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Highlights:

- Xpert Ultra detects tuberculosis in children with a superior sensitivity compared to Xpert.
- The sensitivity of Xpert Ultra is considerably lower in children than in adults.
- The decreased specificity of Xpert Ultra warrants further investigations.
- Xpert Ultra might improve speed and reliability of tuberculosis diagnosis in children.

Xpert MTB/RIF Ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study

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Abstract

Objectives: We evaluated the diagnostic performance of the novel next-generation Xpert MTB/RIF Ultra (Xpert Ultra) in comparison to Xpert MTB/RIF (Xpert) assay for the detection of paediatric pulmonary tuberculosis in high burden settings.

Methods: From May 2011 to September 2012, children with suspected pulmonary tuberculosis were enrolled at two Tanzanian sites and sputum samples were examined using sputum smear, Xpert and culture. Xpert Ultra was tested between January and June 2017 using sputum pellets, which had been stored at -80°C. The diagnostic accuracy of Ultra versus Xpert was determined using well-defined case definitions as reference standard.

Results: In total, 215 children were included. The median age was 5.4 years, the HIV prevalence was 52% and 13% had culture-confirmed pulmonary tuberculosis. When only the first available sample of each patient was analysed, the sensitivity of Xpert Ultra was 64.3 % (95% CI: 44.1 to 81.4) while that of Xpert was 53.6% (95%CI: 33.9 to 72.5). The specificity of Xpert Ultra based on analysis of all available samples was 98.1% (95%CI: 93.4 to 99.7), that of Xpert was 100%.

Conclusions: Xpert Ultra was found to have a higher sensitivity, but slightly reduced specificity compared to Xpert in detecting pulmonary tuberculosis in children.

Introduction

The World Health Organisation (WHO) estimated one million new tuberculosis (TB) cases and 250,000 TB-related deaths among children younger than 15 years in 2016 [1]. Childhood TB is often underdiagnosed and underreported due to suboptimal diagnostic methods and weak national reporting systems [2,3]. Microbiological confirmation is rare, because of difficulties in obtaining adequate samples from children, the low bacillary loads and missing host biomarker [4]. Consequently, the diagnosis of paediatric TB in most high TB burden settings is based on clinical symptoms, TB contact information and, if available, chest radiography [5,6].

The Xpert MTB/RIF assay (Xpert; Cepheid Inc., Sunnyvale, CA, USA) enables detection of both *Mycobacterium tuberculosis* (MTB) complex and rifampicin resistance in pulmonary and extrapulmonary samples. Since 2013, the WHO strongly recommends the assay as a first diagnostic test in children suspected of having MDR-TB or HIV-associated TB while a conditional recommendation is made for all children suspected of having TB [7]. Unfortunately, the expectations for substantially improved TB detection in children are only partly met. According to a recent meta-analysis, Xpert has a suboptimal sensitivity of 62% in expectorated or induced sputum samples compared to culture [8].

The novel Xpert MTB/RIF Ultra assay (Xpert Ultra; Cepheid Inc., Sunnyvale, CA, USA) has been developed to overcome the shortcomings of Xpert with regard to limited sensitivity for MTB complex detection in patients with paucibacillary disease [9] and occasionally false-positive and -negative signals for rifampicin resistance [10,11]. Xpert Ultra is equipped with two additional molecular targets (IS6110 and IS1081), a larger chamber for DNA amplification, fully nested nucleic acid amplification, faster thermal cycling, and improved assay chemistry [12]. These

technical improvements result in a lower limit of detection of the MTB H37Rv strain in sputum of 15.6 CFU/ml for Xpert Ultra versus 112.6 CFU/ml for Xpert [12].

A prospective, multicentre, diagnostic accuracy study in adults demonstrated a higher sensitivity of Xpert Ultra compared to Xpert in clinical use, especially for paucibacillary samples (i.e. from patients with smear-negative TB and/or HIV infection). However, the increased sensitivity comes at the cost of decreased specificity [13]. In 2017, the WHO declared Xpert Ultra non-inferior to Xpert and gave guidance on the interpretation of Xpert Ultra semi-quantitative 'trace' results which are considered the main reason for the reduced specificity and often represent non-viable bacilli particularly in patients with a recent history of TB [14].

Data on the diagnostic performance of Xpert Ultra in children are scarce. We conducted a comparative diagnostic accuracy study of Xpert Ultra versus Xpert for the detection of pulmonary TB and rifampicin resistance in children suspected of having TB.

Methods

Study population and case definition categories

This diagnostic accuracy study was performed at two Tanzanian research sites, the NIMR-Mbeya Medical Research Center, Mbeya, and the Ifakara Health Institute, Bagamoyo in accordance with STARD guidelines (Supplementary table 1) [15]. From May 2011 to September 2012, children suspected of having TB, between six months and 16 years of age were consecutively enrolled and followed up to a maximum of nine months. At least one of the following eligibility criteria had to be met: i) persistent, non-remitting cough of more than 14 days not responding to antibiotics, ii) repeated episodes of fever within the last 14 days not responding to antibiotics, after malaria has been excluded, ii) weight loss or failure to thrive during the previous three months, iv) signs and symptoms suggestive of extrapulmonary TB. Children who had received anti-TB treatment in the past 12 months were excluded.

Based on clinical examinations and microbiological evaluations of all available sputum samples, children were classified into five groups: culture confirmed TB (at least one sputum sample was culture-positive for MTB), highly probable TB (chest radiograph consistent with TB confirmed by two independent reviewers, histology/cytology typical for TB, or fluorescent/acid-fast bacilli on microscopy), probable TB (clinically suspected TB without objective findings as above), "not TB" (alternative diagnosis established and clinical resolution without anti-TB treatment), unknown TB status (any other possible combination of results and/or loss
to follow up after recruitment) [16].

Clinical and Laboratory Procedures

Demographic information and results of medical history, clinical examination, anthropometric measurements, HIV testing, CD4+ T cell count, tuberculin skin test, and chest radiography, assessed by two independent experts, were recorded at enrolment. The study protocol scheduled for collection of at least two induced or three expectorated sputum samples. However, in some cases the type and also the number of sputum samples collected varied between one and five according to clinical requirements.

After decontamination with N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH), all sputum sample pellets were examined by light microscopy after Ziehl-Neelsen staining. The pellet of at least one sample was inoculated into liquid (MGIT; BACTEC MGIT 960, Becton Dickinson, USA) and onto solid Loewenstein-Jensen (LJ) culture media. Cultures positivity for growth of acid-fast bacilli was confirmed by MPT64 antigen or molecular tests (Genotype MTBC or CM, Hain Lifescience, Germany). Phenotypic (DST in MGIT using SIRE kit) and genotypic (MTBDRplus, Hain Lifescience, Germany) tests were applied for drug resistance testing. Xpert was used to test at least one of the child's sputum samples.

Xpert Ultra testing was performed between 7th January and 25th July 2017 at both sites using decontaminated sputum pellets which were stored at -80°C. At least one sample from each participant with the TB classification culture confirmed, highly probable, probable, or "not TB" was assigned to Xpert Ultra testing. Only a randomly selected sub-group with unknown TB status was analysed due to cost restrictions. The GeneXpert instruments provided automated readouts for detection of MTB complex (detected, not detected, or error message) and rifampicin resistance (detected, not detected, or indeterminate). Semi-quantitative results for Xpert Ultra

were recorded as follows: trace, very low, low, medium, or high. All laboratory tests were performed blinded to information about clinical course and/or case definition categories.

Statistical Analysis

Xpert Ultra results were retrieved from the machine export files. All other data were double entered into Microsoft Access databases (Microsoft Corp, Redmond, WA), compared and corrected for data entry errors. All statistical analyses were performed using Stata (version 15.1, StataCorp, College Station, TX).

To characterize the study population, we report percentages for categorical variables and medians and interquartile ranges for continuous variables, since none of them was normally distributed. Calculation of diagnostic parameters (sensitivity, specificity, positive and negative predictive value) of the tests only included children with culture confirmed TB and children where TB was excluded ("not TB"), respectively. This reference standard is based on all available culture results for each child, including samples which were not available for Xpert Ultra testing, whereas the calculation of diagnostic accuracy in this article are only based on samples that were Ultra tested. Diagnostic accuracy parameters were calculated in three ways: 1. Per patient/1st sample, including only the first available sample tested with Ultra; 2. Per patient/all samples, including all samples tested with Ultra for each patient and regarding the patient as test positive if any of these samples was positive; 3. Per sample, including all tested samples individually with Ultra. Exact (Clopper-Pearson) 95% confidence intervals were calculated and were adjusted for within person clustering in the per sample analysis.

To examine the potential influence of other factors (HIV status, gender, age etc.) on Xpert Ultra sensitivity, we performed log-binomial regression adjusted for within person clustering [17].

Ethical Approval and Informed Consent

The study protocol was approved by the Mbeya Medical Research and Ethics Committee, the Institutional Review Board of the Ifakara Health Institute, and the Medical Research Coordinating Committee of Tanzania. Written informed consent was obtained from a literate parent or legal guardian, including consent to store samples for future diagnostic evaluation. In case of illiteracy, informed oral consent was attested by an independent witness. Children older than seven years of age additionally provided assent for participation.

Results

In total, 293 children were enrolled at the two sites. Subsequently, 16 children were excluded from the analysis due to lack of stored sputum (n=12), the participants died during enrolment (n=2) or the children had solely extrapulmonary TB (n=2). Another 62 participants were excluded because of their unknown TB status. Thus, 215 children were included for analysis of the Xpert Ultra diagnostic performance. The STARD diagram shows the flow of participants according to Xpert Ultra results and case definition categories (Figure 1).

Overall, 13% (28/215) of the children had culture confirmed, 4 % (9/215) highly probable, 24 % (52/215) probable and 50% (107/215) "not TB". Furthermore, 9% (19/215) belonged to the unknown TB status group. The median age was 5.4 years (IQR, 1.5 to 9.9). The overall HIV prevalence was 52 % (110/212). The highest HIV prevalence was found in the probable TB and the unknown TB status classification group amounting to 77% (40/52) and 71% (12/17), respectively. According to the WHO immunological classification, severe immunodeficiency was found in 40% (42/105) of the HIV-infected children. Detailed demographic and clinical characteristics are presented in table 1.

Xpert Ultra testing was performed on 520 sputum samples with 517 valid results (Supplementary figure 1). Corresponding results for culture (MGIT and/or LJ), smear microscopy and Xpert were available in 98% (505/517), 98% (506/517) and 81% (417/517), respectively. The proportion of samples with valid Xpert Ultra, Xpert and culture (MGIT and/or LJ) results is shown in Supplementary table 2. The Xpert Ultra assay was positive in 43 samples from 24 patients. In these samples, Xpert Ultra showed either a negative (33/43) or an indeterminate (10/43) result

for rifampicin resistance. Of the 28 samples that were MTB positive in both Xpert and Xpert Ultra testing, 25 were not RIF resistant in both tests, and three were not resistant in the Xpert, but indeterminate in the Ultra.

Per patient sensitivity and specificity analyses used the categories culture confirmed and "not TB" as reference standard. Taking only the first available sputum sample into account, the sensitivity of Xpert Ultra was 64.3 % (95% CI: 44.1 to 81.4), while the sensitivity amounted to 75.0% (95%CI: 55.1 to 89.3) when all samples tested by Xpert Ultra were included into the analysis (Table 2). In comparison, per patient sensitivity for Xpert and smear microscopy was 53.6% (95%CI: 33.9 to 72.5) and 35.7% (95%CI: 18.6 to 55.9) when test results from only the first sample was used and 60.7% (95%CI: 40.6 to 78.5) and 42.9% (95%CI: 24.5 to 62.8) when test results from all samples were included. The specificity of Ultra based on the analysis of the first available sample was 100.0% (95%CI: 96.6 to 100) and based on all available samples it decreased to 98.1% (95%CI: 93.4 to 99.7), whereas Xpert and smear had a specificity of 100% in both scenarios (Table 2).

Xpert Ultra was positive in three study participants without culture confirmed TB. Firstly, a trace-positive Xpert Ultra result was recorded in one sputum sample of a one-year old HIV-positive boy with probable TB who received anti-TB treatment based on clinical suspicion. Secondly, a negative Xpert, but a high positive Xpert Ultra result was found in a nine-year old, HIV-positive girl with a history of TB at the age of six. The girl was diagnosed with bronchitis at enrolment and showed no clinical signs of TB at month 5 and 9 follow up visits. Finally, a trace result was found in an 11-year-old HIV-negative boy who was finally diagnosed of having asthma. The latter two study participants had been classified as having "not TB".

A stratified analysis showed that both Xpert Ultra and Xpert had a higher sensitivity in HIV-positive than HIV-negative children; this difference was not statistically significant. Both Xpert Ultra and Xpert had a higher sensitivity in children with smear-positive compared to children with smear-negative TB. The difference in sensitivity between Xpert Ultra and Xpert was highest within the HIV-positive subgroup (88.9% vs. 66.7%) followed by the smear-negative subgroup (44.4% vs. 27.8%) (Table 3).

Associations between Xpert Ultra sensitivity and different potential predictors were assessed in uni- and multivariable regression models. Only HIV-positivity was significantly associated with Xpert Ultra sensitivity in the multivariable model (Supplementary table 3).

Per sample sensitivity within the group of culture confirmed TB cases showed a sensitivity of 64.3% for Xpert Ultra and 53.6% for Xpert, while the sensitivity for LJ and MGIT combined was 75.0% when only the first collected sample was included. (Supplementary table 4). A head-to-head comparison of results for samples which were tested by all diagnostics (n=407), i.e. Xpert Ultra, Xpert, smear microscopy and culture (MGIT and/or LJ), showed that Xpert Ultra identified TB in seven Xpert negative samples belonging to four different children who were all smear negative, and in three culture negative samples. Culture was the only positive test in 12 samples and smear in one sample (Figure 2).

The semi-quantitative readout for positive Xpert Ultra samples was as follows: 12% high, 49% medium, 5% low, 14% very low and 21% trace. These results agreed well with the semi quantitative results of Xpert (Supplementary table 5).

Discussion

Our data show a higher sensitivity of Xpert Ultra (64.3%) compared to Xpert (53.6%) in children with culture confirmed TB. This is in line with a recent publication, where 67.5% of TB culture positive children were detected by Xpert Ultra with a difference in sensitivity between Xpert Ultra and Xpert of maximal 10%, depending on the reference standard [18]. In our study, as in an earlier multicentre diagnostic study in adults [13], the difference in sensitivity among these two tests was especially pronounced in smear-negative children, with 44.4% for Xpert Ultra versus 27.8% for Xpert. Compared to Xpert, the new Xpert Ultra detected four additional TB cases, three children with the first available sample and a fourth case if all available sputa were tested. All additional TB cases were smear-negative.

The improved sensitivity of Xpert Ultra might not only lead to larger proportion of confirmed childhood TB cases but also to earlier TB diagnosis and treatment initiation particularly compared to culture-based algorithms. Importantly, in the per sample analysis the sensitivity of Xpert Ultra was similar to that of solid culture (64.3%) and only moderately lower compared to liquid and solid culture combined (75.0%) when only one sample per child was tested. Further, as the reference standard for culture confirmed TB was based on up to five sputum culture results, which even includes sputa that could not be Ultra tested, the overall diagnostic sensitivity of Xpert Ultra versus culture might be underestimated as a maximum of only four samples per child were tested with Xpert Ultra. Our data suggest that the overall sensitivity of Xpert Ultra increases with the number of sputum samples tested. This could not be sufficiently analysed because second, third or fourth samples were unavailable for many children. Though, the performance of

only one Ultra test is most realistic for resource-poor settings considering the cost of about 10 USD per test cartridge.

Although the sensitivity of Xpert Ultra was higher than that of smear and Xpert, Xpert Ultra did not detect TB among children with highly probable TB. Like other microbiological tests, Xpert Ultra depends on the presence of mycobacteria in the diagnostic sample. Due to the paucibacillary nature of pulmonary TB in children, sputum samples are often no ideal specimen for TB diagnosis [19,20]. Further paediatric studies are needed to evaluate the diagnostic performance of Xpert Ultra in alternative specimens as blood, stool or cerebrospinal fluid. The poor performance of all assays in the highly probable TB group underlines the relevance of systematic clinical investigations and medical expertise for the diagnosis childhood TB and also calls for novel sputum-independent tests for paediatric TB [4,20–22].

The presence of false-positive Xpert Ultra results among three children with no culture confirmed TB might constitute a further challenge to TB diagnosis in clinical routine. As in adults [13], the specificity of Xpert Ultra (98.1%) was reduced compared to Xpert (100%) in our study, but only when all available samples were included in the specificity analysis. One child in the probable TB group might have been correctly identified as an active TB case only by Xpert Ultra. In a second child, who was classified as “not TB” but had been treated for TB three years ago, Xpert Ultra might have detected MTB DNA only as remnant of a previous active TB episode [14,24]. However, cross-contamination or sample mix-up cannot be excluded. In a third child the false “trace” detection of TB by Xpert Ultra cannot be explained.

Thus, the specificity of Xpert Ultra compared to culture was higher in our paediatric compared to an adult study [13], most likely because the proportion of participants with a history of TB was much smaller (7% versus 21%). To increase specificity, Dorman et al. [13] discussed scenarios for re-classification of Xpert Ultra trace results in adults. In our study, nine samples had a trace-positive Xpert Ultra result. Seven were collected from children with confirmed TB, one belonged to a child with probable, but clinically diagnosed TB, and only one to a child with "not TB". Moreover, the only child with history of TB who was incorrectly detected by Xpert Ultra had a high-positive, not a trace-positive result. Consequently, reclassifying all trace-positive results as TB-negative cannot be recommended.

We observed a substantial difference in sensitivity of more than 22% between Xpert Ultra and Xpert in the HIV-positive children. This can be explained by the reduced limit of detection of Xpert Ultra which is especially relevant for paucibacillary TB in HIV-positives. The sensitivity of Xpert Ultra was remarkably higher in HIV-positive (88.9%) versus HIV-negative TB cases (52.6%). This seems counterintuitive and can only be explained by a study-specific composition of the group with culture confirmed TB, e.g. the proportion of smear-positives was higher in HIV-positive children than in HIV-negative children. In the previous Xpert Ultra studies [13,18] and earlier Xpert studies in children [16,25] no statistically significant difference in the performance of Xpert Ultra and Xpert among HIV-positive and -negative TB suspects was found.

Sub-optimal sensitivity *M. tuberculosis* culture undermines its suitability as a reference standard in paediatric diagnostic accuracy studies. Therefore, we have to assume -with high degree uncertainty- that the sensitivity of Ultra in our study would be in fact lower, if clinical and

radiological along with microbiological criteria are applied to define the reference standard. Further limitations of our study include the small number of participants with confirmed TB which may have led to less accurate estimates for diagnostic performance parameters, especially in subgroups analyses. The exclusion of children with unknown TB might have hypothetically introduced selection bias. The retrospective approach of Xpert Ultra evaluation, using a limited sub-set of stored sputum samples, may have hampered the detection of MTB by Xpert Ultra. Although it seems that Xpert Ultra can reliably detect also small amounts of DNA in sputum samples even from dead bacteria [12,13], it is not known yet how the decontamination process with NALC-NaOH as well as the long term storage at -80°C influences the performance of the test. Also, we were not able to assess the detection of rifampicin resistance as all strains were drug-susceptible.

In summary, this study demonstrated a higher sensitivity of Xpert Ultra compared to Xpert in children. When only one sample per child was tested, Xpert Ultra found three additional TB cases, the majority of them with trace-positive Ultra results. The reduction in specificity of Xpert Ultra seems to be less pronounced in children than in adults. Xpert Ultra thus has the potential to increase the reliability and speed of TB diagnosis in children from settings with a high burden of TB.

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Contributors

IS, AR and KR conceived and designed the study. IS, AR, PC, NEN, FH and KR were overseeing enrolment, patient care and data collection. DM, MS and LK were responsible for the laboratory work. ES performed the data analysis. MH and DHP provided expert advice on data interpretation. IS, AR, ES and KR wrote the draft of the manuscript. All authors contributed to final interpretation of data and the critical review of the final article. The authors approved the current version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of interests

The authors have no conflict of interest to declare.

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Figure 1: STARD diagram with Xpert Ultra results by TB case definitions

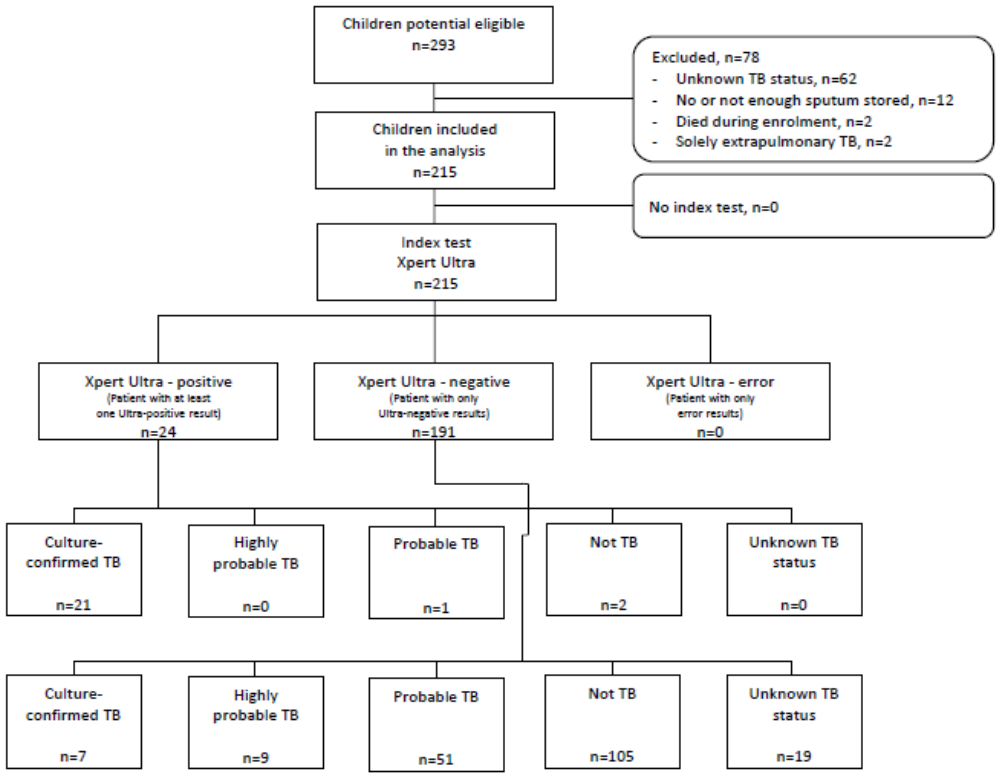
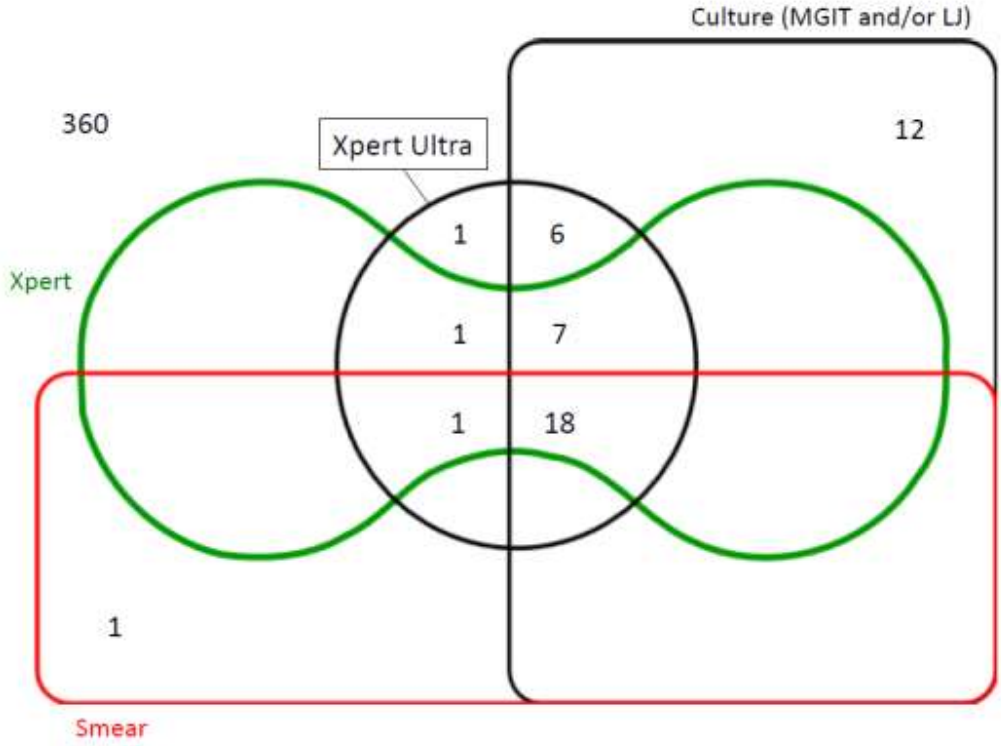


Figure 2: Four-way Venn diagram of Xpert Ultra, Xpert, smear microscopy and/or culture results



Analysis based on per sample analysis (includes only samples with results for all four tests available; n=407)

Table 1: Demographics and clinical characteristics of study participants by classification group

	Culture confirmed TB	Highly probable TB	Probable TB	Not TB	Unknown TB status	All Children
N children (N samples with valid Ultra results)	28 (69)	9 (20)	52 (139)	107 (270)	19 (19)	215 (517)
Age Median years (IQR)	5.7 (1.9; 10.2)	7.9 (2.2; 11.8)	4.1 (1.3; 9.7)	6.0 (2.0; 10.2)	3.2 (1.0; 11.1)	5.4 (1.5; 9.9)
Gender Female, n/N (%)	13/28 (46)	6/9 (67)	23/52 (44)	53/107 (50)	8/19 (42)	103/215 (48)
HIV status						
Negative HIV test, n/N (%)	19/28 (68)	4/9 (44)	12/52 (23)	62/106 (58)	5/17 (29)	102/212 (48)
Positive HIV test, n/N (%)	9/28 (32)	5/9 (56)	40/52 (77)	44/106 (42)	12/17 (71)	110/212 (52)
No data, n	0	0	0	1	2	3
Antiretroviral treatment						
On ART at enrolment, n/N (%)	3/7 (43)	2/5 (40)	15/31 (48)	13/32 (41)	6/11 (55)	39/86 (45)
Not on ART at enrolment, n/N (%)	4/7 (57)	3/5 (60)	16/31 (52)	19/32 (59)	5/11 (45)	47/86 (55)
No data, n	2	0	9	12	1	24
Immune suppression status of HIV-positive children						
No suppression, n/N (%)	3/8 (38)	0/5 (0)	13/37 (35)	18/43 (42)	2/12 (17)	36/105 (34)
Mild suppression, n/N (%)	0/8 (0)	1/5 (20)	3/37 (8)	6/43 (14)	1/12 (8)	11/105 (10)
Moderate suppression, n/N (%)	2/8 (25)	0/5 (0)	6/37 (16)	5/43 (12)	3/12 (25)	16/105 (15)
Severe suppression, n/N (%)	3/8 (38)	4/5 (80)	15/37 (41)	14/43 (33)	6/12 (50)	42/105 (40)
No data, n	1	0	3	1	0	5
Weight for age (< 10 years only) Median Z score (IQR)	-2.2 (-3.9; -1.4)	-2.5 (-4.5; -1.9)	-2.9 (-3.9; -1.8)	-2.0 (-3.0; -0.9)	-3.1 (-4.3; -1.1)	-2.5 (-3.7; -1.2)
Height for age Median Z score (IQR)	-2.8 (-3.7; -1.8)	-3.3 (-3.6; -1.3)	-3.4 (-4.1; -1.9)	-1.9 (-3.1; -0.8)	-3.4 (-4.1; -2.2)	-2.5 (-3.6; -1.4)
Weight for height (<120 cm only) Median Z score n (IQR)	-1.6 (-2.9; -0.7)	-2.3 (-4.4; -0.8)	-1.3 (-2.6; -0.3)	-1.3 (-2.1; -0.3)	-1.7 (-2.4; -0.3)	-1.4 (-2.3; -0.4)
Body mass index for age Median Z score (IQR)	-1.7 (-2.7; -0.8)	-2.7 (-4.4; -1.5)	-1.3 (-2.3; -0.3)	-1.3 (-2.1; -0.4)	-1.7 (-3.4; -0.8)	-1.4 (-2.5; -0.5)
Tuberculin skin test						
Positive in HIV-negative children, n/N (%)	13/18 (72)	1/4 (25)	2/12 (17)	10/59 (17)	1/5 (20)	27/98 (28)
Positive in HIV-positive children, n/N (%)	5/9 (56)	0/5 (0)	6/37 (16)	2/40 (5)	0/12 (0)	13/103 (13)
Positive + indeterminate HIV test, n/N (%)	0/0	0/0	0/0	0/0	0/1 (0)	0/1 (0)
No data, n	1	0	3	8	1	13
TB contact in last 12 months						
Yes, n/N (%)	12/26 (46)	2/7 (29)	14/46 (30)	28/99 (28)	3/16 (19)	59/194 (30)
No, n/N (%)	14/26 (54)	5/7 (71)	32/46 (70)	71/99 (72)	13/16 (81)	135/194 (70)
Unknown, n	2	2	6	8	3	21

Table 2: Per patient diagnostic accuracy in the culture confirmed TB and "not TB" groups (n-positive or n-negative = number of patients tested positive or negative. N = number of patients tested, PPV= Positive Predictive Value, NPV = Negative Predictive Value).

	Sensitivity % (95 % CI), n-positive/N	Samples per patient Mean (SD)	Specificity % (95 % CI), n-negative/N	Samples per patient Mean (SD)	PPV % (95 % CI)	NPV % (95 % CI)
Xpert Ultra						
First available sample	64.3 (44.1 to 81.4), 18/28	1	100.0 (96.6 to 100.0), 107/107	1	100.0 (81.5 to 100.0)	91.5 (84.8 to 95.8)
All samples	75.0 (55.1 to 89.3), 21/28	2.46 (0.74)	98.1 (93.4 to 99.7), 105/107	2.52 (0.69)	91.3 (72.0 to 98.9)	93.8 (87.5 to 97.6)
Xpert						
First available sample	53.6 (33.9 to 72.5), 15/28	1	100.0 (96.5 to 100.0), 105/105	1	100.0 (78.2 to 100.0)	89.0 (81.9 to 94.0)
All samples	60.7 (40.6 to 78.5), 17/28	2.11 (0.92)	100.0 (96.5 to 100.0), 105/105	1..90 (0.93)	100.0 (80.5 to 100.0)	90.5 (83.7 to 95.2)
Smear microscopy						
First available sample	35.7 (18.6 to 55.9), 10/28	1	100.0 (96.6 to 100.0), 107/107	1	100.0 (69.2 to 100.0)	85.6 (78.2 to 91.2)
All samples	42.9 (24.5 to 62.8), 12/28	2.39 (0.74)	100.0 (96.6 to 100.0), 107/107	2.47 (0.68)	100.0 (73.5 to 100.0)	87.0 (79.7 to 92.4)

Table 3: Per patient sensitivity of Ultra and Xpert in children with culture confirmed TB, stratified by HIV-status and smear result (n-positive = number of patients tested positive. N = number of patients tested).

	Sensitivity Ultra 1st available sample % (95 % CI), n-positive/N	**Sensitivity Xpert** 1st available sample % (95 % CI), n-positive/N
HIV-negative	52.6 (28.9 to 75.6), 10/19	47.4 (24.4 to 71.1), 9/19
HIV-positive	88.9 (51.8 to 99.7), 8/9	66.7 (29.9 to 92.5), 6/9
Sensitivity difference	36.3 (5.8 to 66.7)	19.3 (-18.8 to 57.4)
Smear-negative	44.4 (21.5 to 69.2), 8/18	27.8 (9.7 to 53.5), 5/18
Smear-positive	100.0 (69.2 to 100.0), 10/10	100.0 (69.2 to 100.0), 10/10
Sensitivity difference	55.6 (32.6 to 78.5)	72.2 (51.5 to 92.9)