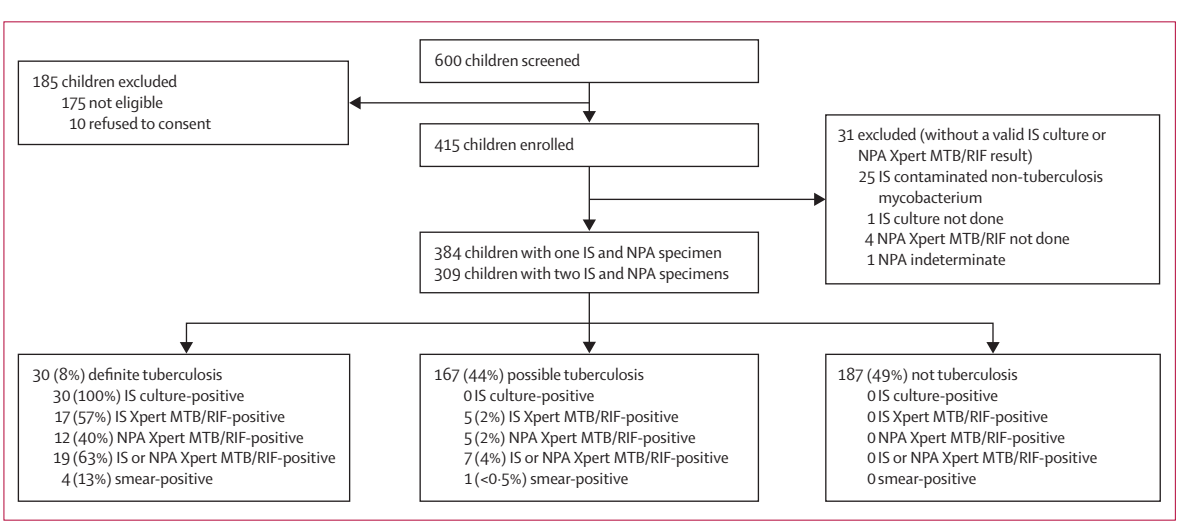


***Figure:*** **Study profile**
IS=induced sputum. NPA=nasopharyngeal aspirate.

Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, and the Provincial Research Ethics Committee of the Western Cape.

### Respiratory specimens

We took two paired induced sputum and nasopharyngeal aspirate specimens at baseline (four specimens in total) and submitted them individually to a central laboratory for Xpert MTB/RIF testing. The induced sputum specimens were also submitted individually for smear and liquid culture because the yield from culture from induced sputum has been shown to be substantially higher than that from nasopharyngeal aspirates.[10]

A nasopharyngeal aspirate was obtained before induced sputum as described elsewhere.[10] Two drops of sterile saline were instilled into each of the patients' nostrils and the nasopharynx was suctioned with a sterile catheter with a mucus trap. A second nasopharyngeal aspirate was obtained the following day or a minimum of 4 h after the first induced sputum procedure.

Sputum induction was done by a trained research nurse at least 30 min after a nasopharyngeal aspirate was obtained, following a 2–3 h fast in a dedicated room, as described elsewhere.[11,12] A second induced sputum specimen was obtained, whenever possible, the following day or a minimum of 4 h after the first specimen. We did baseline arterial pulse oximetry and monitoring during the induced sputum procedure and for 30 min thereafter. Caregivers were reimbursed for transport costs for a second visit, when the second paired induced sputum and nasopharyngeal aspirate specimens were taken the following day.

### Laboratory assays

Nasopharyngeal aspirate and induced sputum specimens were transported within 2 h of collection to an accredited centralised laboratory and processed individually using standardised protocols by trained laboratory technicians. For both specimens, after decontamination with N-acetyl-L-cysteine and sodium hydroxide (1% final concentration), centrifuged deposits were resuspended in 1·5 mL of phosphate buffer. A drop of induced sputum sediment was used for fluorescent acid-fast smear microscopy. For Xpert MTB/RIF, 1·4 mL of specimen reagent was added to 0·7 mL of resuspended sputum or nasopharyngeal aspirate pellet and processed as reported elswhere.[6] Automated liquid culture (BACTEC MGIT; Becton Dickinson, MD, USA) was done using 0·5 mL of resuspended pellet on sputum specimens. Cultures were incubated for up to 6 weeks. Positive cultures were identified by acid-fast staining followed by MTBDR*plus* testing (Hain Lifesciences, Hehren, Germany) to confirm the presence of *Mycobacterium tuberculosis* and rifampicin and isoniazid resistance.[13] Because culture results were

| | Overall (N=384) | Definite tuberculosis (N=30) | Possible tuberculosis (N=167) | Not tuberculosis (N=187) | Odds ratio (95% CI) |
|---|---|---|---|---|---|
| Age in months (median [IQR]) | 38·3 (21·2 to 56·5) | 39·8 (26·8 to 56·5) | 37·2 (22·0 to 55·0) | 40·1 (19·8 to 59·4) | 1·00 (0·99 to 1·02) |
| Sex (male) | 181 (47%) | 17 (57%) | 81 (41%) | 83 (45%) | 1·5 (0·71 to 3·21) |
| HIV infection | 31 (8%) | 5 (16%) | 14 (8%) | 12 (6%) | 2·5 (0·9 to 7·1) |
| WHO HIV clinical staging | | | | | 4·86 (1·62 to 14·58) |
| Stage 1 | 11 (36%) | 0 | 0 | 11 (92%) | .. |
| Stage 2 | 1 (3%) | 0 | 1 (7%) | 0 | .. |
| Stage 3 | 19 (61%) | 5 (100%) | 13 (93%) | 1 (8%) | .. |
| Stage 4 | 0 | 0 | 0 | 0 | .. |
| HIV CDC immune category | | | | | 4·03 (0·41 to 40·03) |
| Mild | 13 (46%) | 4 (67%) | 5 (42%) | 4 (40%) | .. |
| Moderate | 11 (40%) | 1 (17%) | 4 (33%) | 6 (60%) | .. |
| Severe | 4 (14%) | 1 (17%) | 3 (25%) | 0 | .. |
| History of tuberculosis | 42 (11%) | 3 (9%) | 15 (9%) | 24 (13%) | 0·94 (0·13 to 2·48) |
| Radiological changes suggestive of tuberculosis* | 314 (82%) | 26 (87%) | 145 (87%) | 143 (77%) | 1·49 (0·50 to 4·41) |
| Started on tuberculosis treatment | 180 (47%) | 28 (93%) | 152 (91%) | 0 | 17·5 (4·10 to 74·63) |
| *Z* scores (median [IQR]) | | | | | |
| HAZ | –0·8 (–1·7 to 0·1) | –0·3 (–1·1 to 0·4) | –0·8 (–1·7 to 0·1) | –0·9 (–1·7 to 0·1) | 1·39 (1·03 to 1·87) |
| WAZ | –0·5 (–1·3 to 0·4) | 0·0 (–1·2 to 0·9) | –0·7 (–1·6 to 0·3) | –0·4 (–1·3 to 0·3) | 1·28 (0·99 to 1·64) |
| WHZ | 0·2 (–0·9 to 1·1) | 0·3 (–0·6 to 1·9) | 0·1 (–1·2 to 0·8) | 0·3 (–0·5 to 1·2) | 1·12 (0·87 to 1·46) |
| Malnutrition† (a WAZ score less than –2) | 50 (13%) | 5 (13%) | 25 (15%) | 21 (11%) | 0·94 (0·31 to 2·79) |
| Tuberculin skin positive‡ | 259 (69%) | 28 (83%) | 121 (73%) | 113 (63%) | 2·39 (0·89 to 6·42) |

Data are median n (%) unless otherwise stated. CDC=US Centers for Disease Control and Prevention. HAZ=height for age *Z* score. WAZ=weight for age *Z* score. WHZ=weight for height *Z* score. *n=304. †n=370. ‡n=376.

***Table 1:*** **Baseline characteristics**

| | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| Xpert MTB/RIF (induced sputum) | 13/30; 43·3% (27·4–60·8) | 351/354; 99·2% (97·5–99·7) | 81·3 | 95·4 |
| Xpert MTB/RIF (nasopharyngeal aspirate) | 9/30; 30·0% (16·7–47·9) | 350/354; 98·9% (97·1–99·6) | 69·2 | 94·3 |
| Xpert MTB/RIF (induced sputum or nasopharyngeal aspirate) | 19/30; 63·3% (45·5–78·1) | 347/354; 98·0% (96·0–99·0) | 73·1 | 96·9 |
| Smear microscopy (induced sputum) | 4/30; 13·3% (5·3–29·7) | 353/354; 99·7% (98·4–100·0) | 80·0 | 93·1 |

Data are n/N; % (95% CI), unless otherwise stated. PPV=positive predictive value. NPV=negative predictive value.

***Table 2:*** **Accuracy of Xpert MTB/RIF compared with liquid culture on induced sputum in children with at least one sputum specimen and one nasopharyngeal aspirate specimen (N=384)**

| | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| Xpert MTB/RIF (first IS specimen) | 12/28; 42·9% (26·5–60·9) | 280/281; 99·6% (98·0–99·9) | 92·3 | 94·6 |
| Xpert MTB/RIF (both IS specimens) | 16/28; 57·1% (39·1–73·5) | 278/281; 98·9% (96·9–99·6) | 84·2 | 95·9 |
| Xpert MTB/RIF (first NPA specimen) | 8/28; 28·6% (15·3–47·1) | 280/281; 99·6% (98·0–99·9) | 88·9 | 93·3 |
| Xpert MTB/RIF (both NPA specimens) | 11/28; 39·3% (23·6–57·6) | 279/281; 99·3% (97·4–99·8) | 84·6 | 94·3 |
| Xpert MTB/RIF (first IS or NPA specimen) | 14/28; 50·0% (32·6–67·4) | 279/281; 99·3% (97·4–99·8) | 87·5 | 95·2 |
| Xpert MTB/RIF (both IS or NPA specimens) | 18/28; 64·3% (45·8–79·3) | 277/281; 98·6% (96·4–99·4) | 81·8 | 96·5 |
| Smear microscopy | 3/28; 10·7% (3·7–27·2) | 280/281; 99·6% (98·0–99·9) | 75·0 | 99·6 |

Data are n/N; % (95% CI) unless otherwise stated.IS=induced sputum. NPA=nasopharyngeal aspirate. PPV=positive predictive value. NPV=negative predictive value

***Table 3:*** **Accuracy of Xpert MTB/RIF and smear compared with liquid culture in children with two paired induced sputum and nasopharyngeal aspirate specimens (N=309)**

not available at the time of Xpert MTB/RIF testing, staff doing and recording Xpert MTB/RIF tests were masked to culture results. If Xpert MTB/RIF identified rifampicin resistance, the corresponding cultured isolate also underwent phenotypic resistance testing by automated liquid MGIT culture.

### Statistical analysis

We assigned children to one of three categories on the basis of clinical and microbiological investigations: definite tuberculosis (any induced sputum culture positive for *M tuberculosis*), not tuberculosis (culture-negative and documented resolution of symptoms and signs at a follow-up visit at 3 months in children who did not receive tuberculosis treatment), or possible tuberculosis (all other children).

The primary reference standard was a positive culture for *M tuberculosis* on induced sputum. We analysed separately patients with at least one paired induced sputum and nasopharyngeal aspirate specimens from those with two paired specimens. For the primary analysis of the specificity of Xpert MTB/RIF we included only children with two interpretable Xpert MTB/RIF and induced sputum culture results, because a single negative culture result is likely to miss a substantial proportion of culture-confirmed cases. We calculated the sensitivity, specificity, and predictive values of the assays with 95% CIs. We used simple descriptive statistics to characterise the study population and summarised normally distributed continuous data as means with 95% CIs and non-normally distributed continuous data as medians with IQRs. We summarised categorical data as proportions with 95% CIs. Statistical tests included two-group test of proportions, univariate logistic regression, and Wilcoxon rank-sum test. Statistical tests were two-sided with an $\alpha$ of 0·05.

We used EpiInfo (version 6) for calculation of nutritional indices and STATA (version 10) for all other statistical analyses.

### Role of the funding source

The sponsors 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

We enrolled 415 children, 384 (93%) of whom had at least one induced sputum specimen and one nasopharyngeal aspirate (figure). Table 1 shows characteristics of participants at baseline. Although few children had severe malnutrition, many had mild nutritional impairment (table 1). 22 (71%) of the 31 children with HIV were on highly active antiretroviral therapy (HAART).

Of the 384 children with at least one induced sputum and nasopharyngeal aspirate specimen, five (1%) had a positive smear, 26 (7%) had a positive Xpert MTB/RIF, and 30 (8%) had a positive culture result (figure). There were 30 (8%) children with definite tuberculosis, 167 (44%) with possible tuberculosis, and 187 (49%) without tuberculosis. Excluding children classified as not having tuberculosis, 30 (15%) of 197 children had culture-confirmed diseases, of whom 19 (63%) were Xpert-MTB/RIF-positive on a respiratory specimen. 180 children were started on tuberculosis treatment, representing 47% of the total sample and 91% of those with definite or possible tuberculosis. The median time from presentation at clinic to treatment initiation was 2 days (IQR 0–9). In children with definite tuberculosis, five (16%) were HIV-positive and 25 (7%) were HIV-negative (odds ratio 2·5, 95% CI 0·86–7·14). 19 HIV-positive

children (61%) and 161 (46%) HIV-negative children were started on tuberculosis treatment (p=0·09). No child was admitted to hospital.

19 of 30 (63%) children with definite tuberculosis had at least one positive MTB/RIF on induced sputum or nasopharyngeal aspirate, as did seven of 167 (4%) children with possible tuberculosis and none of 187 children without tuberculosis (figure).

Table 2 shows the accuracy of Xpert MTB/RIF on respiratory specimens in children with at least one paired induced sputum and nasopharyngeal aspirate. In children with definite tuberculosis, there was at least one positive Xpert MTB/RIF result in 13 (43%) of 30 induced sputum specimens and in nine (30%) of 30 nasopharyngeal aspirates. In those with possible tuberculosis, a positive Xpert MTB/RIF occurred in three (2%) of 167 induced sputum specimens and in four (2%) of 167 nasopharyngeal specimens. We recorded no positive Xpert MTB/RIF in the not tuberculosis group. The sensitivity of smear on induced sputum specimens (four of 30 specimens; 13%, 95% CI 5–30) was lower than Xpert MTB/RIF (19 of 30 specimens; 63%, 46–78; p<0·0001). Xpert MTB/RIF on respiratory specimens detected all four smear-positive cases (100%, 51–100) and 15 of 26 smear-negative cases (58%, 39–75). The median time from taking respiratory specimens to communicating an Xpert MTB/RIF result to staff at the primary care site was 1 day. This was possible due to the efficient transfer of specimens to the laboratory, real-time performance of Xpert MTB/RIF, and electronic communication between the lab and study staff at the site.

309 children (81%) had a valid second induced sputum and nasopharyngeal aspirate specimen, 28 (9%) of whom had culture-confirmed disease. Two paired induced sputum and nasopharyngeal aspirate specimens were obtained on the same day in 148 (48%) of the 309 children, while the rest had a second paired specimen the following day. A single Xpert MTB/RIF test on induced sputum or nasopharyngeal aspirate detected half of cases (table 3), including all three smear-positive cases (100%, 44–100) and 11 of 25 smear-negative cases (44%, 27–63). The sensitivity of two Xpert MTB/RIF tests on induced sputum specimens (16 of 28; 57%, 39–75) was higher but not significantly different to that on two nasopharyngeal aspirates (11 of 28; 39%, 24–58; p=0·18; table 3). Xpert MTB/RIF was more than five times as sensitive as smear microscopy on induced sputum specimens (table 3).

A second induced sputum increased the detection rate for Xpert MTB/RIF by 33% (from 12 cases to 16 cases; table 3); the incremental yield of a second induced sputum culture was four cases (a 17% increase), from 24 cases to 28 cases (data not shown). A second nasopharyngeal aspirate increased the sensitivity of Xpert MTB/RIF by three cases (38%), from eight cases to 11 cases (table 3).

Seven children classified as having possible tuberculosis had a positive Xpert MTB/RIF and negative culture results (figure). Three of these seven children had a single negative culture and four had two negative cultures; all were treated for pulmonary tuberculosis and had a good clinical response.

When results for rifampicin susceptibility testing were available from culture (followed by MTBDR*plus*) and Xpert MTB/RIF, all were susceptible by Xpert MTB/RIF and MTBDR*plus*. On a per specimen analysis, the sensitivity and specificity of Xpert MTB/RIF for rifampicin resistance were each 100%. Xpert MTB/RIF provided faster results (time from obtaining specimen to reporting to clinician) than culture (median 1 day [IQR 1–1] *vs* 16 days [13–19]; p=0·0001). The number of Xpert MTB/RIF tests recorded as failures or invalid (12 of 1754 [<0·5%]) was smaller than the number of cultures recorded as contaminated (58 of 1128 [1%]; p=0·0001).

## Discussion

In this study, Xpert MTB/RIF on respiratory specimens detected within a day almost two-thirds of children with culture-confirmed tuberculosis who were not admitted to hospital. Xpert MTB/RIF was much more sensitive than smear microscopy, detecting almost five times the number of pulmonary tuberculosis cases. Furthermore, Xpert MTB/RIF was useful for diagnosis of tuberculosis in both HIV-infected and HIV-uninfected children. Xpert MTB/RIF identified an additional group of children in the possible-tuberculosis group who probably had tuberculosis based on clinical features and response to therapy but with negative cultures. Assuming that these were true positive cases, the overall yield of culture (30 cases) and Xpert MTB/RIF (26 cases) in this cohort were similar. This finding suggests that MTB/RIF can identify additional cases over culture in children with milder illness and paucibacillary disease.

This is the first paediatric study to show the usefulness of Xpert MTB/RIF in primary care settings, enabling timely treatment of children with appropriate drugs. Our findings also show the feasibility of obtaining respiratory specimens in primary care even in young children, as indicated by the high success rate for induced sputum and nasopharyngeal aspirate procedures. This finding is consistent with our previous report in which induced sputum was successfully obtained in almost all children (269 of 270) with suspected pulmonary tuberculosis at another clinic.[11]

The culture positivity rate in this study was lower than those in previous hospital-based studies;[6,10,12] but similar to that in a paediatric study of induced sputum at another tuberculosis clinic in the same area.[11] Children presenting in primary care are less ill than hospitalised populations, as indicated by the need for ambulatory treatment only, in all children in the present study. Also, unless Xpert MTB/RIF is done at the point of care, operational issues such as transport of specimens can be more challenging in primary care than they are in hospitals.

The sensitivity of Xpert MTB/RIF was also lower than that reported in studies of children admitted to hospital

*Panel:* **Research in context**

**Systematic review**

When planning the study, we searched PubMed for articles published in English up to Dec 31, 2009, using the search terms "child", "tuberculosis", "diagnosis", "Xpert", and "MTB/RIF". We identified no articles. For the writing of the paper, we repeated this search with an additional term "primary care" for articles published up until March 30, 2013. We identified five hospital-based studies of use of Xpert for diagnosis of childhood tuberculosis, but there were no studies done in a primary care setting.

**Interpretation**

This study provides the first evidence that Xpert MTB/RIF on respiratory specimens (induced sputum or nasopharyngeal aspirates) can be useful for rapid diagnosis of pulmonary tuberculosis in children in a primary care setting. Our findings show that the yield from culture or Xpert MTB/RIF was lower than that reported for children in hospital, suggesting a lower bacillary load associated with less severe illness. Consistent with hospital studies, repeated specimens for Xpert MTB/RIF resulted in a higher diagnostic yield. Xpert MTB/RIF was much more sensitive than was smear microscopy, and results were available faster than they were for culture. The previous evidence base suggested that Xpert MTB/RIF done on respiratory specimens is effective for diagnosis of pulmonary tuberculosis in children admitted to hospital; this study suggests that use of Xpert MTB/RIF is also feasible and useful in primary care settings.

in South Africa, Zambia, Uganda, and Tanzania, with sensitivities of 72–90% on sputum specimens.[6–10] Findings from a Zambian study showed a sensitivity of 90% on spontaneously produced sputum, but children were older than were those in the present study, and a higher proportion (51%) had HIV.[9] In younger children, the sensitivity of Xpert MTB/RIF on gastric lavage was reported as 65%, which was similar to our finding for tests done on induced sputum (57%).[9] Findings from a hospital-based study of Ugandan children showed that Xpert MTB/RIF on induced sputum detected 79% of culture-confirmed cases.[8] An age of greater than 5 years was independently associated with a positive Xpert MTB/RIF; almost a third were older than 5 years, by contrast with the present study in which the median age was 38 months.

Children with less severe illness might have a lower bacillary load, further accounting for the lower sensitivity of Xpert MTB/RIF in ambulatory settings. This notion is lent support by the very small number of smear-positive cases in the present study, which was also lower than that seen in children admitted to hospital.[6,10] The sensitivity of Xpert MTB/RIF was higher in smear-positive than it was in smear-negative cases, as has also been shown in children in hospital.[6,7,10] Findings from a Cochrane review of Xpert MTB/RIF in adults with suspected tuberculosis showed a similar pooled sensitivity of 68% for smear-negative cases.[14] Therefore, the sensitivity of Xpert MTB/RIF in this study, in which most children had negative smear microscopy results, is consistent with that reported in both adults and children with smear-negative disease. Development of a more sensitive PCR diagnostic test than Xpert MTB/RIF, which has a limit of detection of 130 colony-forming units per mL,[15] could be especially useful for the detection of less severe cases of pulmonary tuberculosis in children.

The sensitivity of Xpert MTB/RIF on induced sputum was higher but not significantly different to that on nasopharyngeal aspirates. This finding is consistent with results from a study done in children in hospital,[10] in which culture yield was substantially higher from induced sputum and the yield from induced sputum Xpert MTB/RIF was higher but not significantly different from nasopharyngeal aspirates. These findings are lent support by paediatric studies done in Uganda, Yemen, and Peru, reporting nasopharyngeal aspirates to be useful for diagnosis using culture or in-house PCR methods.[16–19] The higher yield from Xpert MTB/RIF on induced sputum than on nasopharyngeal aspirates could, however, be clinically significant; further study with a larger sample size is needed to address this. Overall, our results suggest that induced sputum specimens are preferable to nasopharyngeal aspirate specimens. In a previous study at another tuberculosis clinic in this area, nursing staff rated induced sputum specimens as easy or very easy to obtain from most children.[11] Induced sputum requires nebulisation with hypertonic saline and suctioning—hypertonic saline nebulisation is now recommended for children with bronchiolitis,[20] and suctioning is routinely done by nurses in primary care. Therefore, more widespread use of induced sputum should be considered in primary care. Infection control procedures such as use of protective masks should be implemented to prevent transmission. However, obtaining induced sputum specimens might not be possible in many primary care clinics in high-tuberculosis, low-resource settings in view of the need for trained health-care workers, oxygen, and electricity to do this procedure. When induced sputum is unobtainable, nasopharyngeal aspirate samples remain useful for Xpert MTB/RIF, especially because they are easily obtained in primary care.

Consistent with findings from our previous studies,[6,10,12] the yield from both culture and Xpert MTB/RIF increased substantially with a second specimen. Therefore, two specimens (whether induced sputum or nasopharyngeal aspirate) should be recommended in children. These results are also consistent with a Tanzanian study that reported an incremental yield with repeated induced sputum in children whether culture, Xpert MTB/RIF, or a combination were used.[7] Further study of the cost-

effectiveness of repeated specimens is needed in children. However, findings from a cost-effectiveness analysis in adults with tuberculosis[21] indicate that replacement of smear microscopy with Xpert MTB/RIF is highly cost effective.[21] Our study adds to the body of evidence that lends support to WHO recommendations that Xpert MTB/RIF replace smear as the initial diagnostic test in suspected HIV-associated tuberculosis or multidrug-resistant tuberculosis, and as a follow-on test in settings where these types of tuberculosis are less of a concern.[22] However, unlike the adult recommendations for a single Xpert MTB/RIF on one sputum sample, these results indicate the need for repeated specimens in children. A repeat visit to collect a second specimen was needed in slightly more than half of children. Such repeat visits would have a negative implication on patient and clinic resources. However, two visits are the standard of care for children with suspected pulmonary tuberculosis because interpretation of skin test results needs a second visit within 48–72 h of placement. A second induced sputum specimen or nasopharyngeal aspirate could therefore be timed to coincide with this visit.

Limitations of this study include the small number of HIV-infected children. Nevertheless, pulmonary tuberculosis was prevalent in HIV-infected children, with 16% having culture-confirmed disease and more than 60% having a clinical diagnosis. Moreover, this clinic is a typical primary care facility situated in a low-income area of South Africa. A further limitation is the absence of on-site testing for Xpert MTB/RIF, which was not done as a point-of-care test. Operational challenges in scaling-up use of Xpert MTB/RIF and in establishing it as a point-of-care test might limit its usefulness.

This study also draws attention to the limitations of the available methods for confirmation of pulmonary tuberculosis in children, especially in primary care settings. Only 17% of children treated for pulmonary tuberculosis were culture-positive, showing the limitations of using culture confirmation as the reference standard for diagnosis. Cases in which children tested Xpert MTB/RIF-positive but culture-negative were probably true cases of pulmonary tuberculosis, because all the children responded to tuberculosis treatment. However, only a few children (4% of the study population) were in this group. Therefore, better diagnostic tests are needed to reliably diagnose children with pulmonary tuberculosis. The assessment of new diagnostics in children is, however, challenging in view of the low sensitivity of culture as the reference standard.

#### Contributors

HJZ and MPN had the idea for the study, obtained funding, and supervised the study. MPN and WZ co-ordinated and oversaw microbiological testing. HJZ led the writing of the paper. LW was responsible for data management and analysis. WI was responsible for clinical oversight of the study. KD provided expert advice and obtained funding for the study.

#### Conflicts of interest

MPN has received funding from the Foundation for Innovative New Diagnostics (Geneva, Switzerland) for studies to assess the performance and effect of Xpert MTB/RIF. All other sauthors declare that they have no conflicts of interest.

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