Sequential and parallel testing for microbiological confirmation of tuberculosis disease in children in five low-income and middle-income countries: a secondary analysis of the RaPaed-TB study

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Summary

Background Despite causing high mortality worldwide, paediatric tuberculosis is often undiagnosed. We aimed to investigate optimal testing strategies for microbiological confirmation of tuberculosis in children younger than 15 years, including the yield in high-risk subgroups (eg, children younger than 5 years, with HIV, or with severe acute malnutrition [SAM]).

Methods For this secondary analysis, we used data from RaPaed-TB, a multicentre diagnostic accuracy study evaluating novel diagnostic assays and testing approaches for tuberculosis in children recruited from five health-care centres in Malawi, Mozambique, South Africa, Tanzania, and India conducted between Jan 21, 2019, and June 30, 2021. Children were included if they were younger than 15 years and had signs or symptoms of pulmonary or extrapulmonary tuberculosis; they were excluded if they weighed less than 2 kg, had received three or more doses of anti-tuberculosis medication at time of enrolment, were in a condition deemed critical by the local investigator, or if they did not have at least one valid microbiological result. We collected tuberculosis-reference specimens via spontaneous sputum, induced sputum, gastric aspirate, and nasopharyngeal aspirates. Microbiological tests were Xpert MTB/RIF Ultra (hereafter referred to as Ultra), liquid culture, and Löwenstein–Jensen solid culture, which were followed by confirmatory testing for positive cultures. The main outcome of this secondary analysis was categorising children as having confirmed tuberculosis if culture or Ultra positive on any sample, unconfirmed tuberculosis if clinically diagnosed, and unlikely tuberculosis if neither of these applied.

Findings Of 5313 children screened, 975 were enrolled, of whom 965 (99%) had at least one valid microbiological result. 444 (46%) of 965 had unlikely tuberculosis, 282 (29%) had unconfirmed tuberculosis, and 239 (25%) had confirmed tuberculosis. Median age was 5·0 years (IQR 1·8–9·0); 467 (48%) of 965 children were female and 498 (52%) were male. 155 (16%) of 965 children had HIV and 110 (11%) children had SAM. 196 (82%) of 239 children with microbiological detection tested positive on Ultra. 110 (46%) of 239 were confirmed by both Ultra and culture, 86 (36%) by Ultra alone, and 43 (18%) by culture alone. 'Trace' was the most common semiquantitative result (93 [40%] of 234). 481 (50%) of 965 children had only one specimen type collected, 99 (21%) of whom had *M tuberculosis* detected. 484 (50%) of 965 children had multiple specimens collected, 141 (29%) of whom were positive on at least one specimen type. Of the 102 children younger than 5 years with *M tuberculosis* detected, 80 (78%) tested positive on sputum. 64 (80%) of 80 children who tested positive on sputum were positive on sputum alone; 61 (95%) of 64 were positive on induced sputum, two (3%) of 64 were positive on spontaneous sputum, and one (2%) was positive on both.

Interpretation High rates of microbiological confirmation of tuberculosis in children can be achieved via parallel sampling and concurrent testing procedures. Sample types and choice of test to be used sequentially should be considered when applying to groups such as children younger than 5 years, living with HIV, or with SAM.

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Research in context

Evidence before this study

Microbiological confirmation is the gold standard to diagnose tuberculosis disease in children, but is hampered by the paucibacillary nature of paediatric tuberculosis and the need for invasive sample collection, which are amplified in subgroups at high risk of dying from tuberculosis (eg, children younger than 5 years, with HIV, and with severe acute malnutrition [SAM]). We searched PubMed on March 21, 2024, for articles published in English between database inception and Feb 29, 2024, using the search terms "TB", "microbiology", "confirmation", "culture", "NAAT", "PCR", "accuracy", and "child", "children", "paediatric", or "pediatric" in various combinations. From this search and cross-referencing the articles, we included studies that reported on microbiological detection and yield in diagnosing tuberculosis disease in children younger than 18 years. Culture has reported low sensitivity, ranging from 7–40%. A 2016 meta-analysis based on respiratory specimens collected from 939 children with tuberculosis estimated a culture sensitivity of 24%. Other microbiological detection methods include WHO-recommended molecular rapid diagnostic tests, such as GeneXpert MTB/Rif Ultra, with reported sensitivities of 44–75% compared with culture. Optimal sampling strategies to achieve high diagnostic yields remain unclear. Studies have reported that both the combination of different specimen types and concurrent sampling have the potential to increase diagnostic sensitivity in children. Most studies that we identified were from a single centre or limited by small sample sizes. Further WHO recommendations are general and not adapted to the high-risk subgroups.

Added value of this study

We report a secondary analysis of data collected in the RaPaed-TB study, namely 2299 samples collected from 965 children younger than 15 years. Microbiological confirmation was obtained in 239 (46%) of 521 children who were classified as having tuberculosis disease. Of note, 38 (49%) of 77 infants younger than 1 year with tuberculosis disease had microbiologically confirmed tuberculosis. 196 (82%) of 239 children with microbiological detection tested positive on GeneXpert MTB/RIF Ultra, with trace being the most common semiquantitative result (the majority of which occurred in culture-negative children). Children with HIV and children with SAM were more often microbiologically confirmed by culture than the overall cohort. Our results suggest that concurrent sampling and testing yields increased confirmation rates, especially in children younger than 5 years, and that optimal testing strategies might differ considerably between high-risk subgroups. Our results provide information about sampling and testing strategies to increase the microbiological detection of tuberculosis in children.

Implications of all the available evidence

To achieve increased yields for microbiological confirmation, optimal sampling and testing strategies might need to be adjusted for specific subgroups of interest, especially considering the 2022 treatment-decision algorithms recommended by WHO to be implemented at low levels of health care in settings with a high burden of tuberculosis. These algorithms aim to overcome the large tuberculosis detection gap in children, which currently do not specify specimen type or testing method and suggest the same testing approaches for all children younger than 10 years. Our data emphasise the need for the development of novel, improved, point-of-care tests and age-adjusted and comorbidity-adjusted testing strategies.

Introduction

Tuberculosis is a major cause of morbidity and mortality in children globally, with 1·2 million cases and 240 000 deaths annually, predominantly in low-income and middle-income countries (LMICs).[1,2] Most deaths from paediatric tuberculosis are due to underdiagnosis.[3] Improved detection with timely initiation of therapy could substantially reduce mortality, as treatment is effective for 90% of children.[4] Confirming a diagnosis in children younger than 18 years remains challenging, especially in subgroups at highest risk of adverse outcomes (eg, children younger than 5 years, with HIV, or with severe acute malnutrition [SAM]), who account for almost 80% of tuberculosis-related childhood deaths.[3]

Despite the unsatisfactory sensitivity and considerable infrastructural demand, microbiological testing is crucial in tuberculosis diagnosis.[5,6] Mycobacterial detection of tuberculosis in children by culture is considered the gold standard, with sensitivity ranging from 7–40%.[7–10] In a 2016 meta-analysis, the yield of culture on respiratory specimens among 939 children with tuberculosis was only 24%.[7] Furthermore, the time to detection on culture can take 6–8 weeks. WHO recommends molecular rapid diagnostic tests, such as Xpert MTB/RIF Ultra.[11,12]

Microbiological confirmation in children is also hampered by poor sampling techniques, the paucibacillary nature of tuberculosis disease, and poor infrastructure for testing. Children younger than 5 years often cannot produce sputum spontaneously, so induced sputum might be needed.[13] Sputum induction has been shown to be useful and feasible in several LMICs and for young children; diagnostic yield improved with repeated samples or a combination of different specimens for parallel testing.[10,14–16] Current WHO recommendations on tuberculosis testing, including WHO-endorsed treatment-decision algorithms, do not specify specimen type or testing method.[6]

We aimed to investigate optimal testing strategies for microbiological confirmation of tuberculosis in children

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See **Online** for appendix

younger than 15 years, including the yield in high-risk subgroups.

Methods

Study design and participants

RaPaed-TB (NCT03734172) was a multicentre diagnostic accuracy study evaluating novel diagnostic assays and testing approaches for tuberculosis in children in five LMICs (ie, Malawi, Mozambique, South Africa, Tanzania, and India).[17] In brief, children presenting with presumptive tuberculosis to five health-care centres (ie, tertiary hospitals in Cape Town, South Africa; Blantyre, Malawi; and Vellore, India and urban health facilities in Mbeya, Tanzania and Maputo, Mozambique) were consecutively enrolled between Jan 21, 2019, and June 30, 2021. Children were included if they were younger than 15 years and had signs or symptoms of pulmonary or extrapulmonary tuberculosis.[17] Children were excluded if they weighed less than 2 kg, had received three or more doses of anti-tuberculosis medication at time of enrolment, were in a condition deemed critical by the local investigator, or if they did not have at least one valid microbiological result (appendix p 2).[17]

Clinical assessments and testing were standardised across sites. At enrolment, all children underwent a chest radiograph and a Tuberculin skin test. HIV status was established following WHO and national guidelines.[17,18] Nutritional status was assigned via weight-for-age, height-for-age, and weight-for-height Z scores according to the WHO 2006 child-growth standards.[19] SAM was defined as weight-for-height Z score, BMI-for-age less than –3 SDs, middle upper-arm circumference less than 115 mm, or presence of bilateral oedema. Treatment decisions were made by the local study team in accordance with local and national guidelines.[17]

The RaPaed-TB study was approved by the ethics committees of coordinators and local investigators (appendix p 4).[17] Before any study-specific procedure was conducted, a parent or guardian gave signed written informed consent and children assented if applicable, following local institutional review boards.[17] If a participant could not read or write, witnessed oral consent or assent was obtained.

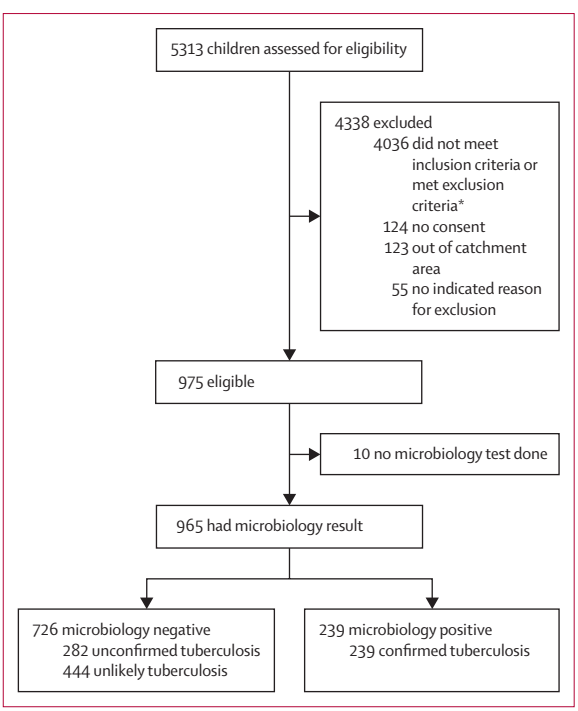

Figure 1: **RaPaed-TB study profile**
*Of 4036 children who did not meet inclusion criteria or met exclusion criteria, 2109 (52·3%) had another diagnosis that was more likely, 1229 (30·5%) had insufficient inclusion criteria, 256 (6·3%) were discharged from facilities before enrolment occurred, 185 (4·6%) had received more than three doses of tuberculosis treatment before screening, 107 (2·7%) were deemed to have a disease that was too critical for enrolment, 100 (2·5%) had no legal guardian available to consent, 37 (0·9%) had social issues precluding enrolment, 12 (0·3%) were enrolled in another study, and one (<1%) had invalid age.

Procedures

We collected tuberculosis-reference specimens via spontaneous sputum, induced sputum, gastric aspirate, and nasopharyngeal aspirates. Two respiratory specimens (defined as spontaneous sputum, induced sputum, or gastric aspirate) were collected from each participant on sequential days or at least 4 h apart. Specimen collection was standardised across sites. Induced sputum and gastric aspirate were collected in children unable to produce spontaneously expectorated sputum. For induced sputum, children were nebulised with hypertonic saline and a bronchodilator and sputum was collected by nasopharyngeal suction via a sterile catheter and mucus trap.[20] Children younger than 5 years had a nasopharyngeal aspirate taken before sputum induction. Extrapulmonary (ie, non-reference) specimens were collected if clinically indicated, depending on local capacity and guidelines (appendix p 3).

Samples were processed at the laboratory of each site following standard operating procedures.[17] Microbiological tests were Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA; hereafter referred to as Ultra), liquid culture (BACTEC mycobacterial growth indicator tube [MGIT] system; BBL MGIT 960, Becton Dickinson Microbiology Systems, Sparks, MD, USA; hereafter referred to as MGIT), and Löwenstein–Jensen solid culture (hereafter referred to as LJ), which were followed by confirmatory testing for positive cultures. The first sample was concentrated before splitting for further processing with Ultra and culture. Confirmatory testing included Ziehl–Neelsen staining followed by a line probe assay (LPA; GenoType MTBDR*plus*, Hain Lifescience, Nehren, Germany). Cultures were incubated for up to 6 weeks and duration until positivity was recorded. The decontaminated pellet from the first sputum or gastric aspirate was split and used for culture and Ultra testing, done as per manufacturer recommendations. The second sputum or gastric aspirate was tested via culture (appendix p 4).

	All (n=965)	Confirmed tuberculosis (n=239)	Unconfirmed tuberculosis (n=282)	Unlikely tuberculosis (n=444)
Country				
South Africa	224 (23%)	77 (32%)	79 (28%)	68 (15%)
Tanzania	230 (24%)	46 (19%)	57 (20%)	127 (29%)
Mozambique	219 (23%)	22 (9%)	86 (30%)	111 (25%)
Malawi	198 (21%)	34 (14%)	56 (20%)	108 (24%)
India	94 (10%)	60 (25%)	4 (1%)	30 (7%)
Sex				
Female	467 (48%)	115 (48%)	125 (44%)	227 (51%)
Male	498 (52%)	124 (52%)	157 (56%)	217 (49%)
Age, years	5·0 (1·8–9·0)	6·2 (1·8–11·6)	4·6 (1·7–8·3)	4·7 (2·0–7·7)
Age group				
0 years to <1 year	124 (13%)	38 (16%)	39 (14%)	47 (11%)
1 year to <5 years	361 (37%)	64 (27%)	111 (39%)	186 (42%)
5 years to <10 years	283 (29%)	55 (23%)	80 (28%)	148 (33%)
10 years to <15 years	197 (20%)	82 (34%)	52 (18%)	63 (14%)
Comorbidities and clinical findings				
HIV*	155 (16%)	24 (10%)	68 (24%)	63 (14%)
Severe acute malnutrition†	110 (11%)	37 (15%)	32 (11%)	41 (9%)
Hospitalised at enrolment	323 (33%)	129 (54%)	85 (30%)	109 (25%)
Recruited at tertiary level	516 (53%)	171 (72%)	139 (49%)	206 (46%)
Recruited at urban facility	449 (47%)	68 (28%)	143 (51%)	238 (54%)
Tuberculosis disease				
Pulmonary tuberculosis only	374 (39%)	139 (58%)	207 (73%)	NA
Extrapulmonary tuberculosis only	59 (6%)	37 (15%)	9 (3%)	NA
Pulmonary tuberculosis and extrapulmonary tuberculosis	98 (10%)	57 (24%)	33 (12%)	NA
Tuberculosis-related clinical findings				
Tuberculin skin test positive	411/867 (47%)	131/215 (61%)	141/257 (55%)	139/395 (35%)
Non-severe tuberculosis disease	160 (17%)	47 (20%)	98 (35%)	NA
Chest-radiograph findings attributable to tuberculosis	262 (27%)	108 (45%)	91 (32%)	63 (14%)
Hospitalised at enrolment	323 (33%)	129 (54%)	85 (30%)	109 (25%)
Tuberculosis treatment initiation				
Treatment for drug-sensitive tuberculosis	472 (49%)	178 (74%)	246 (87%)	48 (11%)
Treatment for drug-resistant tuberculosis	54 (6%)	37 (15%)	12 (4%)	5 (1%)
No treatment initiated‡	387 (40%)	24 (10%)‡	22 (8%)	341 (77%)

Data are n (%), n/N (%), or median (IQR). NA=not applicable. *HIV status not available for 22 participants across the three groups (appendix p 5). †Nutritional status was assigned according to WHO 2006 child-growth standards. ‡Tuberculosis treatment not initiated due to loss to follow-up (n=3), death (n=2), or the decision of the treating physician (single trace positive with definite alternative diagnosis n=9; response to alternative treatment n=1; treatment initiation after end of study n=3; missing data n=6).

Table 1: **Demographic and clinical characteristics of children recruited in RaPaed-TB, by clinical category**

Sex data, options male or female, were collected from hospital records and verified by self-report from participant, parent, or guardian.

Statistical analysis

The RaPaed-TB study was designed as a diagnostic accuracy study to evaluate novel diagnostic assays and was powered accordingly.[17] Recruitment was initially planned for a 2-year period with an assumed cohort size of 1000 children with tuberculosis, including 250 children with confirmed tuberculosis. A sample of 250 children would allow the detection of a sensitivity increase from 62% (Xpert MTB/RIF) to 82%, with more than 90% power at the 95% confidence level. This Article is a secondary analysis.

Children were categorised retrospectively according to US National Institutes of Health consensus definitions, adapted to include children with extrapulmonary tuberculosis.[21] We categorised children as having confirmed tuberculosis if culture or Ultra positive on any sample, unconfirmed tuberculosis if clinically diagnosed, and unlikely tuberculosis if neither of these applied (appendix p 3).[21]

Stratifying by clinical case category, normally distributed continuous variables are summarised with mean (SD) and non-normally distributed variables are

	All	Induced sputum	Spontaneous sputum	Gastric aspirate*	Nasopharyngeal aspirate†	Non-reference sample‡
Microbiological testing						
Any positive	355/2299 (15·4%)	163/1273 (12·8%)	65/389 (16·7%)	35/151 (23·2%)	20/332 (6%)	72/154 (46·8%)
MGIT positive	222/1935 (11·5%)	113/1261 (9·0%)	53/388 (13·7%)	23/144 (16·0%)	0/2	33/140 (23·6%)
LJ positive	106/1502 (7·1%)	28/930 (3·0%)	35/354 (9·9%)	25/140 (17·9%)	0/0	18/78 (23·1%)
Ultra positive	234/1470 (15·9%)	91/716 (12·7%)	32/191 (16·8%)	25/94 (26·6%)	20/332 (6%)	66/137 (48·2%)
Ultra semi-quantitative result, if positive						
Trace	93/234 (39·7%)	35/91 (38·5%)	10/32 (31·3%)	8/25 (32·0%)	11/20 (55·0%)	29/66 (43·9%)
Very low	40/234 (17·1%)	19/91 (20·9%)	1/32 (3·1%)	7/25 (28·0%)	3/20 (15·0%)	10/66 (15·2%)
Low	50/234 (21·4%)	17/91 (18·7%)	4/32 (12·5%)	5/25 (20·0%)	4/20 (20·0%)	20/66 (30·3%)
Medium	30/234 (12·8%)	13/91 (14·3%)	7/32 (21·9%)	3/25 (12·0%)	2/20 (10·0%)	5/66 (7·6%)
High	18/234 (7·7%)	5/91 (5·5%)	9/32 (28·1%)	2/25 (8·0%)	0/20	2/66 (3·0%)

Data are n/N (%). LJ=Löwenstein–Jensen solid culture. MGIT=BACTEC mycobacterial growth indicator tube system and BBL MGIT 960. Ultra=Xpert MTB/RIF Ultra. *Done only at the Indian site. †Done only in children aged <5 years. ‡Predominantly extrapulmonary specimens, collected at South African and Indian sites according to local guidelines and capacity.

Table 2: **Microbiological results of all samples collected in RaPaed-TB**

summarised with median (IQR). Categorical values are presented as n (%). Test positivity by testing method was analysed per specimen type for reference and non-reference specimens. Samples with contaminated or indeterminate results were excluded from analyses. Criteria for test positivity were further described in a per-patient analysis and an intersection graph was created to show the frequencies of combinations of positive results from different specimen types (appendix pp 10–14). Finally, the incremental yield for different respiratory reference specimens (ie, spontaneous sputum, induced sputum, or gastric aspirate) was calculated for children with at least one serial Ultra and culture, following the actual sequence of samples taken. Per protocol, the first sample was processed first on Ultra and subsequently on culture; the second sample underwent culture testing. For children younger than 5 years, Ultra on nasopharyngeal aspirate was included as a first sample. If a subsequent second respiratory sample was available, the additional yield of a second culture was calculated. Here, children with sole extrapulmonary tuberculosis were excluded.

Use of nasopharyngeal aspirate in children younger than 5 years as an easy-to-collect specimen was explored by conducting a per-patient, direct comparison, including children for whom one Ultra result from a nasopharyngeal aspirate was available on the same day as one MGIT culture and one Ultra was done on sputum (appendix p 14).

Data were analysed with Stata version 17.0 and R version 4.1.3.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. However, Cepheid, which supplied testing kits at no cost, was given the opportunity to comment on the manuscript before submission.

Results

Of 5313 children screened, 975 were enrolled between Jan 21, 2019, and June 30, 2021, of whom 965 (99%) had at least one valid microbiological result (figure 1). Children who were not enrolled largely had some symptoms suggestive of tuberculosis but did not meet inclusion criteria as defined by the study protocol (appendix p 2).[17] Of 965 included, 444 (46%) had unlikely tuberculosis, 282 (29%) had unconfirmed tuberculosis, and 239 (25%) had confirmed tuberculosis. Microbiological confirmation was thus obtained for 239 (46%) of 521 children classified as having tuberculosis disease (ie, 239 children with confirmed tuberculosis and 282 with unconfirmed tuberculosis). Of note, 38 (49%) of 77 infants younger than 1 year with tuberculosis disease had microbiologically confirmed tuberculosis (table 1; appendix p 5). Median age was 5·0 years (IQR 1·8–9·0); 467 (48%) of 965 children were female and 498 (52%) were male. 155 (16%) of children had HIV, and 82 (53%) of 155 children with HIV were taking antiretroviral therapy at time of enrolment. 110 (11%) of 965 children had SAM.

Tuberculosis treatment was initiated in 526 (55%) of 965 children after study inclusion, of whom 54 (10%) were treated for drug-resistant tuberculosis. 37 (69%) of 54 children treated for drug-resistant tuberculosis had confirmed tuberculosis. Samples from 12 (22%) of 54 children had drug resistance detected by either Ultra or LPA. Six (50%) of these 12 children had rifampicin monoresistance, four (33%) had multidrug-resistant tuberculosis, and two (17%) had isoniazid monoresistance. Five (42%) of these 12 had rifampicin resistance detected on Ultra alone and two (17%) had rifampicin resistance detected on LPA alone. Of seven children with a valid result for rifampicin resistance on both Ultra and LPA, agreement between methods was seen in three (43%).

2299 samples collected from 965 children had results on Ultra or culture. Most were sputum (1273 [55%] induced sputum and 389 [17%] spontaneous sputum), 332 (14%) were nasopharyngeal aspirate, and 151 (7%) were gastric aspirate (table 2). 154 (7%) of 2299 samples were non-reference specimens, predominantly tissue, cerebrospinal fluid, or pleural fluid (appendix p 6).

Overall, 355 (15%) of 2299 of samples tested positive for *Mycobacterium tuberculosis*; specifically, 163 (13%) of 1273 induced-sputum samples, 65 (17%) of 389 spontaneous-sputum samples, 20 (6%) of 332 nasopharyngeal aspirates, 35 (23%) of 151 gastric aspirates, and 72 (47%) of 154 non-reference samples. A total of 1927 MGIT cultures were conducted; 335 (17%) were contaminated and 222 (12%) tested positive. Of 1502 LJ cultures analysed, 105 (7%) were positive and 25 (2%) were contaminated. Of 1466 Ultras, 234 (16%) were positive and none were indeterminate; the semiquantitative result trace was most frequent (93 [40%] of 234), followed by low (50 [21%] of 234; table 2). Visualisation of proxies of bacterial load in the same sample suggested an almost linear association between time-to-positivity in MGIT and Ultra semiquantitative readout for induced sputum, spontaneous sputum, and gastric aspirate, contrary to non-reference standard samples (appendix p 7).

196 (82%) of 239 children with microbiological detection tested positive for *M tuberculosis* on Ultra. Of children who were positive, 110 (46%) of 239 were confirmed by both Ultra and culture, 86 (36%) by Ultra alone, and 43 (18%) by culture alone (figure 2). Numbers of Ultras and cultures in these three groups were similar. Children with tuberculosis confirmed by both culture and Ultra had a median of 1·5 (IQR 1·0–2·0, range 1·0–4·0) Ultra cultures, 2·0 (2·0–2·0, 1·0–4·0) MGIT cultures, and 2·0 (2·0–3·0, range 1·0–5·0) LJ cultures. Children with tuberculosis confirmed by Ultra only had a median of 2·0 (1·0–2·0, 1·0–4·0) Ultra cultures, 2·0 (2·0–3·0, 1·0–4·0) MGIT cultures, and 2·0 (2·0–3·0, 1·0–4·0) LJ cultures. Children with tuberculosis confirmed by culture only had a median of 1·0 (1·0–2·0, 1·0–4·0) Ultra cultures, 2·0 (2·0–2·0, 1·0–6·0) MGIT cultures, and 2·0 (2·0–2·0, 1·0–3·0) LJ cultures. Although numbers of cultures and Ultra conducted per group were similar, semiquantitative Ultra results differed between groups. Of note, 63 (73%) of 86 confirmed by Ultra alone were confirmed on trace and 58 (92%) of those 63 were confirmed on a single result, 33 (52%) were younger than 5 years, and one (2%) had previous tuberculosis.

To assess the value of concurrent sampling and testing, we summarised the numbers of samples and number of different specimens taken (appendix p 7). Children with confirmed tuberculosis had more samples taken (3·0, IQR 2·0–3·0) than children with unconfirmed tuberculosis (2·0, 2·0–3·0) and with unlikely tuberculosis (2·0, 2·0–3·0). The number of different specimen types collected did not differ between the groups.

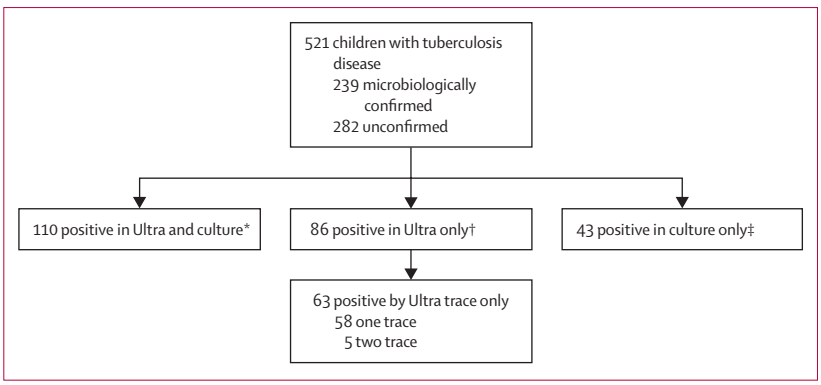

Figure 2: **Overview of microbiological confirmation**
LJ=Löwenstein–Jensen solid culture. MGIT=BACTEC mycobacterial growth indicator tube system and BBL MGIT 960. Ultra=Xpert MTB/RIF Ultra. *Maximum semiquantitative Ultra result: 14 (13%) of 110 trace, 20 (18%) very low, 35 (32%) low, 22 (20%) medium, 17 (16%) high, and two (2%) missing. †Maximum semiquantitative Ultra result: 63 (73%) of 86 trace, 14 (16%) very low, six (7%) low, two (2%) medium, and one (1%) high. ‡Culture positivity: MGIT 30 (70%) of 43, MGIT and LJ nine (21%), and LJ four (9%).

481 (50%) of 965 children had only one specimen type collected (figure 3), 99 (21%) of whom had *M tuberculosis* detected. Of the 484 (50%) of 965 children with multiple specimens collected, 141 (29%) were positive on at least one specimen type (figure 3). For both groups, proportions of positive specimen types varied greatly between children younger than 5 years, children with HIV, and children with SAM (appendix p 9).

Of 239 children with confirmed tuberculosis, 212 (89%) tested positive on one specimen type alone (ie, sputum 142 [67%] of 212, gastric aspirate 12 [6%], nasopharyngeal aspirate five [2%], non-reference specimen 49 [23%]). Of 27 (11%) of 239 children with *M tuberculosis* detected in more than one specimen type, 13 (48%) were positive on induced sputum and nasopharyngeal aspirate. Only five (25%) of the 20 children who tested positive on nasopharyngeal aspirate were positive on nasopharyngeal aspirate alone (appendix p 10).

Of the 102 children younger than 5 years with *M tuberculosis* detected, 80 (78%) tested positive on sputum. 64 (80%) of 80 children who tested positive on sputum were positive on sputum alone; 61 (95%) of 64 were positive on induced sputum, two (3%) of 64 were positive on spontaneous sputum, and one (2%) was positive on both. 15 (19%) of 80 children who tested positive on sputum were positive on both sputum and nasopharyngeal aspirate. Four (21%) of 19 children with *M tuberculosis* detected on nasopharyngeal aspirate were positive on nasopharyngeal aspirate alone. 17 children had *M tuberculosis* detected in a non-reference specimen, of whom 15 (88%) were positive in that specimen alone (appendix pp 9, 11).

Of the 24 children with HIV and confirmed tuberculosis, 22 (92%) were positive on sputum. 19 (86%) of these 22 children were positive on sputum alone. 13 (68%) of 19 were positive on induced sputum, five (26%) were positive on spontaneous sputum, and one (5%) was positive on both. All children with HIV with a positive

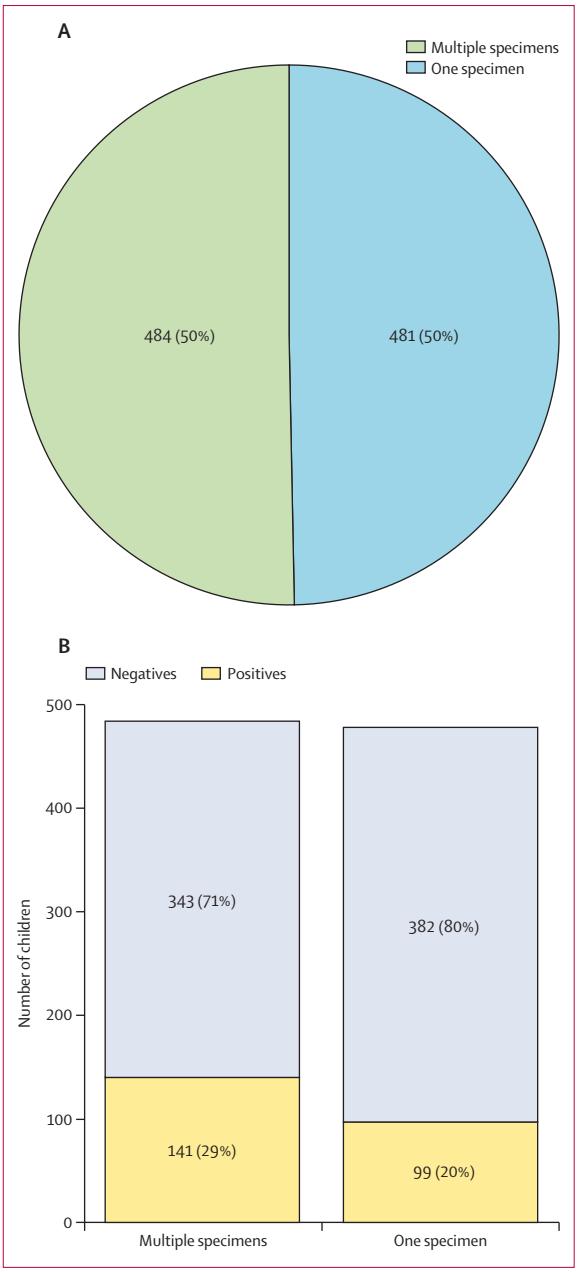

***Figure 3: Mycobacterium tuberculosis* results for the overall cohort by number of specimen types collected**
(A) Distribution of specimen collection count. (B) Proportion of *Mycobacterium tuberculosis* results by number of specimen types collected.

nasopharyngeal aspirate were also positive on induced sputum (appendix pp 9, 12).

A total of 37 children with SAM had *M tuberculosis* infection, with 25 (68%) detected on sputum. Of these 25, 20 (80%) were positive on sputum only. 12 (60%) of these 20 were positive on induced sputum, seven (35%) were positive on spontaneous sputum, and one (5%) was positive on both. Of three children testing positive on nasopharyngeal aspirate, all were also positive on induced sputum. By contrast, among seven children with *M tuberculosis* detected on non-reference specimens, six (86%) were only positive on that sample (appendix pp 9, 13).

Considering the proportion of samples with *M tuberculosis* detected on Ultra alone, culture alone, or Ultra and culture, the distribution of positivity of specimen types was similar for the overall cohort (both 110 [46%] of 239, Ultra only 86 [36%], culture only 44 [18%]) and children younger than 5 years (Ultra only 45 [44%] of 102, both 36 [35%], culture only 16 [16%]), in whom most samples tested positive on Ultra. However, in children with HIV (culture only ten [40%] of 25, Ultra only eight [33%] of 24, both six [25%] of 24) or SAM (both 21 [57%] of 37, Ultra only six [16%], culture only three [8%]), the largest proportion was positive on culture (appendix pp 10–13).

To assess the value of concurrent sampling and testing in children with pulmonary tuberculosis disease (ie, confirmed and unconfirmed pulmonary tuberculosis), we calculated incremental yield of one respiratory sample (sputum or gastric aspirate) tested on one Ultra and one culture and additionally of a second culture, if available (table 3). Considering any respiratory specimen among 447 children with tuberculosis in the overall cohort, 124 (28%) were identified by the first Ultra on the first sample. Culture detected an additional 21 (5%) of 447 children when done on the first sample and an additional 22 (5%) when done on a second sample. In 129 children younger than 5 years with pulmonary tuberculosis disease (ie, confirmed and unconfirmed pulmonary tuberculosis), there were 19 (15%) with Ultra-positive tests on the first nasopharyngeal aspirate. Ultra on a first sputum detected an additional 22 (17%) of 129 children and two (2%) children were detected by culture on the first sputum. Another seven (5%) of 129 children were confirmed by culture of a second sputum. Trends were similar for children with HIV and with SAM (table 3).

Of 205 children younger than 5 years with one Ultra result from a nasopharyngeal aspirate available on the same day as one MGIT culture and one Ultra done on sputum, sputum was positive in 17 (8%) on Ultra and in 12 (6%) on MGIT. Of those 29, seven (24%) children were positive on Ultra only and one (3%) child was positive on culture only. There were ten (5%) of 205 Ultra-positive tests on nasopharyngeal aspirate, four (40%) of which tested negative on sputum MGIT and Ultra.

Discussion

In this secondary analysis of the RaPaed-TB multicentre diagnostic accuracy, 239 of 965 children had microbiologically confirmed tuberculosis via standardised testing with Ultra and culture. High yields were achieved by respiratory specimens, including for children younger than 5 years, whereas nasopharyngeal-aspirate sampling was less sensitive. Most children had tuberculosis confirmed via Ultra, most commonly trace, except in

	All children				Children with HIV				Children with severe acute malnutrition			
	Total	Test positive	Additional yield	Cumulative yield	Total	Test positive	Additional yield	Cumulative yield	Total	Test positive	Additional yield	Cumulative yield
All children with pulmonary tuberculosis disease												
Any respiratory specimen types combined												
First Ultra	447	124	NA	28%	87	11	..	13%	62	26	..	42%
First culture	447	94	21	32%	87	12	6	20%	62	19	2	45%
Second culture*	447	93	22	37%	87	9	2	22%	62	18	2	48%
Sputum												
First Ultra	420	112	NA	27%	86	10	..	12%	54	19	..	35%
First culture	420	79	15	30%	86	12	6	19%	54	12	1	37%
Second culture*	420	80	22	36%	86	9	2	21%	54	13	2	41%
Gastric aspirate												
First Ultra	27	21	NA	78%	1	1	..	100%	8	8	..	100%
First culture	27	15	2	85%	1	0	0	100%	8	7	0	100%
Second culture*	27	8	0	85%	1	0	0	100%	8	3	0	100%
Children aged <5 years in the overall cohort												
Sputum												
First Ultra on nasopharyngeal aspirate	129	19	NA	15%	14	3	..	21%	13	3	..	23%
First sputum Ultra	129	37	22	32%	14	4	1	29%	13	5	2	39%
First sputum culture	129	23	2	33%	14	3	0	29%	13	4	0	39%
Second sputum culture*	129	23	7	39%	14	3	0	29%	13	3	0	39%

We calculated the incremental yield for sputum and gastric aspirate for children with pulmonary tuberculosis disease, both confirmed and unconfirmed, and at least one serial Ultra and culture each; children with extrapulmonary tuberculosis disease only were excluded. For the first three specimen types (ie, sputum, gastric aspirate, and any combined), the denominator is the number of children who had a result for the first Ultra and the first culture. For children aged <5 years, the denominator is the number of children who had a result for the first Ultra on nasopharyngeal aspirate, the first sputum Ultra, and the first sputum culture. NA=not applicable. Ultra=Xpert MTB/RIF Ultra. *If available.

Table 3: **Incremental yields of Ultra, first, and second cultures on serial samples in children categorised as having pulmonary tuberculosis disease and in children aged <5 years in the overall cohort**

children with HIV or SAM, for whom microbiological confirmation by culture predominated. Concurrent sampling and testing increased detection in children younger than 5 years, especially those with SAM. The high microbiological confirmation rates we identified might be partly attributed to standardised procedures and well trained study staff, including frequent monitoring and retraining if needed.[17] There was a similar high rate of 28% in a diagnostic accuracy study of South African children, of whom 160 (82%) of 195 in the overall cohort were younger than 5 years.[10]

The combination of different specimen types has the potential to increase diagnostic sensitivity, and less invasive sampling approaches are of increasing interest. Previous diagnostic studies have reported similar yields between two nasopharyngeal-aspirate samples, one nasopharyngeal aspirate and one stool sample, or two gastric aspirates or induced-sputum samples.[10,22] In a diagnostic accuracy study of South African children, the combination of one Ultra on nasopharyngeal aspirate and one Ultra on induced sputum increased the sensitivity to 80% in children confirmed to have tuberculosis by culture compared with 46% in testing one Ultra on nasopharyngeal aspirate alone.[10] In RaPaed-TB, the sensitivity of Ultra on nasopharyngeal aspirate alone increased from 34% to 72% if one Ultra on induced sputum was added. Although nasopharyngeal aspirate is less invasive and easier to implement than procedures such as sputum induction or gastric aspirate, yield of microbiological confirmation is lower.[10,11,23]

Benefits of concurrent sampling in children has been described by studies reporting improved yields of 8–50%, but sample sizes were small and often highlight the difficulties in establishing a microbiological diagnosis in high-risk subgroups.[10,16,23] In a cross-sectional diagnostic study in Kenya of 300 children with multiple microbiological assessments, including up to two samples per specimen type (of which six were assessed), only 10% had confirmed tuberculosis,[22] although this study used Xpert MTB/RIF instead of Ultra in children younger than 5 years, a quarter of whom were children with HIV. In children with HIV, microbiological diagnosis is infrequent due to paucibacillary disease.[24,25] In a diagnostic accuracy study of 272 children with HIV from eight hospitals in Burkina Faso, Cambodia, Cameroon, and Viet Nam, only 11% had microbiologically confirmed tuberculosis, despite extensive diagnostic investigations.[26] In this secondary analysis, a quarter of children with HIV had tuberculosis that was microbiologically confirmed. Children with SAM are another recognised high-risk group for tuberculosis, although data remain scarce.[7,27,28] In RaPaed-TB, almost half of children with

SAM and presumed tuberculosis had microbiological confirmation. That culture provided the highest yield in children with HIV and SAM in this analysis is interesting.

The implementation of parallel sampling and concurrent testing in settings with high tuberculosis burden and low resources might be limited by the need for associated infrastructure and costs, especially considering technically trained personnel for additional specimen types and additional consumables for collection.[29] Furthermore, incremental costs of processing added specimens might hamper uptake in low-resource settings.[29] These limitations are especially true for culture-related analyses, as these require biosafety level 3 facilities. Pooling of serial samples for testing via molecular methods might be a cost-effective option, but requires further studies including in high-risk groups.

A quarter of children had tuberculosis microbiologically confirmed with Ultra trace only and thus did not have any drug-susceptibility testing. The proportion of children in our study initiated on treatment for drug-resistant tuberculosis was higher in children with confirmed tuberculosis compared with children with unconfirmed tuberculosis. This observation highlights the challenge in addressing the wide detection gap of drug-resistant tuberculosis in children globally, especially in children younger than 5 years.[4]

RaPaed-TB has one of the largest cohorts in the past 20 years, conducted in five centres across five countries. However, there are limitations. Despite training and monitoring of study procedures, considerable heterogeneity existed between study centres, with differing levels of underlying epidemiology, available infrastructure, and local capacities. Recruitment was mainly focused on tertiary care and aimed at including a large proportion of children with confirmed tuberculosis to estimate the diagnostic accuracy of novel assays, so our results might not be generalisable to decentralised care or less severe forms of tuberculosis disease. Sampling strategies differed between study centres; notably, gastric aspirate was predominantly collected at the Indian site, which contributed smaller numbers of children but higher confirmation rates overall. The RaPaed-TB study was not powered to compare different microbiological-testing approaches, and the results in this Article do not include incremental diagnostic accuracy that accounts for false positives or negative results, as defining a robust and reliable reference standard is difficult (a limitation inherent to all disease with an imperfect gold standard). Furthermore, uncertainties around the reduced specificity of Ultra trace for tuberculosis disease persist.[11,30] We did not control for factors that might influence specimen quality, such as time of sampling or interval between samples. Furthermore, some specimen types and subgroups, such as children with HIV or SAM, were small. Nevertheless, there were important findings in these specific subgroups regarding optimal diagnostic strategies. Finally, although urine and stool testing were done, they were not endorsed by local guidelines and thus not reported to the clinical teams.

In summary, our results highlight the benefit of concurrent sampling and testing to improve the detection of *M tuberculosis* in children. The study findings have contributed to updated WHO recommendations on the diagnosis of paediatric tuberculosis, which now strongly indicate concurrent sampling and testing. Although the rates of microbiological detection in this Article are among the highest reported in a diagnostic study, they emphasise the need for novel, improved, point-of-care tests and age-adjusted and comorbidity-adjusted testing strategies.

Contributors

NH, LO, MH, IS, NEN, HJZ, VPV, JSM, ELC, MN, and CG acquired funding. LO, RSo, SMG, PN, AT, IS, CG, NH, and HJZ designed the RaPaed-TB study. LO, ZF-S, HJZ, and LL designed this secondary analysis. ZF-S, DB, CK, IS, NEN, RSe, MN, HJZ, VPV, JSM, MMN, ELC, CG, AR, ES, MPN, and TDM conducted the RaPaed-TB study and collected data. LO, AR, LL, and NH curated data. LO, LL, ZF-S, NH, and HJZ did the formal analysis and wrote the original draft of the manuscript. LL and NH accessed and verified the data. LO, NH, AR, and MH did project administration. LO, AR, and NH were supervisors. LL and LO visualised data. HJZ and NH critically reviewed data. All authors revised and approved the final version of the manuscript, had full access to all data in the analysis, and had final responsibility for the decision to submit for publication.

Declaration of interests

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Data sharing

De-identified data collected for the RaPaed-TB study, including individual participant data and a data dictionary defining each field in the set, is available at https://data.mendeley.com/datasets/9zb8pjzfgf/1. The study protocol has been previously published[17] and informed consent forms and the statistical analysis plan will be made available with publication upon reasonable request to the corresponding author with investigator support and a signed data access agreement.

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References

1 Dodd PJ, Yuen CM, Sismanidis C, Seddon JA, Jenkins HE. The global burden of tuberculosis mortality in children: a mathematical modelling study. *Lancet Glob Health* 2017; **5:** e898–906.

2 WHO. Global tuberculosis report 2023. 2023. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023 (accessed June 28, 2024).

3 WHO. Roadmap towards ending TB in children and adolescents. 2018. https://www.who.int/publications/i/item/9789241514668 (accessed June 28, 2024).

4 WHO. Roadmap towards ending TB in children and adolescents, 3rd ed. 2023. https://www.who.int/publications/i/item/9789240084254 (accessed June 28, 2024).

5 Marcy O, Borand L, Ung V, et al. A treatment-decision score for HIV-infected children with suspected tuberculosis. *Pediatrics* 2019; **144:** e20182065.

6 WHO. WHO consolidated guidelines on tuberculosis: module 5: management of tuberculosis in children and adolescents. 2022. https://www.who.int/publications/i/item/9789240046764 (accessed June 28, 2024).

7 DiNardo AR, Detjen A, Ustero P, Ngo K, Bacha J, Mandalakas AM. Culture is an imperfect and heterogeneous reference standard in pediatric tuberculosis. *Tuberculosis (Edinb)* 2016; **101S:** S105–08.

8 Thomas TA. Tuberculosis in children. *Thorac Surg Clin* 2019; **29:** 109–21.

9 Nicol MP, Zar HJ. Advances in the diagnosis of pulmonary tuberculosis in children. *Paediatr Respir Rev* 2020; **36:** 52–56.

10 Zar HJ, Workman LJ, Prins M, et al. Tuberculosis diagnosis in children using Xpert Ultra on different respiratory specimens. *Am J Respir Crit Care Med* 2019; **200:** 1531–38.

11 Kay AW, Ness T, Verkuijl SE, et al. Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children. *Cochrane Database Syst Rev* 2022; **9:** CD013359.

12 WHO. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. 2017. https://www.who.int/publications/i/item/WHO-HTM-TB-2017.04 (accessed June 28, 2024).

13 Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. *Lancet Infect Dis* 2003; **3:** 624–32.

14 Marais BJ. Childhood tuberculosis: epidemiology and natural history of disease. *Indian J Pediatr* 2011; **78:** 321–27.

15 Zar HJ, Tannenbaum E, Hanslo D, Hussey G. Sputum induction as a diagnostic tool for community-acquired pneumonia in infants and young children from a high HIV prevalence area. *Pediatr Pulmonol* 2003; **36:** 58–62.

16 Zar HJ, Workman L, Isaacs W, Dheda K, Zemanay W, Nicol MP. 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. *Lancet Glob Health* 2013; **1:** e97–104.

17 Olbrich L, Nliwasa M, Sabi I, et al. Rapid and accurate diagnosis of pediatric tuberculosis disease: a diagnostic accuracy study for pediatric tuberculosis. *Pediatr Infect Dis J* 2023; **42:** 353–60.

18 WHO. Consolidated guidelines on HIV testing services (2015). 2015. https://www.who.int/publications/i/item/9789241508926 (accessed Sept 12, 2024).

19 WHO. WHO child growth standards and the identification of severe acute malnutrition in infants and children. 2009. https://www.who.int/publications/i/item/9789241598163 (accessed June 28, 2024).

20 Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. *Lancet* 2005; **365:** 130–34.

21 Graham SM, Cuevas LE, Jean-Philippe P, et al. Clinical case definitions for classification of intrathoracic tuberculosis in children: an update. *Clin Infect Dis* 2015; **61** (suppl 3)**:** S179–87.

22 Song R, Click ES, McCarthy KD, et al. Sensitive and feasible specimen collection and testing strategies for diagnosing tuberculosis in young children. *JAMA Pediatr* 2021; **175:** e206069.

23 Zar HJ, Workman L, Isaacs W, et al. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. *Clin Infect Dis* 2012; **55:** 1088–95.

24 Kay AW, Rabie H, Maleche-Obimbo E, Sekadde MP, Cotton MF, Mandalakas AM. HIV-associated tuberculosis in children and adolescents: evolving epidemiology, screening, prevention and management strategies. *Pathogens* 2021; **11:** 33.

25 Kay AW, González Fernández L, Takwoingi Y, et al. Xpert MTB/RIF and Xpert MTB/RIF Ultra assays for active tuberculosis and rifampicin resistance in children. *Cochrane Database Syst Rev* 2020; **8:** CD013359.

26 Marcy O, Ung V, Goyet S, et al. Performance of Xpert MTB/RIF and alternative specimen collection methods for the diagnosis of tuberculosis in HIV-infected children. *Clin Infect Dis* 2016; **62:** 1161–68.

27 Vonasek BJ, Radtke KK, Vaz P, et al. Tuberculosis in children with severe acute malnutrition. *Expert Rev Respir Med* 2022; **16:** 273–84.

28 Wagnew F, Worku W, Dejenu G, Alebel A, Eshetie S. An overview of the case fatality of inpatient severe acute malnutrition in Ethiopia and its association with human immunodeficiency virus/tuberculosis comorbidity-a systematic review and meta-analysis. *Int Health* 2018; **10:** 405–11.

29 Brent AJ, Mugo D, Musyimi R, et al. Bacteriological diagnosis of childhood TB: a prospective observational study. *Sci Rep* 2017; **7:** 11808.

30 Signorino C, Votto M, De Filippo M, Marseglia GL, Galli L, Chiappini E. Diagnostic accuracy of Xpert Ultra for childhood tuberculosis: a preliminary systematic review and meta-analysis. *Pediatr Allergy Immunol* 2022; **33** (suppl 27)**:** 80–82.