Diagnosing childhood pulmonary tuberculosis using a single sputum specimen on Xpert MTB/RIF at point of care

N Gous,1 MSc Med; L E Scott,1 PhD; S Khan,1 G Reubenson,7 MB BCh, FCPaed, DCH, DTM&H; A Coovadia,2 MB BCh, FCPaed, DCH, Dip HIV Man; W Stevens,1,3 MB BCh, MMed (Haem), FCPath (Haem)

1 Department of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
2 Rahima Moosa Mother and Child Hospital and Department of Paediatrics and Child Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
3 National Health Laboratory Service, Johannesburg, South Africa, and National Priority Programme, South Africa

Corresponding author: N Gous (natasha.gous@gmail.com)

Background. The GeneXpert MTB/RIF (Cepheid, USA) (Xpert) has proved successful for pulmonary tuberculosis (TB) diagnosis on decontaminated/concentrated induced sputum specimens from children. Capacity to perform induction in many settings is limited.

Objectives. To assess: (i) volumes of ‘routinely obtained’ sputum in a district-level academic hospital; (ii) whether sputum specimens not meeting Xpert-required testing volumes could still be tested; and (iii) performance of Xpert on a single paediatric sputum specimen at point of care (POC).

Methods. Two sputa were collected from paediatric TB suspects (≤14 years) at Rahima Moosa Mother and Child Hospital, Johannesburg, South Africa. One specimen was weighed at POC; if the volume was ≥0.1 mL but <0.5 mL, it was increased to 0.5 mL using saline. On-site Xpert testing (G3 cartridge) was performed by a dedicated laboratory technician. The second specimen was referred for TB smear microscopy and culture as per standard of care (SOC).

Results. A total of 484 patients presumed to have TB (median age 24 months) were eligible for this study, performed between June 2011 and May 2012. Xpert could not be used on 4.1% of specimens because of volumes <0.1 mL, and 62.8% required addition of saline prior to Xpert testing. Xpert generated a 2.2% error and 3.7% invalid rate, compared with the SOC that rejected 2.3% because of insufficient volume and 2.3% that were contaminated. The diagnostic performance compared with culture was 62.5% (95% confidence interval (CI) 24.7 - 91) and 99.1% (95% CI 97.4 - 99.8) sensitivity and specificity, respectively, for Xpert (n=345) and 33.3% (7.9 - 69.9) and 99.5% (98.1 - 99.9) sensitivity and specificity, respectively, for smear microscopy (n=374).

Conclusions. Up to 67% of ‘routinely obtained’ sputum specimens from children (≤14 years) are below the required volume for Xpert testing but can be ‘topped up’ with saline. Xpert MTB/RIF performed better than microscopy and generated clinically relevant, timeous results, but sensitivity did not reach the same levels as culture in children.


Additional challenges are often the poor quality[29] and low quantity of specimen yield that hamper bacteriological confirmation of disease.[30] It is for this reason that no universal diagnostic algorithm for TB in children exists, diagnosis relying on a combination of clinical signs and nonspecific tests.[31] Clearly, a stronger emphasis needs to be placed on advancing the research and development of more effective diagnostic strategies for childhood TB detection.

Over half a million children are diagnosed with tuberculosis (TB) every year. The majority of these infections occur in 22 high-burden countries[32] and account for 6% of the global TB burden.[33] The World Health Organization (WHO) reported a staggering 74 000 deaths in HIV-negative children <15 years of age in 2012 alone, making TB one of the top ten leading causes of death in children.[40]

Control programmes are hampered by the increasing spread of multidrug-resistant TB (MDR-TB); globally 40 000 new cases of MDR-TB are reported annually in paediatric populations.[33] In South Africa (SA), children aged ≤14 years are estimated to account for approximately 15 - 20% of the total TB disease burden.[43]

These global statistics may grossly underestimate the TB burden,[35] as microbiological confirmation of TB is challenging in children. Typically, the type of specimen obtained for testing depends on the age of the child and the clinical presentation.[46] Specimens other than sputum most frequently include nasopharyngeal aspirates (NPAs), gastric aspirates, induced sputum, throat swabs, fine-needle lymph node aspiration, bone marrow, urine, stool and aseptic fluids (ascitic, pleural and cerebrospinal fluid). With the exception of the bone marrow and lymph node, owing to the paucibacillary nature of the specimens, multiple sampling over several days is required.[49]
studies are limited and most have focused on induced sputa, where sensitivities ranging from 33.3% to 70% in smear-negative, culture-positive paediatric patients are reported. Increased sensitivities of up to 75.9% could be achieved if two induced sputa were tested. Further studies are now concentrating on alternative specimen types such as NPAs, gastric lavage aspirates, bronchoalveolar lavage and stool.

In all Xpert studies on paediatric populations, specimens underwent laboratory decontamination and concentration prior to Xpert testing. The aim of this study was threefold: (i) to assess the volume of ‘routinely obtained’ sputum (i.e. no induced sputum facility available) from children ≤14 years of age at a district-level academic hospital; (ii) to determine whether sputum specimens not meeting the Xpert minimum required testing volume could be manipulated before testing; and (iii) to determine the feasibility of performing Xpert MTB/RIF on raw paediatric sputum specimens by a dedicated staff member, at the point of care (POC).

Methods

Patient recruitment

Paediatric patients accessing care at the Rahima Moosa Mother and Child Hospital (RMMCH), Johannesburg, SA, were eligible for inclusion if their treating clinician suspected TB. RMMCH is a district-level academic hospital that provides paediatric care to the population of the west of Johannesburg. The study received approval from the University of the Witwatersrand Human Ethics Committee (Protocol number: M110496).

Study procedures

At the time of the study, the routine procedure at RMMCH was to collect two sputum specimens; one specimen was sent to the NHLS Braamfontein laboratory for liquid culture (MGIT) and drug susceptibility testing (DST) when necessary. A GeneXpert instrument was placed on site in an existing POC laboratory designated for sample processing (specimen sorting, centrifugation, rapid HIV testing, data capture) at RMMCH. This allowed specimens to be tested on site.

Specimen collection

A physiotherapist collected two sputum specimens concurrently, using clinic standard of care methodology, in standard sputum collection containers from children ≤14 years of age who were suspected of having TB. Respiratory samples were obtained following administration of chest physiotherapy (at the discretion of the individual physiotherapist). One of these specimens was randomly selected for on-site Xpert testing (G3 version cartridge) by a trained laboratory technician (to replace the first smear) and the second specimen was sent to the NHLS Braamfontein TB laboratory as per routine, for decontamination and concentration (NALC-NaOH), smear microscopy, MGIT culture and DST, when indicated.

Xpert MTB/RIF testing

Specimens were weighed (in their containers) on a bench-top precision balance (Sigma-Aldrich, USA) to estimate the volume of the specimen. If any specimen was >0.1 mL but less than the minimum required volume of 0.5 mL (estimated at 0.5 g), sterile saline was added using a standard Finnpipette (Thermo Fisher Scientific, USA) and sterile pipette tips to increase the volume to at least 0.5 mL. Testing of specimens on the Xpert MTB/RIF assay was performed on the same day as specimen collection. However, if any specimen could not be tested the same day as a result of late specimen receipt, it was stored in a 4°C fridge overnight, followed by testing the next day as per manufacturer’s instructions. All specimens were processed as per standard Xpert MTB/RIF protocol by addition of Sample Reagent (SR) buffer in a 3:1 or 2:1 ratio to take the final volume to 2 mL (Cepheid, USA). The on-site result obtained from the Xpert MTB/RIF assay was reported to the treating clinicians.

Statistical analysis

Specimen volumes for Xpert testing were described. Non-parametric tests were used to determine associations between age and volume, Xpert results and volume, and age and pre-processing, at 95% confidence interval (CI). The sensitivity and specificity and positive and negative predictive values (PPV and NPV) were calculated for the Xpert MTB/RIF assay and smear microscopy with a 95% CI using MGIT culture as the gold standard.

Results

Data summary

A flow diagram illustrates the processing of patient samples (Fig. 1). A total of 484 eligible children (median age 24 months) had samples processed between June 2011 and May 2012 (interquartile range (IQR) 12 - 60). Two sputum specimens were collected by physiotherapy from 484 patients (none required nebulisation); one sample was sent for on-site Xpert testing and the other was sent for routine laboratory testing. The laboratory rejection rate due to insufficient volume for smear and culture processing was 2.3% (11/484). Culture reported a 2.3% (11/473) contamination rate, which was excluded from the quantitative analysis (Fig. 1). After study commencement, culture and smear results for 86 patients could not be retrieved from the laboratory information system and were therefore excluded from final quantitative analysis.

### Fig. 1. Flow chart of specimen analysis.
Diagnostic performance analysis

Sputum specimen volumes received from children for Xpert testing are shown in Table 1. A total of 20/484 specimens (4.1%) had sputum volumes of <0.1 mL and could not be tested. Of the 464 Xpert tests performed on site, overall error and invalid rates of 2.2% (10/464) (1 volume error and 9 code 5011 errors) and 3.7% (17/464), respectively, were reported (Fig. 1).

Using Spearman’s correlation coefficient (owing to skewness of the data), an overall weak correlation was observed between age and volume of specimens received (rho=0.49). No association was observed between the volume of specimen received and the Xpert result (p=0.14). However, an association was observed between age and Xpert results (p=0.004), with more TB-positives detected in the older age group (median age 132 months).

While prior specimen manipulation by addition of sterile saline before Xpert testing did not affect the error/invalid rate (p=0.19) reported on the Xpert, more positive results were generated from specimens that did not require the addition of saline; however, this did not reach significance (p=0.18).

Table 2 further categorises the specimens by age and volume and details the performance by Xpert compared with liquid culture as the reference. Sputum volume clearly increased with age and it appears that, on average, specimens from children aged ≥25 months would require less addition of saline prior to Xpert testing. The minimum age group in which MTB was detected by Xpert was 13 months, with total reported MTB positivity of 2.7% (12/437).

Smear microscopy also only detected MTB from 13 months onwards, reporting 7/389 positive specimens (1.8%) (Table 3). MGIT culture initially reported a positivity rate of 4.0% (15/376); however, after follow-up DST in all culture-positive specimens using the Hain MTBDrplus version 1 assay (Hain LifeScience, GmbH, Germany), only 9/15 were confirmed as MTB complex (2.4%) (Table 3). The other six specimens were probably non-tuberculosis mycobacteria, but were not definitively identified. Culture remained most sensitive in detecting MTB in the lower age group, detecting 1 positive in each age group <24 months and 6 positives in the >60-month category.

Performance data for MGIT culture, smear microscopy and the Xpert MTB/RIF are shown in Table 3.

Smear microscopy was able to identify 3 of the 9 culture-confirmed MTB-positive cases but additionally reported 2 culture-negative (and Xpert-negative) specimens as positive. Compared with culture on 374 specimens, smear generated a sensitivity and specificity of 33.3% (95% CI 7.9 - 69.9) and 99.5% (95% CI 98.1 - 99.9), respectively. Relative to smear, Xpert detected 3 additional MTB-positive cases.

Of the 12 Xpert-positive cases reported, corresponding culture results could not be found in 4 cases. Xpert detected 5/9 culture-confirmed MTB-positive specimens and an additional 3 cases. Analysis of 345 patients with both Xpert and culture results demonstrated an Xpert sensitivity of 62.5% (95% CI 24.7 - 91) and high specificity of 99%. Overall the Xpert was able to detect 66.7% of smear-positive, culture-positive cases (2/3) and 50% of smear-negative, culture-positive cases (3/6).

The Xpert MTB/RIF reported only a single specimen (8.3%, 1/12) with rifampicin resistance, but the MTBDrplus v1 reported rifampicin and isoniazid susceptibility in this specimen. Two additional specimens were reported mono-isoniazid-resistant by MTBDrplus v1.

Discussion

Moving the Xpert MTB/RIF assay to the POC has been shown to be feasible, to save
A trained healthcare worker was required to perform the induction of sputum specimens received from children. The rate of salivary contamination was relatively low, at 2.3%, but some specimens required additional processing. The study found that a larger number of MTB-positive cases were identified using Xpert than smear microscopy or MGIT culture, with sensitivity and specificity values of 62.5% and 99.5%, respectively. However, the performance varied among clinics and centres.

The study also evaluated the costs associated with Xpert testing and found that the cost savings were significant, especially for low-volume testing. An additional 1.6% of TB positives could be detected by Xpert compared to smear microscopy, with a cost savings of USD3.28 per case. This highlights the potential cost-effectiveness of Xpert in resource-limited settings.

**Table 3. Performance of smear microscopy, MGIT culture (with LPA confirmation) and the Xpert MTB/RIF for diagnosis of childhood TB**

<table>
<thead>
<tr>
<th>Assay performance</th>
<th>Culture</th>
<th>Smear microscopy</th>
<th>Xpert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimens, n</td>
<td>376</td>
<td>389</td>
<td>437</td>
</tr>
<tr>
<td>MTB-positive, n (%)</td>
<td>9 (2.4)</td>
<td>7 (1.8)</td>
<td>12 (2.7)</td>
</tr>
<tr>
<td>MTB-negative, n (%)</td>
<td>367 (97.6)</td>
<td>382 (98.2)</td>
<td>425 (96.3)</td>
</tr>
<tr>
<td>MTB RIF-sensitive, n</td>
<td>9</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>MTB RIF-resistant, n</td>
<td>0</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

**Performance analysis vs. culture as the reference**

<table>
<thead>
<tr>
<th>Specimens, n</th>
<th>Comparator test</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparator</td>
<td>374</td>
<td>33.3 (7.9 - 69.9)</td>
<td>99.5 (98.0 - 99.9)</td>
<td>66.0 (15.4 - 93.5)</td>
<td>98.4 (96.5 - 99.4)</td>
</tr>
<tr>
<td>Xpert</td>
<td>345</td>
<td>62.5 (24.7 - 91.0)</td>
<td>99.1 (97.4 - 99.8)</td>
<td>62.5 (24.7 - 91.0)</td>
<td>99.1 (97.4 - 99.8)</td>
</tr>
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LPA = line probe assay.

An advantage of performing on-site Xpert testing specifically for children is the rapid turnaround of results. However, on-site Xpert testing on inadequate specimen volumes may increase the chances of obtaining errors and invalid results that cannot be repeated on the same sample. In our study, Xpert MTB/RIF outperformed TB microscopy but did not demonstrate acceptable sensitivity in this real-world setting and cannot currently replace TB culture in the diagnosis of paediatric/childhood TB. Alternative specimen types, which are easier to obtain and not subject to volume limitations, such as stool, may provide a better diagnostic option.

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References