
Out of the
Department for Infectious Diseases & Tropical Medicine, Klinikum der Universität München, LMU

# Evaluation of new diagnostics for Tuberculosis in children

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at the Medical Faculty of
Ludwig-Maximilians-Universität, Munich

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# Table of content

## Key Words

Tuberculosis

Childhood tuberculosis

pulmonary tuberculosis

diagnostics evaluation

Xpert MTB/RIF Ultra

# Abstract

**Background:** Tuberculosis (TB) remains a significant cause of death in children. Availability of a rapid and accurate diagnostic test for TB is recognized as an important tool of the END TB strategy yet delayed or missed diagnosis is still common in children. This work report findings of the Reach4Kids Africa (R4KA) study cohort on the diagnostic accuracy of Xpert MTB/RIF Ultra (Ultra) for the diagnosis of pulmonary tuberculosis (PTB) in children. Further analysis on diagnostic performance characteristics and semiquantitative readouts categories of Ultra results have been described in the RaPaed-AIDA-TB cohort.

**Methods:** From the R4KA study cohort, between July 2017 to December 2019, children with presumed TB were prospectively enrolled at clinical sites in three African countries. Children were assessed using history, physical examination and chest X-rays. Sputum or gastric aspirate samples were analysed using Ultra, and culture. The diagnostic accuracy of Ultra was calculated against culture as the reference standard. Analysis of semiquantitative readout categories of Ultra results and the incremental yield from second sample was assess in children enrolled in the RaPaed-AIDA-TB project which is being conducted in three sites in Africa and one site in India.

**Results:** From the R4KA study cohort, a total of 547 children were included. The median age was 4.7 years, 77 (14.1%) were HIV infected, and 77 (14.1) had bacteriologically confirmed TB. The sensitivity of Ultra was 66.3% (95% CI, 47-82), and the specificity was 95.4% (95% CI, 89-99) when assessed against culture. Ultra, detected an additional 20 cases in the group of children with negative culture results. In the RaPaed-AIDA-TB cohort, 786 children were included, and the median age was 5.7 years. In 234 children who were diagnosed with confirmed TB, 85 (36.3%) were confirmed by Ultra alone.

**Conclusion:** Despite the improved performance of Ultra as compared to reports of the previous cartridge version, Xpert MTB/Rif, its sensitivity remains sub-optimal for the detection of TB in children. Findings from the RaPaed-AIDA-TB study, add important data on the diagnostic accuracies of novel assays.

# List of figures

# List of tables

# List of abbreviations

| | |
|---|---|
| BCG | Bacillus Calmette–Guérin |
| CD4 | Cluster of Differentiation 4 |
| CFU | Colony Forming Unit |
| CIH | Centre for International Health |
| CRF | Case Report Form |
| CSF | cerebrospinal fluid |
| DNA | Deoxyribonucleic acid |
| DR | Drug resistance |
| eCRF | Electronic Case Report Form |
| eMRS | Extended Microbiological Reference Standard |
| ERC | Expert review committee |
| GA | Gastric Aspirate |
| GCP | Good Clinical Practice |
| HIV | Humman Immunodeciency Syndrome |
| ICF | Informed Consent Form |
| ICH | International Council for Harmonisation |
| IRB | Institution Review Board |
| LAM | *Lipoarabinomannan* |
| LMU | Ludwig-Maximilians-University |
| LTFU | Lost to follow up |
| MDR | Multi-drug resistant |
| MMRC | Mbeya Medical Research Center |
| MRS | Microbiological Reference Standard |
| MTB | *Mycobacterium tuberculosis* |
| MUAC | Mid Upper Arm Circumference |
| NIMR | National Institute for Medical Research |
| NTPs | National TB Programs |
| OR | Odd Ratio |
| PCR | Polymerase Chain Reaction |
| POC | Point of Care |
| PTB | Pulmonary Tuberculosis |
| R4KA | Reach for Kids Africa |

| | |
|---|---|
| Rapid-TB | Rapid and Accurate Diagnosis of Paediatric TB |
| RIF | Rifampicin |
| RNA | Ribonucleic acid |
| SDG | Sustainable Development Goals |
| SI | Sputum Induction |
| SOP | Standard Operating Procedure |
| STARD | Standards for Reporting Diagnostic accuracy studies |
| TAM | T-cell activation and maturation marker |
| TB | Tuberculosis |
| TPPs | Target Product Profiles |
| TST | Tuberculin Skin Test |
| Ultra | Xpert MTB/RIF Ultra® |
| WHO | World Health Organization |
| Xpert | Xpert® MTB/RIF® |
| ZN | Ziehl-Neelsen |

# 1. Introduction

## 1.1 Global Situation of Childhood Tuberculosis

Childhood Tuberculosis (TB) contributes a significant proportion of the total global TB burden. In the 2021 Global TB report, among the HIV negative people it was estimated that children below 15 years of age represented 11% (1.1 million cases) of the 10 million total TB incident cases and 16% (239,000 deaths) of the total TB deaths.[1] The highest TB burden occurs in children less than 5 years old and in children with HIV, and still a large proportion remain missed without being treated or notified to the health systems. It was projected that up to 69% of missed cases, occur in children under five years of age.[2] This underlines the challenges of diagnosis and the difficulties of reporting the true magnitude of the burden of TB disease among children.

Sustainable development goal (SDG) number 3 calls for ensuring healthy lives and promoting wellbeing for all at all ages. This goal highlights the importance of investing in prevention and treatment for children and adolescents as an effective strategy for reducing the global burden of TB. Furthermore, the 2018 United Nations General Assembly High Level Meeting on the Fight Against TB committed to diagnosing and treating 3.5 million children with TB by 2022, but still, only 1.4 million (41%) children with TB were diagnosed and treated by 2020 (Figure 1.1).[1] The challenges of diagnosing TB in children calls for global effort to invest in capacity building focused on improving skills and infrastructures especially in developing countries for timely recognition of TB; improving diagnostic support, treatment and preventative measures as key measures to ensuring good outcomes in children with TB.

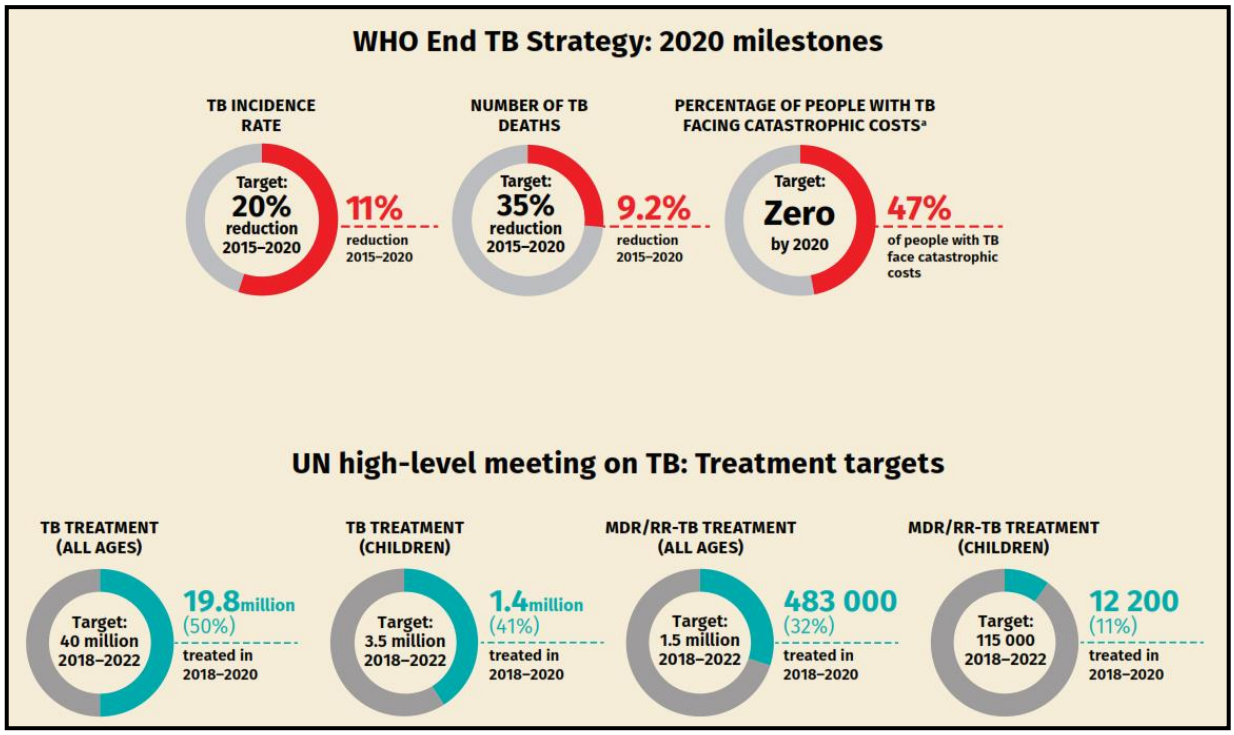


*Figure 1.1: Global tuberculosis report 2021; WHO End TB strategy: 2020 milestones; from Global tuberculosis report 2021*

Availability of a rapid and accurate diagnostic test for TB is recognized as an important tool to enable early diagnosis and early initiation of treatment in children as the slow progress towards achieving paediatric TB targets is partly due to diagnostic challenges. Children often present with non-specific clinical and radiological findings [3] and obtaining samples for microbiological confirmation is always difficult in children. As a result, sample collection is often not attempted and most children are initiated on TB treatment based on clinical grounds alone.[4,5]

Increased attention and advocacy towards evaluating novel TB tests has led to a number of new tests being developed with potential to become game-changers for childhood TB diagnostics. The WHO target product profiles (TPPs) that were developed in 2014, define priority needs of the end users with the optimal performance characteristics of the new tests that test developers should meet.[6] The identified four priority needs for new TB diagnostics are: a rapid sputum-based test for detecting TB at the microscopy-center level,

a rapid biomarker-based non sputum test for detecting TB that should also be suitable for children, a triage test of referral test for identifying patients suspected of having TB, and a test for next-generation drug-susceptibility testing. Recently, further guidance on the design of diagnostic accuracy studies for evaluation of novel TB tests that meet WHO TPPs in order to facilitate evidence generations in the country of intended use have been published.[7–10]

## 1.2 Literature Review

### 1.2.1 The challenges of TB Diagnosis in children

Tuberculosis (TB) in children often paucibacillary in nature and present with non-specific clinical and radiological features which make clinical diagnosis difficult. Obtaining samples for microbiological confirmation of TB diseases is usually challenging due to inability of the child to cough out sputum. Sputum Induction (SI), Nasopharyngeal aspiration (NPA), and gastric aspiration (GA) are the common sample recovery methods in children but are not straight forward to perform, very unpleasant to the child, and have sup optimal microbiological yield when used with smear, culture or molecular methods.[11,12] This often lead to diagnosis delays which contribute to the difficulties of controlling TB diseases in children.

In most high TB burden settings, the diagnosis of TB in children is usually based on history of TB contacts, positive Tuberculin Skin Test (TST) and radiological findings suggestive of TB. However, the contact history is often not clear, the tuberculin test can be false positive due to routine BCG immunization, and chest X-rays are non-specific and difficult to interpret, which make clinical diagnosis difficult. Common viral and bacterial infections of the lung may also present with similar symptoms and radiological features.[13,14] Therefore, reliance on clinical or radiological findings alone may result in either under or over

treatment which may lead to poor outcome and or unnecessary use of costly anti - TB drugs in children, and missed alternative diagnoses.

Furthermore, a significant proportion of paediatric patients may be asymptomatic during the initial stages of the disease (subclinical diseases), and in those who are symptomatic the symptoms may include cough, fever, weight loss, night sweats, and wheezing. Previous studies were done to attempt to determine signs and symptoms based score charts to aid childhood TB diagnosis but results showed poor sensitivity and specificity because symptoms can be similar to symptoms of other diseases such as pneumonia, and asthma.[15,16]

### 1.2.2 Currently available tools and the needs for new TB diagnostics in children

*Smear microscopy and sputum culture*

Confirmation of *Mycobacterium tuberculosis* (MTB) in PTB can be performed by smear microscopy, culture or identification of MTB cellular components such as nucleic acid or cell wall in biological samples. The introduction of fluorescent microscopy with Auramine staining has improved the sensitivity of microscopy and reduced operator time when compared with traditional acid-fast stains, such as Ziehl-Neelsen (ZN). However, this advance did not improve significantly the performance of smear microscopy which currently detects only 10-15% of tuberculosis cases in children.[17]

Sputum culture is the commonly used reference standard for microbiological confirmation of TB. Despite the high specificity of sputum culture, its sensitivity is relatively poor in children. In one study, the sensitivity of culture ranged from 30% to 40% when compared with the clinical reference standard for the detection of TB in children[17] although this could be attributable to low specificity of the clinical reference standard itself. Despite the imperfectness of the clinical reference standards this finding suggests that even with culture,

a significant proportion of TB in children goes undetected. The other limitations of the traditional culture technique are slow turnaround time, high cost of the equipments and the need for highly trained personnel, making it difficult to use as a routine test in a setting with limited resources.

*Molecular tests for TB diagnosis in children*

Several commercial molecular tests were developed to be used in the diagnosis of MTB and determination of drug resistance (DR). Line probe assay technology was the first molecular tests to be endorsed by the WHO for detection of TB and simultaneously detect DR.[18] The test uses polymerase chain reaction to rapidly identify MTB, and DR related mutations simultaneously. In 2010, the WHO endorsed Xpert MTB/RIF assay (Xpert; Cepheid Inc, CA, USA), the first near point of care and fully automated assay used for TB diagnosis that relies upon PCR techniques for detection of TB and rifampicin resistance. In 2013, Xpert assay was further recommended to be used as the initial diagnostics tests for TB in children.[19] Early evaluation studies supported this recommendation by demonstrating that Xpert has improved accuracy for detection of TB, compared to smear microscopy in children.[20,21] Despite considerable advantages of Xpert assay for offering rapid diagnosis for TB, it has a higher limit of detection (112.6 CFU/ml) as compared to liquid culture [22] which reduce its sensitivity in samples with low bacterial load such as in immunocompromized patients and children. Therefore, the initial WHO recommendations improved the detection of TB disease mostly in adults, but as observed in several previous studies [23–27] the sensitivity remained suboptimal in children. In previous systematic reviews and meta-analysis, the pooled sensitivity of Xpert in children was 62% [27] compared to 89% [28] in adults, pointing out the continued need for a new test with improved diagnostic accuracy in children.

In 2017, Xpert MTB/RIF Ultra assay (Ultra) (Cepheid Inc, CA, USA), an advanced version of the Xpert MTB/RIF assay with improved sensitivity for simultaneous detection of TB and Rif resistance was introduced. Specifically, Ultra is equipped with two additional molecular targets (IS6110 and IS 1081), a large chamber for DNA amplification, fully nested nucleic acid amplification, faster thermos cycling, and improved assay chemistry.[29] The Ultra assay runs on the Genexpert platform and its limit of detection is equivalent to the 15.6 CFU/ml limit of detection of liquid culture versus 112.6 CFU/ml for Xpert.[30] In a multicenter evaluation study (by FIND) in adult patients with PTB, Ultra demonstrated higher sensitivity than Xpert in smear negative and HIV infected patients and improved accuracy for detection of Rifampicin resistance.[31] Available data from limited studies in children also indicated a higher sensitivity for Ultra.[32–34] In a recent meta-analysis of Xpert and Ultra assays for active TB and Rif resistance in children, Ultra demonstrated a pooled sensitivity of 72.5% and specificity of 97.5%.[35] After endorsement of Ultra by WHO for detection of TB disease and Rif resistance, the same recommendations that apply for Xpert, has been adopted by the guidelines.[36] Recently WHO has further recommended the use of Ultra in GA and stool samples for the diagnosis of TB in children.[37] However, the evidence base to support this practice in children remains small; only few studies using archived frozen samples and limited by small sample size have been published to date.[32–34] Data from large prospective studies especially using freshly collected samples are still lacking.

The recent development of the GeneXpert® Omni by Cepheid ((Sunnyvale, CA, USA) resulted in a platform that allows for placement in microscopy centres. This will leverage existing infrastructure around smear microscopy and enable earlier diagnosis and treatment for TB. The Omni runs the same PCR based cartridges and the GeneXpert plat-

forms therefore was expected to have similar diagnostic accuracies as the previous GeneXpert platforms. Unfortunately, Cepheid has halted the commercialization of the GeneXpert Omni therefore it is unlikely that it will be available for evaluation in future studies.[38]

Very recently, a new PCR based assay, the Truenat (Molbio Diagnostics, Goa, India) testing system was introduced. The system is designed to be operated in peripheral laboratories with minimal infrastructure. In 2020 WHO reviewed the performance characteristics of this molecular point-of-care test for TB and endorsed the test for TB diagnosis. The Truenat testing systems takes about 25 minutes for DNA extraction and another 35 minutes to detect TB. The Truenat testing system uses portable, battery-operated devices to rapidly detect MTB and RIF resistance. The instruments are compact, portable and can be operated with a battery-pack, making them ideal for use at the point of care. The limit of detection was determined to be 100 CFU/ml sputum sample which is relatively similar to the widely used Xpert.[39–41] In a large multi-center, which included 1807 adult participants with suspected PTB from sites in India, Peru, Ethiopia and Papua New Guinea, the overall sensitivity estimates of Truenat was 73%.[39] The sensitivity was much lower in smear-negative, culture-positive participants (36%), which raises concerns on it performance in children who usually have paucibacillary TB disease. Unlike the Xpert assay, there is a pipetting step needed after extraction which may make this assay more vulnerable to cross-contamination. The Truenat assay has not yet been evaluated in paediatric studies.

*Urine Lipoarabinomannan (LAM) for detection of TB in children*

Detection of Mycobacterial Lipoarabinomannan (LAM) antigen was introduced since 2015. LAM is a constituent of glycolipid from MTB released from metabolically active or degenerating mycobacterial cells into urine. The Alere Determine (AlereLAM; Abbott, Chi-

cago, IL, USA) was the first commercially available assay using lateral flow for LAM detection in TB patients. In a previous meta-analysis,[42,43] the sensitivity of AlereLAM was 45% in people with HIV, with higher sensitivity (56%) in patients with CD4 counts of 100 cells per µL or lower. Despite this sub optimal sensitivity, AlereLAM reduces mortality when implemented for the immunosuppressed patients with HIV.[44] For this reason, WHO recommends the use of AlereLAM in critically ill patients with HIV and in those with a CD4 count equal or less than 100 cells per µL. This suboptimal sensitivity of AlereLAM may influence poor uptakes among clinicians in routine clinical practice.

Recently, another urine based test which also detect LAM in urine, the FujiLAM SILVAMP TB LAM (FujiLAM; Fujifilm, Tokyo, Japan) was introduced. FujiLAM performance includes a 5-steps procedure according to manufactures instructions and takes around 50-60 minutes from the time of sample collection to results communication to the patient, therefore it is considered a true point of care test as results could be communicated to the patient on the same day. Earlier evaluation studies using frozen samples from adults with HIV in South Africa, FujiLAM showed promising results. In this study, while maintaining higher specificity, the sensitivity of FujiLAM was 70.4.% as compared to 42.3% of AlereLAM.[45] In the a recent study involving children less than 15 years with presumed TB from the R4KA cohort, the sensitivity of FujiLAM showed higher sensitivity of 64.9% while that of AlereLAM was 30.7%.[46] The specificity of FujiLAM was similar to that of AlereLAM.

Given the substantial improved sensitivity of FujiLAM, it has the potential for being an important test for TB diagnosis in HIV infected patients and in children who are known to be paucibacillary in nature. Unpublished reports suggest that urine LAM tests are prone to false positive results if stool is contaminated into urine which could be an issue for smaller children who cannot collect midstream urine. Therefore, further studies to assess the utility of Urine LAM tests for the diagnosis of TB in children are still needed.

## 1.3 Rationale and Objectives

Accurate and consistent diagnosis of childhood TB is critical for effective management of TB, for measuring the precise burden of childhood TB, the TB-related morbidity and mortality, and to establish drug susceptibility patterns. Microbiological confirmation of childhood TB often rely on tests from sputum samples which apart from its pauci pacillary nature, it is difficult to obtain in children.[13] The introduction of the Xpert assay which was endorsed by WHO as initial diagnostic test for children, showed improved sensitivity against gold standard however, its sensitivity was inferior to that of culture, a problem especially in pauci-bacillary specimens mainly from children and HIV-coinfected persons.[21,47] Due to these difficulties, bacteriological confirmation is often not even attempted in most of the children. These challenges point out the need for new test with improved sensitivity overall, even above the current gold standard.[48] Furthermore, the availability of tests which work on samples that are easier to obtain than sputum and which will have sensitivity and specificity comparable to the current gold standard, may solve the problem associated with sputum collection and therefore improve the overall detection of TB cases in children.[31] In this study we aimed at evaluating the diagnostic accuracies of Ultra assay for the diagnosis of TB using fresh respiratory specimens in children with presumed TB. Furthermore, we present the RaPaed-AIDA-TB study microbiology results as a result of the joint consortium work, to discuss study design work presented in this thesis, and to allow a discussion of the R4K results in perspective. In this we presented the diagnostic performance characteristics of Ultra results including the semiquantitative readouts categories. Furthermore, the potential new TB candidate tests under evaluation in the RaPaed-AIDA-TB project have been discussed.

# 2. Material and Methods

This study was partly carried out as a sub study within the Reach4Kids Africa (R4KA) study cohort which recruited participants in four African Countries namely Tanzania, The Gambia, Mali, and Nigeria. The primary objective of R4KA was to establish a repository of samples for testing performance of existing diagnostics and to validate novel assays in order to develop reliable diagnostic tools for childhood TB. The PhD sub study involved participants from sites in Tanzania, Mali, and The Gambia.[46] The Nigerian site was not included due to lack of sputum culture capacity.

To further characterize the diagnostics performance of Ultra and describe the methodological approaches for the evaluation of new TB diagnostics in children, this PhD project was extended into the RaPaed-AIDA-TB study which had the primary objectives to evaluate the sensitivity and specificity of new candidate tests for detecting childhood TB and calculate positive and negative predictive values in the study population.

## 2.1 PhD student's own contribution

I was the principal investigator (PI) in Tanzania for the primary R4KA study which generated data for the PhD sub study. I was responsible for the oversight and overall conduct of the study in Tanzania, I participated in the conceptualization of the primary study design, writing the protocol, and data collection. I was responsible for the design of the PhD sub study and performed data analysis with the support of a statistician.

Furthermore, I was the Co-principal investigator for the RaPaed-AIDA-TB study, and I was part of the expert group that designed the inclusion and exclusion criteria and data analysis pathway for the study and participated in data analysis and publication. The results of this study are presented as output of the consortium work, mainly to put the R4KA and study design work into context.

## 2.2 Reach4Kids Africa study cohort

### 2.2.1 Study Sites

In Tanzania, the study was led by the National Institute for Medical Research, Mbeya Centre (NIMR-MMRC) and participant recruitment is conducted at the outpatient and in-patient department of the Mbeya Regional Referral hospital. In the Gambia, the study was led by The Medical Research Council Unit The Gambia at the London school of Hygiene and Tropical Medicine (MRCG at -LSHTM) in Fajara, The Gambia in the Greater Banjul area, where two thirds of all TB patients diagnosed and notified in the country, and in Mali, study was led by the University Teaching Hospital Gabriel Toure (CHU-Gabriel Toure), Bamako, Mali. NIMR – MMRC was responsible for coordination of this PhD sub study across all sites.


*Figure 2.1: Countries in which participants were recruited for the Reach4kids Africa study cohort (Tanzania, Nigeria, Mali, and the Gambia). The Nigerian site was not involved in the PhD sub study.*

The selected three study sites had adequate clinical and partner facilities for recruitment and follow up of participants and all are equipped with Genexpert instruments, liquid and solid culture and drug resistance testing. All sites have established link with the respective National TB Programs (NTPs) in respective countries.

### 2.2.2 Study design

A cross sectional study of prospectively recruited children presenting with symptoms suggestive of active TB.

### 2.2.3 Study population

Data from children below 15 years of age who had symptoms compatible with active TB (cough persisting for more than 2 weeks, failure to thrive, and persistent unexplained fever) were abstracted from the R4KA study database.

Between July 2017 and December 2019, children aged below 15 years with presumed TB were consecutively enrolled and followed up at a dedicated outpatient childhood TB clinics at each the respective institutions. Children were eligible for enrolment if they had symptoms suggestive of PTB characterised by persistent or unremitting cough for more than two weeks and any of weight loss/failure to thrive, or persistent unexplained fever.

### 2.2.4 Sampling

All enrolled children at the Tanzanian, Mali, and The Gambian sites were included in the analysis. There was no sample size calculation due to secondary nature of the study. For the primary study it was anticipated that 75 children will be diagnosed with active TB and will be commenced on TB treatment. Based on previous experience, we anticipated an estimated 30% of the TB cases will be confirmed TB by culture and/or Ultra; the rest will be classified as unconfirmed TB. The remaining children were categorized as “unlikely TB”. HIV testing were carried out in all children following counselling and those found to be HIV-infected were referred to the national HIV program for care. All children were followed up at 1, 3 and 6 months to record any changes in initial classification. Through clinical assessment and microbiological evaluations, children were categorised as having Confirmed TB, Unconfirmed TB, or Unlikely TB by experienced clinicians based on the NIH consensus statement.[49] Children who were diagnosed with TB disease (“confirmed” or “unconfirmed TB”) received appropriate treatment as the per the National TB Programme guidelines. Children with a confirmed alternative diagnosis received appropriate

non-TB therapy and were included in the “unlikely-TB” group and were only clinically followed-up. Children from whom samples were not obtained or had no valid Ultra or culture results were not included in the diagnostic category.

| Diagnostic classification | Description of definition |
|---|---|
| **Confirmed tuberculosis** | Bacteriological confirmation obtained<br>*Requires Mycobacterium tuberculosis to be confirmed (culture or Xpert® MTB/RIF (Ultra®) assay) from at least 1 specimen* |
| **Unconfirmed tuberculosis** | Bacteriological confirmation NOT obtained AND at least 2 of the following:<br>• Symptoms suggestive of TB<br>• CXR consistent with TB<br>• Recent exposure or immunologic evidence of MTB infection (TST and/or IGRA positive)<br>• Positive response to TB treatment<br>*Requires documented positive clinical response on tuberculosis treatment - no time duration specified*<br><br>With M. tuberculosis infection<br>*Immunological evidence of M. tuberculosis infection (TST and/or IGRA positive)*<br><br>Without M. tuberculosis infection<br>*No immunological evidence of M. tuberculosis infection* |
| **Unlikely tuberculosis** | Bacteriological confirmation NOT obtained AND criteria “unconfirmed TB” not met<br><br>With M. tuberculosis infection<br>*Immunological evidence of M. tuberculosis infection (TST and/or IGRA positive)*<br><br>Without M. tuberculosis infection<br>*No immunological evidence of M. tuberculosis infection* |

*Figure 2.2: Case definitions by based on the NIH consensus; reproduced from Graham SM, et al. Clin Infect Dis. 2015;61*

### 2.2.5 Clinical and Laboratory Procedures

Clinical and laboratory data were obtained from the R4KA database. Information obtained from children with presumptive TB comprised of medical history of participants, standardised clinical examination with collection of detailed anthropometric data such as middle upper arm circumference (MUAC), weight, height, HIV testing using National guideline

and chest radiography. Study information and informed consent procedures was conducted through the main R4KA study.

Sputum or gastric aspirate samples were collected from all children as per respective site standard operating procedures. In Tanzania we collected only one sample type, i.e sputum and 2 samples were obtained per child by SI. In The Gambia, one sample was obtained by SI and when not feasible, a gastric aspirate (GA) sample was collected, while in Mali one sample was obtained by GA.

In brief, SI was performed in younger children who were not able to expectorate spontaneously. After pre-treatment with an inhaled bronchodilator, nebulization with hypertonic (5%) saline was performed, and secretions were obtained by suctioning or by expectoration in older children. Precautions were taken to prevent nosocomial transmission during sputum induction. The procedures were performed in a well-ventilated room, and sufficient time were allowed between procedures. Appropriate particulate respirators (N95 or FFP2) were provided to staff who performed by the procedures. Early morning GA were the alternative method used to obtain sample for mycobacteriological confirmation. In this method, specimens were taken in the early morning before breakfast to achieve optimal yields.

At all sites, sputum samples were obtained by spontaneous expectoration in older children who were able to expectorate.

Obtained samples were then tested for fluorescence microscopy, Ultra, and culture on mycobacteria Growth Indicator Tubes (MGIT liquid culture) and solid culture in Lowenstein Jensen medium (LJ) (Beckton and Dickson Company, MD, USA) following the manufacturer's instructions. Growth of M. tuberculosis in culture was confirmed by ZN stain, lateral flow antigen test (MPT64, Beckton and Dickson Company, MD, USA) or

MTBDRplus line probe assays (Hain Lifesciences, Nehren, Germany). All laboratory tests results were performed blinded to information about clinical course at the study laboratory of the respective sites.

### 2.2.6 Data management and data analysis

All data were double entered into password protected Microsoft Access database (Microsoft Cop. Redmond, WA), compared and corrected for data entry errors and were analysed using R statistical software (version 4.1.1).

Data were summarized by frequency and proportions for categorical variables and medians and interquartile ranges for continuous variables. In the primary analysis, the diagnostic sensitivity and specificity of Ultra were calculated against culture as a Microbiological reference standard (MRS). We also calculated the sensitivity of Ultra against an extended microbiological reference standard (eMRS) defined as positive results of both Ultra and/or culture, in children with confirmed TB as was recently suggested.[50] With eMRS, information on specificity could not be presented to avoid inclusion bias as positive Ultra results led to decision to initiate TB treatment, and therefore, children with corresponding negative culture results could not be classified as unlikely TB disease. Statistical significance was accepted at p-values of $<0.05$.

For subgroup analysis stratified by country, smear results, HIV status, age, nutritional status, we estimated the pooled sensitivity, specificity, and 95% CI using the Bayesian bivariate random-effects meta-analysis to account for the possible effects of heterogeneity across the subgroups.

### 2.2.7 Ethical Considerations

Ethical clearance and approval to conduct the study were obtained from the respective ethics committees of the participating sites namely the Tanzanian Medical Research Co-ordinating Committee, the Gambian Government and MRC Joint Ethics committee, and Ethics committee of the Faculty of Medicine, Pharmacy and Dentistry of the University of Sciences, Techniques and Technologies of Bamako (USTTB). The PhD proposal was in addition reviewed by the Institutional Review Board (IRB) of the University of Munich. Prior to any study-specific procedure, signed written consent/assent or witnessed oral consent/assent in the case of illiteracy is obtained. Data obtained from the parent study were treated in a confidentiality manner, and unauthorized persons had no access to the collected data. No personal identification information was captured in the R4KA study database; therefore, participant was identified only by study identification number, which were kept confidential throughout the study.

## 2.3 Rapid and accurate diagnosis of paediatric TB disease (RaPaed-AIDA-TB study) cohort

### 2.3.1 Study sites

The Rapaed-AIDA-TB study is not yet closed and is recruiting a population similar to the R4KA study. Participants are enrolled at (1) NIMR-MMRC, Tanzania, (2) University of Cape Town Lung Institute (UCTLI), South Africa (3) University of Malawi College of Medicine, Malawi (4) Instituto Nacional de Saúde (INS), Mozambique, and at the Paediatrics Unit-III & Pediatric Infectious Diseases, Christian Medical College Hospital, Vellore, India. NIMR-MMRC in Tanzania is the only site that participated in both cohorts.

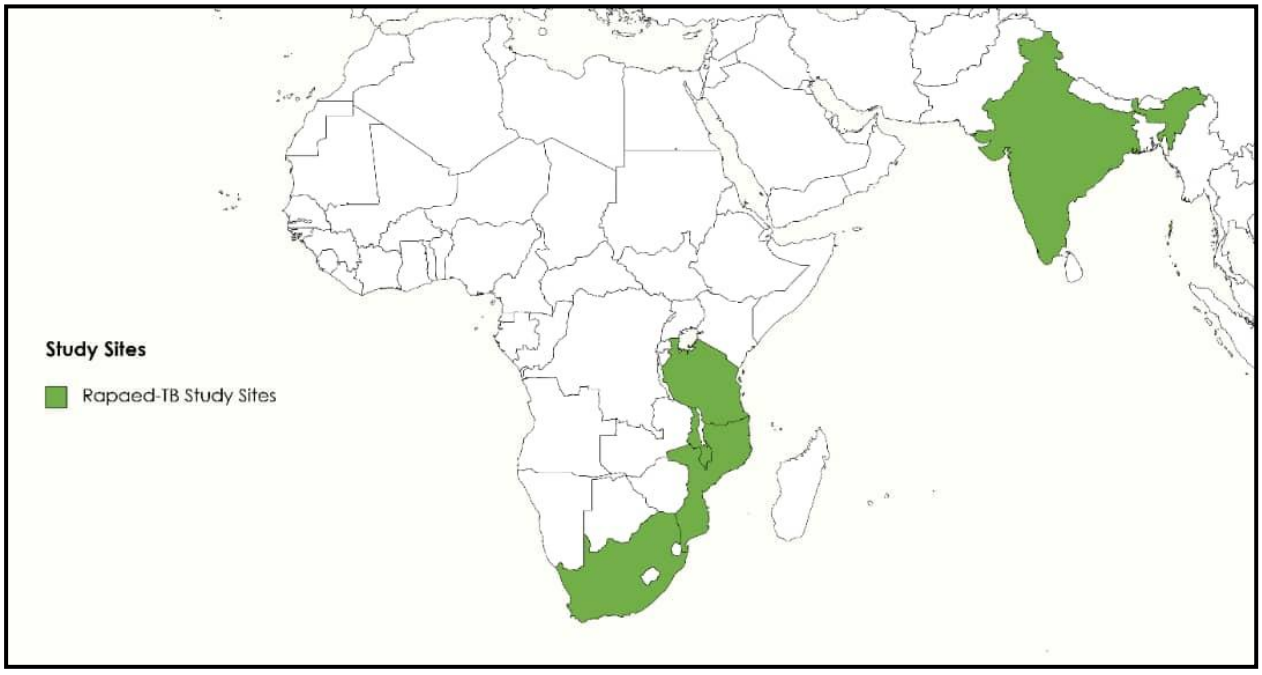


*Figure 2.3: Country in which participants were recruited for the RaPaed-AIDA-TB study cohort (Tanzania, Mozambique, Malawi, South Africa, and India)*

### 2.3.2 Study design

RaPaed-AIDA-TB is a prospective diagnostic accuracy study evaluating several novel index tests and diagnostic approaches in the target population of children below 15 years suspected of having TB.

### 2.3.3 Study population

Children below 15 years of age are enrolled based on the on two major inclusion criteria namely: i) prior confirmed TB disease, i.e., referred for study assessment following initial microbiological confirmation of active TB disease in a non-study screening, or ii) symptoms and signs of pulmonary and extrapulmonary TB. Children who are critically ill such as those with hypovolemic shock, or anaemia with tachycardia or tachypnoea per discretion of the attending clinician are excluded. Children who have body weight <2kgs or are already taking TB medication for more than 72h (treatment or prophylaxis) were also excluded from participation.

### 2.3.4 Sampling

The study aimed to recruit 1,000 paediatric presumptive TB patients across five sites (200 each) over a two-year period. A 25% microbiological confirmation was be attempted, which will allow to enrol at least 250 participants with confirmed TB across all sites. The number of 250 participants with confirmed TB will allow detection of a sensitivity similar to the increase from 62% (Xpert MTB/RIF®) to 82%, with more than 90% power at the 95% confidence level. This sample of 250 participants with confirmed TB disease will allow meaningful subgroup analyses within and between age groups, HIV, and nutrition status. At enrolment, TB diagnosis is based on microbiological samples tested positive for the presence of MTB complex (culture and/or Ultra®) and information on demographic and clinical data are captured. The study consists of baseline visit and follow up visits at month 1 and month 3, and in children on TB treatment or clinically unwell are additional visit at month 6 are performed. Similar to R4KA cohorts, children were categorised as having Confirmed TB, Unconfirmed TB, or Unlikely TB by experienced clinicians based on the NIH consensus statement.[49] Participant recruitment was initiated in January 2019.

### 2.3.5 Clinical and Laboratory procedures

At baseline visit, informed consent is obtained, and data from standard diagnostic procedures are recorded in the electronic Case Report Forms (eCRFs). The baseline visits include collection of demographic information, medical history, and physical examination. Clinical investigation includes HIV testing, tuberculin skin tests (TST), and chest X-ray or other radiology if clinically indicated. At each follow up visit, assessment of TB symptom, physical examination, CXRs, and TB treatment documentation are performed. Collection of sputum samples are performed if clinically indicated.

Respiratory samples including (SI, GA, or NPA) are collected from all children are in a standardized way across all sites. Other samples such as blood, urine and stool samples were also collected. In all African sites, consecutive sputum samples are collected in sterilized, well lockable labelled sputum container with scaling. If possible, spontaneous (i.e., voluntarily expectorated) sputum samples are collected; in younger children, sputum induction or gastric lavage is performed. Sputum induction is carried out as previously described.[12,51] At the Indian site, GA was the standard procedures for recovery of respiratory samples and the Indian investigators opted to continue using the same procedure during the study.

All laboratory procedures are performed at each study site inline to the procedures outlined in the study specific laboratory manual. The standard TB diagnostic tests which include Ultra assay, Growth Indicator Tubes (MGIT liquid culture) and solid culture in Lowenstein Jensen medium (LJ) (Beckton and Dickson Company, MD, USA) are performed following the manufacturer's instructions. Growth of M. tuberculosis in culture was confirmed by ZN stain, lateral flow antigen test (MPT64, Beckton and Dickson Company, MD, USA),[52] and at least one GenoType MTBDRplus V2® line probe assay (Hain Lifescience, GmbH, Germany).

### 2.3.6 Data management and data Analysis

Participant's demographic, clinical, and laboratory data are recorded on paper work-sheets and concurrently entered onto electronic Case Report Forms (eCRFs) using OpenClinica® software (OpenClinica® Community Edition Version: 3.12; OpenClinica LLC, Waltham, MA, USA). Data entry is monitored and reviewed regularly during the course of the study, including comparing source data with entries in OpenClinica®. Data cleaning is performed on a regular basis.

Primary Endpoint of the study is the confirmation/rule-out of TB disease, with the degrees of certainty "confirmed TB", "unconfirmed TB" or "unlikely TB" (Figure 2.2). Children are classified using information on microbiological testing, clinical signs and symptoms suggestive of TB, radiological findings, TB contact history, determination of MTB infection, and treatment response.

Stratifying by clinical case category, normally distributed continuous variables are summarised using mean and standard deviation (SD) for normally distributed variables or median and inter-quartile-range (IQR) for non-normally distributed variables. Categorical values are presented as counts, percentages and/or centiles and their respective 95% confidence intervals (95%CI).

Assessment of MRS tests including the determination of the yield of culture and Ultra for SI, spontaneous sputum, NPA, and GA. Patients were defined as having confirmed TB when having positive test result from any sample of a particular specimen (speciation available and positive). All data manipulation and statistical analyses were performed using Stata version 16 and version 17 (StataCorp, Texas, USA).

### 2.3.7 Ethical Considerations

This study is being performed in accordance with the study protocol, the declaration of Helsinki,[53] as well as any other applicable national and other regulatory guidelines. The protocol and the informed consent document used in this study were submitted to the coordinators and the responsible investigators' ethics committees for approval. Written documentation of approval of both the protocol and the informed consent were provided to the sponsor before initiation of the study. Prior to any study-specific procedure, signed written consent/assent or witnessed oral consent/assent in the case of illiteracy is obtained.

# 3. Results

## 3.1 Diagnosis of Tuberculosis in children using Xpert MTB/RIF Ultra on fresh respiratory samples: R4KA study cohort

### 3.1.1 Baseline characteristics

In total, 671 children with presumed TB were recruited. Subsequently, 134 children were excluded from the analysis including 14 with unknown TB status, 37 with no valid Ultra and/or culture results, and 83 in whom culture had not been performed. Ultra and culture results were obtained in 547 children comprising 189 from Tanzania, 116 from Mali, and 242 from The Gambia. The study flow diagram shows the flow of participants according to TB test results and case definition categories (Figure 3.1).

The median age of enrolled children was 4.7 years and 307 (56.1%) were males. Overall, 77/547 (14.1%) of the enrolled children were HIV infected and 112 (20.9%) were severely malnourished. Overall, 86.8% had at least some malnutrition. Children enrolled from Mali had the highest prevalence for both HIV (26.7%), and severe malnutrition (36%). Among all enrolled children, 460 (84.3%) had TB tests performed on sputum samples, while 86 (15.5%) TB tests were performed on gastric aspirate samples. Detailed demographic and clinical characteristics are presented in Table 3.1.

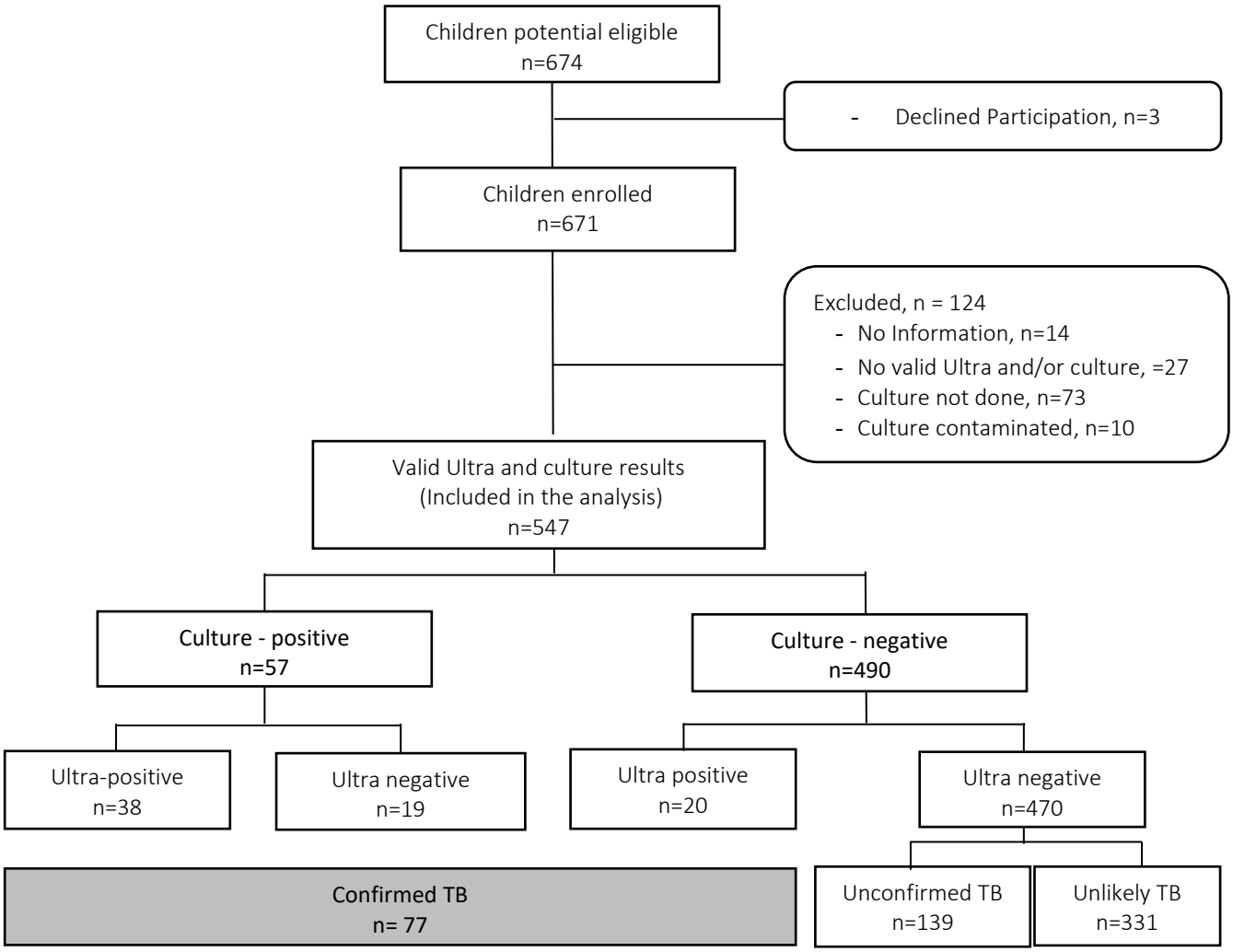


*Figure 3.1: Study flow diagram by TB case definition*

*Children were categorized using the revised classification of intrathoracic TB for evaluation of studies in children as follows: Confirmed TB group defined as a sub group of children who tested positive for TB on culture, Ultra, or both; Unconfirmed TB group defined as a sub group of children with no bacteriological confirmation but had suggestive signs and symptoms or suggestive radiological findings; and Unlikely TB group defined as a sub-group of children with no bacteriological confirmation (by culture, Ultra, or both) and no clinical diagnosis of TB.*

| Characteristics | Tanzania (N=189) | Mali (N=116) | Gambia (N=242) | Total (N=547) |
|---|---|---|---|---|
| Age in years, median, [IQR] | 3.4(1.5-7.2) | 5 (2.2-10) | 5.6(2.3-8.5) | 4.7[2.0 – 8.5] |
| Age, n (%) | | | | |
| 0–1 years | 31 (16.4) | 12(10.3) | 20 (8.3) | 63(11.5) |
| 1– 5 years | 92 (48.7) | 39(33.6) | 93 (38.4) | 224(41.0) |
| 5–10 years | 40 (21.2) | 34(29.3) | 90 (37.2) | 164(30.0) |
| 10–14 years | 26(13.8) | 31(26.7) | 39 (16.1) | 96(17.5) |
| Male Sex, n (%) | 103(54.5) | 72(62.1) | 132(54.6) | 307 (56.1) |
| HIV Status, n (%) | | | | |
| Positive | 21 (11.1) | 31 (26.7) | 25 (10.3) | 77 (14.1) |
| Negative | 163 (86.2) | 85 (73.3) | 211 (87.2) | 459 (83.9) |
| Unknown | 5 (2.7) | 0 | 6 (2.5) | 11 (2.0%) |
| BCG scar, n (%) | | | | |
| Yes | 170(90.0) | 0 | 193(79.8) | 363 (66.4) |
| No | 19(10) | 0 | 44(18.2) | 63 (11.5) |
| No information | 0 | 116(100) | 5(2.1) | 121(22.1) |
| Malnutrition Status, n (%) | | | | |
| Severe | 32(16.9) | 40 (36.0) | 40 (16.9) | 112(20.9) |
| Moderate | 24 (12.7) | 24 (21.6) | 57 (24.1) | 105 (19.6) |
| Mild | 87 (46.0) | 41 (36.9) | 121 (51.1) | 249 (46.4) |
| Normal | 46 (24.3) | 6 (5.4) | 19 (8.0) | 71 (13.2) |
| Previously treated TB, n (%) | | | | |
| Yes | 7 (3.7) | 4 (3.5) | 5 (2.1) | 16 (2.9) |
| No | 182 (96.3) | 112 (96.5) | 237 (97.9) | 531 (97.1) |
| Type of Specimen, n (%) | | | | |
| Spontaneous | 48 (25.5) | 31 (26.7) | 36 (14.9) | 115 (21.0) |
| Induced | 140 (74.5) | 0 | 201 (83.1) | 341 (62.3) |
| Gastric aspirate | 0 | 81 (69.8) | 5 (2.1) | 86 (15.8) |

*Table 3.1: Baseline Characteristics of study participants*

### 3.1.2 Clinical and laboratory presentation of enrolled participants

Overall, 77 (14.1%) had culture confirmed TB, 139 (25.4%) unconfirmed TB, and 331 (60.5%) unlikely TB. There were no positive results for drug resistance either by Ultra or

culture. Among 547 children, Ultra was positive in 58 children (10.6%), and culture was positive in 57 (10.4%) children. Among children with positive Ultra results, 38 were in the culture positive group, while 20 were in the culture negative group (Figure 3.1). Of those with positive Ultra but negative culture results, 8 were HIV positive, and 2 were previously treated for TB. Among the 2 children who were previously treated for TB, one was a HIV positive, 13 years old from Tanzania who was treated for TB in the past one year, and the other was HIV positive 8 years old from Mali who was previously treated for TB in the past 5 years at the time of enrolment.

| | **Tanzania (N=189)** | **Mali (N=116)** | **Gambia (N=242)** | **Total (N=547)** |
|---|---|---|---|---|
| Culture positive, n (%) | 21(11.1) | 23(19.8) | 13(5.4) | 57(10.4) |
| Ultra positive, n (%) | 19(10.1) | 23(19.8) | 16(6.6) | 58(10.6) |
| Diagnostic classification, n (%) | | | | |
| Confirmed TB | 25(13.2) | 31(26.7) | 21(8.7) | 77(14.1) |
| Unconfirmed TB | 23(12.2) | 69(59.5) | 47(19.4) | 139(25.4) |
| Unlikely TB | 141(74.6) | 16(13.8) | 174(71.9) | 331(60.5) |

*Table 3.2: Microbiological yield and diagnostic classification of TB*

Among all participants, cough, fever, and weight loss, were the most common symptoms reported at baseline with 80.5% (420 participants), 73.7% (403 participants), and 68.5% (374 participants) respectively. Haemoptysis was the least frequent symptom, constituting only 4.8% (26 participants). TB symptoms declined sharply both all groups regardless of the TB treatment status. At month six, cough (5.6%), fever (4.2%), and failure to gain weight (3.4%) were remained more prevalent in the unlikely TB group than in the confirmed TB and unconfirmed TB groups (Table 3.3).

| TB Symptoms | | Baseline | | | Month 6 | | |
|---|---|---|---|---|---|---|---|
| | All | Confirmed | Unconfirmed | Unlikely | Confirmed | Unconfirmed | Unlikely |
| Cough, n (%) | 420 (80.5) | 67 (91.8) | 105 (80.2) | 248 (78.0) | 2 (3.3) | 4 (5.3) | 15 (5.7) |
| Fever, n (%) | 403 (73.7) | 49 (63.6) | 104 (74.8) | 250 (75.5) | 2 (3.3) | 1 (1.3) | 11 (4.2) |
| Failure to gain weight, n (%) | 374 (68.5) | 63 (81.8) | 106 (76.8) | 205 (61.9) | 1 (1.6) | 2 (2.6) | 9 (3.4) |
| Night sweat, n (%) | 330 (60.6) | 53 (68.8) | 88 (63.8) | 189 (57.3) | 2 (3.3) | 2 (2.6) | 7 (2.6) |
| Chest Pain, n (%) | 214 (40.2) | 36 (46.8) | 55 (40.7) | 123 (38.3) | 0 | 0 | 6 (2.3) |
| Difficult in breathing, n (%) | 187 (34.6) | 39 (50.7) | 66(48.2) | 82 (25.1) | 1 (1.6) | 0 | 4 (1.5) |
| Hemoptysis, n (%) | 26 (4.8) | 4 (5.2) | 7 (5.1) | 15 (4.6) | 0 | 0 | 0 |

*Table 3.3: Clearance of TB symptoms by TB category in the confirmed and unconfirmed TB group*

### 3.1.3 Radiological presentation of enrolled participants

Chest X-ray (CXR) was performed in 498 (94.5%) participants. Among them 70 (14.1%) had microbiologically confirmed TB, 125 (25.1%) unconfirmed TB, and the rest were categorized as unlikely TB. Overall, CXR abnormalities were more prevalent in the confirmed TB and in the unconfirmed TB group and less prevalent in the unlikely TB group. Peri-hilar adenopathy were present in 21 (30%) participants and 30 (24.0%) participants in the confirmed TB and unconfirmed TB groups respectively. Only 9.9% (29 participants) of the unlikely TB group had peri-hilar adenopathy. Cavities and spine abnormalities were only present in the confirmed TB and Unconfirmed TB group, and none in the unlikely TB group. Table 3.4 describe details of radiological presentation in all children and by diagnostic categories.

| Characteristics | Confirmed TB n=70 | Unconfirmed TB n=125 | Unlikely TB n=294 |
|---|---|---|---|
| Lobar consolidation, n (%) | 44 (62.9) | 85 (68.0) | 38 (12.9) |
| Bronchopneumonic, n (%) | 37 (52.9) | 77 (61.6) | 34 (11.6) |
| Peri-hilar adenopathy, n (%) | 21 (30) | 30(24.0) | 29 (9.9) |
| Hyperinflation, n (%) | 4 (5.7) | 4 (3.2) | 26 (8.8) |
| Interstitial changes, n (%) | 23 (32.9) | 39 (31.2) | 7 (2.4) |
| Nodula infiltration, n (%) | 3 (4.3) | 7(5.6) | 6 (2.0) |
| Calcification in the lung, n (%) | 2 (2.9) | 5 (4.0) | 4 (1.4) |
| Fibrosis, n (%) | 5 (7.1) | 3 (2.4) | 4 (1.4) |
| Cavity, n (%) | 9 (12.9) | 6 (4.8) | 0 |
| Peri-hilar infiltration, n (%) | 35 (50) | 70 (56.0) | 125 (42.5) |
| Para tracheal nodes, n (%) | 7 (10) | 8 (6.4) | 7 (2.4) |
| Airway compression, n (%) | 3 (4.3) | 6 (4.8) | 1 (0.3) |
| Pleural fluid, n (%) | 6 (8.6) | 10 (8) | 4 (1.4) |
| Tracheal deviation, n (%) | 6 (8.6) | 14 (11.3) | 6 (2.0) |
| Mediastinal deviation, n (%) | 5 (7.1) | 6 (4.8) | 4 (1.4) |
| Spine abnormality, n (%) | 3 (4.3) | 2 (1.6) | 0 |
| Displaced diaphragm, n (%) | 8 (11.4) | 6 (4.8) | 2 (0.7) |

*Table 3.4: Radiological features in children with confirmed and unconfirmed TB*

### 3.1.4 Sensitivity and Specificity of Ultra

Using culture as MSR, the sensitivity of Ultra was 66.3% (95% CI 47-82), and specificity was 95.4% (95% CI 89-99) (Table 3.5). Using eMRS, we calculated the sensitivity of Ultra in a subgroup of children with positive culture and/or Ultra results combined. With this approach the sensitivity of Ultra against eMRS was 76% (95% CI: 61%-88%), while that of culture was 74% (95% CI: 53%-89%).

In the stratified analysis of sensitivity and specificity of Ultra against MRS. By country, the sensitivity of Ultra was 69.0% (95% CI 19.0-97.0) in Tanzania, 60.0% (95% CI 12.0-95.0) in The Gambia and 64.0% (95% CI 15.0-96.0 in Mali. By HIV status the sensitivity of Ultra highest in the HIV negative children (76.6%; 95% CI: 51.9-80.8) than in the HIV positive

group (57.1%; CI 24.4-85.4). The sensitivity of Ultra was higher in children aged 5-10 years (87.5%; 95% CI 58.4-98.7) and 10-14 years (78.4%; 95% CI 56.2-92.9). By nutrition status, the sensitivity of Ultra was higher in severely malnourished children (76.0%; 95% CI 56.0-91.0). However, the differences in sensitivities between groups were not statistically significant. Concerning the specificity of Ultra, it was lower in children enrolled in Mali (89.0%; 95% CI: 50-99.0), in HIV positive children (88.4 955 CI: 76.8-95.9), and children who produced GA samples (90.0%; 95% CI: 79.0-97.0). Table 3.5 demonstrate the diagnostic sensitivity and specificity in details.

| Characteristics | Sensitivity (95% CI) | n-positive/N | Specificity (95% CI) | n-negative/N |
|---|---|---|---|---|
| Overall | | | | |
| Ultra | 66.3(47.0-82.2) | 38/57 | 95.4(88.7-98.7) | 470/490 |
| Country | | | | |
| Gambia | 60.0(12.0-95.2) | 8/13 | 94.8(72.0-99.7) | 221/229 |
| Mali | 64(14.5-95.7) | 15/23 | 88.9(49.7-99.2) | 85/93 |
| Tanzania | 69.1(19.0-96.8) | 15/21 | 96.1(79.3-99.8) | 164/168 |
| HIV Status | | | | |
| HIV Positive | 57.1(24.4-85.4) | 4/7 | 88.4(76.8-95.9) | 62/70 |
| HIV Negative | 67.6(51.9-80.8) | 34/50 | 97.0(93.9-98.9) | 397/409 |
| Age | | | | |
| 0-1 years | 58.7(20.6-89.8) | 3/5 | 97.8(90.7-99.8) | 57/58 |
| 1-5 years | 47.9(26.3-69.9) | 11/23 | 97.2(92.6-99.3) | 196/201 |
| 5-10 years | 87.5(58.4-98.7) | 9/10 | 93.1(84.3-98.0) | 144/154 |
| 10-14 years | 78.4(56.2-92.9) | 15/19 | 94.8(86.3-98.9) | 73/77 |
| Malnutrition Status | | | | |
| Severe | 76.0(56.0-91.0) | 17/22 | 93.0 (86.0-98.0) | 84/90 |
| Moderate | 47.0 (21.0-75.0) | 5/11 | 97.0 (92.0-99.0) | 92/94 |
| Mild | 64.0 (42.0-83.0) | 13/20 | 95.0 (90.0-98.0) | 218/229 |
| No malnutrition | 71.0 (26.0-97.0) | 3/4 | 98.0 (92-100) | 66/67 |
| Type of Specimen | | | | |
| Spontaneous sputum | 74.0 (54.0-88.0) | 20/27 | 94.0 (87.0-98.0) | 83/88 |
| Induced | 68.0 (42.0-88.0) | 11/16 | 97.0 (94.0-99.0) | 320/329 |
| Gastric aspirate | 48.0 (21.0-75.0) | 7/14 | 90.0 (79.0-97.0) | 66/72 |

*Table 3.5: Diagnostic accuracy of Ultra using culture as Microbiological Reference Standard*

## 3.2 Diagnostic performance characteristics and semiquantitative readouts of Ultra Results: RaPaed-AIDA-TB study cohort

### 3.2.1 Baseline characteristics

A total of 974 participants were enrolled into the Rapaed-AIDA-TB study in four sites in Africa (Tanzania, Mozambique, Malawi, South Africa) and one centre in India. Among them 188 (24.9%) were excluded from the analysis due to missing data at the time of this analysis, as detailed in figure 3.2. Of the 786 participants who could be classified into one of the diagnostic categories, 195 (25.0%) were enrolled from South Africa, 220 (28.2%) from Tanzania, 121 (15.5%) from Mozambique, 158 (20.3%) from Malawi, and 86 (11.0%) from India. The sites in South Africa (32.9%) and India (25.2%) contributed higher proportions of microbiologically confirmed TB.

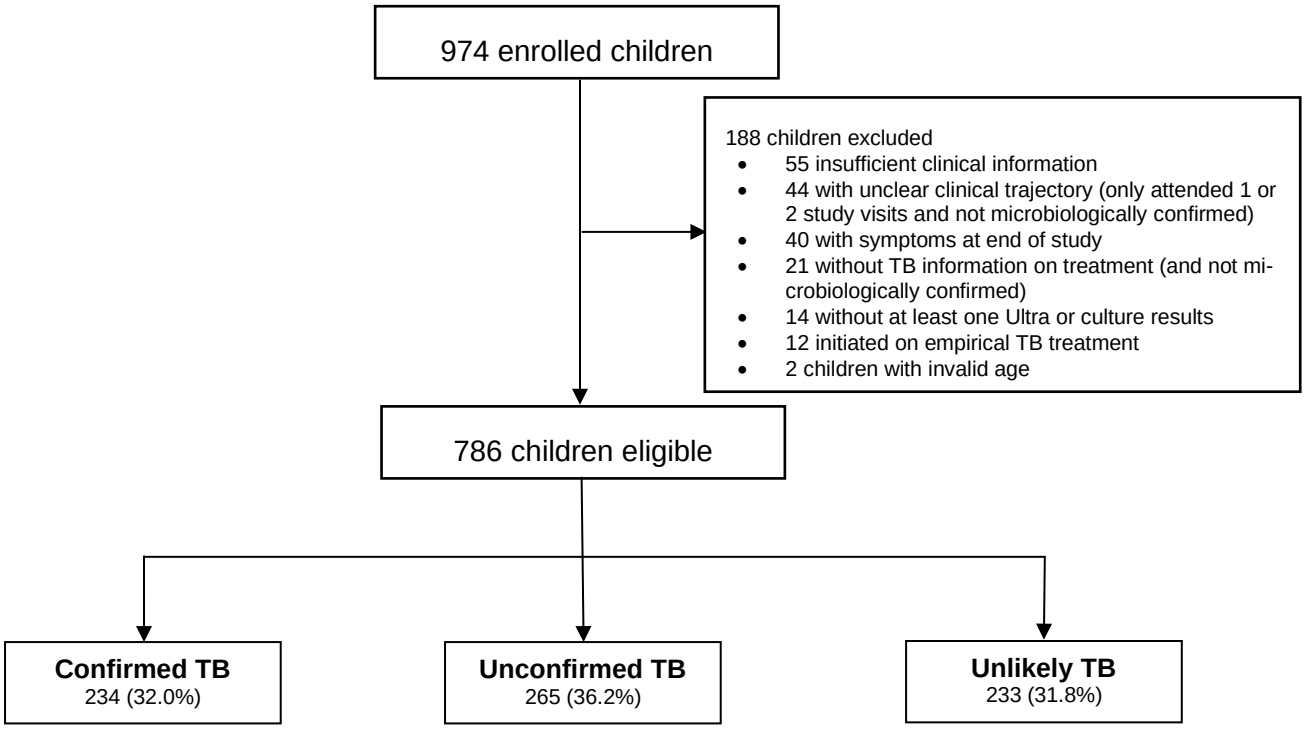


*Figure 3.2: Flow diagram of study participants*

Among participants that were eligible for diagnostic classification, 234 (32.0%) were categorized as confirmed TB, 265 (36.2%) as unconfirmed TB, and 233 (31.8%) as unlikely

TB. The median age was 5.7 years (SD, 4.2), 374 (47.9%) were females, and 116 (15.2%) were HIV infected. Overall TST positivity was 49.6% (62.0% in the unconfirmed TB, 49.0 in unconfirmed TB, and 40.4% in the unlikely TB group). The proportion of previously treated TB participants were 2.6% with no obvious differences between the diagnostic categories. Demographics information have been described in detail in table 3.6.

| | **All** **N = 780** | **Confirmed TB** **n = 234 (30.0%)** | **Unconfirmed TB** **n = 264 (33.8%)** | **Unlikely TB** **n = 282 (36.2%)** |
|---|---|---|---|---|
| **Female** | 374 (47.9) | 111 (47.4) | 122 (46.2) | 141 (50.0) |
| **Age** | | | | |
| Age in years, mean (SD) | 5.7 (4.2) | 6.7 (4.9) | 5.4 (4.0) | 5.3 (3.7) |
| 0-<1 years | 96 (12.3) | 37 (15.8) | 34 (12.9) | 25 (8.9) |
| 1-<5 years | 289 (37.1) | 63 (26.9) | 102 (38.6) | 124 (44.0) |
| 5-<10 years | 230 (29.5) | 55 (23.5) | 83 (31.4) | 92 (32.6) |
| 10-<15 years | 165 (21.2) | 79 (33.8) | 45 (17.0) | 41 (14.5) |
| **Site** | | | | |
| South Africa | 195 (25.0) | 77 (32.9) | 85 (32.2) | 33 (11.7) |
| Tanzania | 220 (28.2) | 45 (19.2) | 53 (20.1) | 122 (43.3) |
| Mozambique | 121 (15.5) | 22 (9.4) | 61 (23.1) | 38 (13.5) |
| Malawi | 158 (20.3) | 31 (13.2) | 55 (20.8) | 72 (25.5) |
| India | 86 (11.0) | 59 (25.2) | 10 (3.8) | 17 (6.0) |
| **HIV Positive** | 116 (15.2) | 20 (9.1) | 67 (26.1) | 27 (10.0) |
| On ART | 56 (52.8) | 10 (47.6) | 25 (43.1) | 21 (77.8) |
| CD4 count (cells/mm3), mean (SD) | 604.2 (456.9) | 448.7 (319.8) | 621.9 (467.8) | 662.0 (497.5) |
| **Previous TB treatment** | 20 (2.6) | 6 (2.6) | 9 (3.4) | 5 (1.8) |
| **TST positive** | 359 (49.6) | 132 (62.0) | 117 (49.0) | 110 (40.4) |
| TST measurement (mm), mean (IQR) | 9.0 (0.0 to 16.0) | 11.1 (0.0 to 18.0) | 8.6 (0.0 to 17.0) | 6.5 (0.0 to 14.0) |
| **Anthropometrics** | | | | |
| Weight for age (z-score), mean (SD) | -1.1 (1.4) | -1.3 (1.5) | -1.2 (1.5) | -1.0 (1.3) |
| Weight for length (z-score), mean (SD) | -0.4 (1.6) | -0.6 (1.7) | -0.3 (1.6) | -0.0 (1.5) |
| BMI for age (z-score), mean (SD) | -0.7 (1.8) | -1.4 (2.1) | -1.0 (1.7) | -0.8 (1.5) |
| MUAC (mm), mean (SD) | 144.8 (20.5) | 141.3 (20.1) | 145.6 (24.0) | 146.3 (16.6) |

*Table 3.6: Clinical characteristics of children at enrolment, by TB category*

Definitions: ART: Antiretroviral therapy, TST: Tuberculin skin test, BMI: Body mass index, MUAC: Mid upper arm circumference

### 3.2.2 Ultra-semiquantitative readouts in children with confirmed TB

In 234 participants with confirmed TB, 109 (46.6%) were diagnosed by both culture and Ultra, 85 (36.3%) by Ultra alone, and 40 (17.1%) by culture alone. In participants who were diagnosed with both culture and Ultra, 14 (12.8) participants had semiquantitative results “trace” and in those who were diagnosed with Ultra alone, 72.9% (62/85) had trace results; in this group 57 (67.9%) participants were diagnosed by a single trace results and 5 (6.0%) where diagnosed with 2 trace results. Details of semiquantitative readout of Ultra results in relation to culture results have been described in detail in figure 3.3.

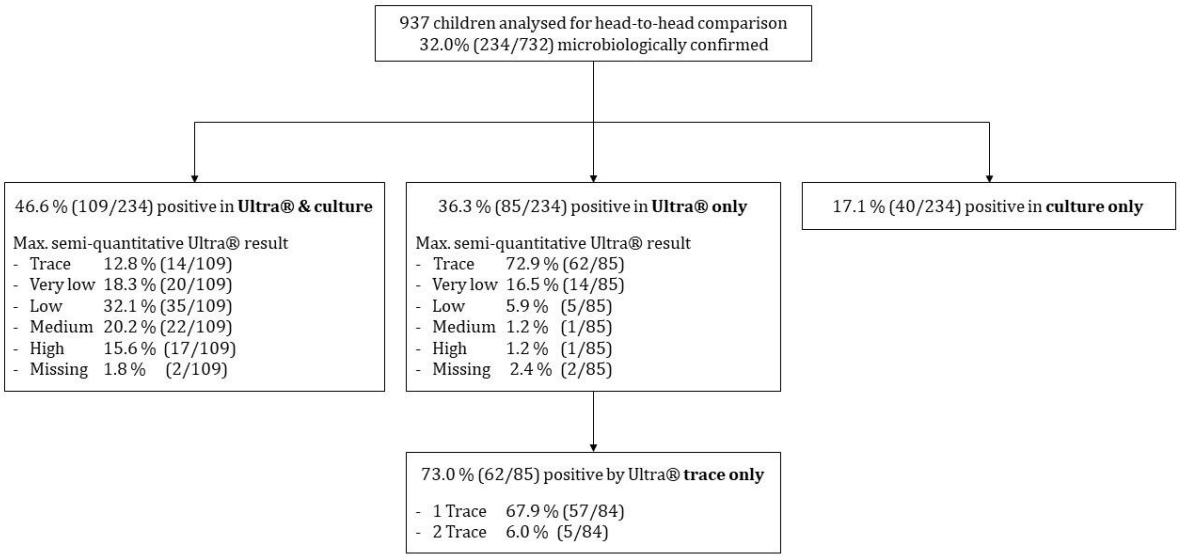


*Figure 3.3:* ***Overview of TB tests results in children with confirmed TB***

### 3.2.3 Incremental yield from a second respiratory sample

Assessment of incremental yield was performed in children who had at least two respiratory samples collected. Overall microbiological yield from the second sample was 4.3%. On per sample type, the highest increment yield was 3.8% for spontaneous sputum samples, 4.8% for SI samples, and 18% in GA samples (table 3.7).

| Specimen type | Specimen | N | Test +, n (%) | Cumulative Yield, n (%) |
|---|---|---|---|---|
| All pulmonary samples | 1st sample | 813 | 85 (10.5%) | 85 (10.5%) |
| | 2nd sample | 813 | 100 (12.3%) | 120 (14.8%) |
| Spontaneous sputum | 1st sample | 130 | 21 (16.2%) | 21 (16.2%) |
| | 2nd sample | 130 | 22 (16.9%) | 26 (20.0%) |
| Induced sputum | 1st sample | 534 | 41 (7.7%) | 41 (7.7%) |
| | 2nd sample | 534 | 55 (10.3%) | 67 (12.5%) |
| Gastric lavage | 1st sample | 56 | 12 (21.4%) | 12 (21.4%) |
| | 2nd sample | 56 | 8 (14.3%) | 13 (23.2%) |

*Table 3.7: Incremental yield of cultures performed on serial pulmonary specimens*

# 4. Discussion

In the R4KA cohort we prospectively enrolled 547 children with presumed PTB in three African Countries namely Tanzania, The Gambia, and Mali. We described the clinical and radiological characteristics by diagnostic classification in children with presumed PTB in the R4KA cohorts and have assessed the clinical outcome of enrolled participants after the month 6 end of study visit. Additionally, we prospectively assessed the performance of Ultra in children with presumed TB using fresh respiratory samples in contrast to the frozen archived samples that were used in many of the available evidence to date on the performance of Ultra in children[32–34,54]. Furthermore, from the RaPaed-AIDA-TB study cohort, we have used the results as an output of the study design work presented in this thesis, and in order to put the R4KA results into context of a second study. The diagnostic performance characteristics of Ultra results including the semiquantitative readouts categories have been presented. It is noteworthy that both studies achieved a high proportion of children with microbiological confirmation of TB.

## 4.1 R4KA cohort characteristics

Overall, 216 children were diagnosed to have PTB (77 confirmed, 139 unconfirmed) and were classified based on the NIH consensus statement. The proportion of children with microbiologic confirmation was 14.1% among all participants included in the analysis, with high variability between sites. The Mali sites demonstrated higher microbiologic confirmation (26.7%) as compared to Tanzania (13.2%) and The Gambia (8.7%). This can be explained by the difference in study population between sites. The Mali site is a tertiary referral hospital and had a high proportion of HIV infected children therefore possibly higher likelihood of microbiologic confirmation due to advanced TB disease, although this study was not powered to establish the TB disease status of children recruited at different

sites. Recruitment in Tanzania and The Gambia was performed in a secondary healthcare hospital and received many referrals from several surrounding primary care health facilities possibly with relatively minimal TB disease. The diagnostic capabilities and referral system at participating hospitals were not further studied. Several studies [54–57] have demonstrated variable microbiologic confirmation rate in children highlighting the existing diagnostic dilemma of TB in children. In our previous study [58] where we used less sensitive eligibility criteria for enrolment and in another study [56] where severe pneumonia was the eligibility for enrolment, the microbiologic confirmation of 5.2% and 6.3% respectively were achieved. In another study where they used a triad of TB symptoms, positive TST and/or suggestive CXR, microbiological confirmation of 27.3% was achieved.[57] In our study, despite the observed differences in microbiologic yield between sites, eligibility assessment for enrolment was not different from what is usually practiced in the routine care. In a very recent study [54] a microbiologic confirmation of 14.2% was achieved similar to 14.1 % in our study.

Diagnosis of TB is usually start with initial evaluation of TB symptoms then followed by radiological and/or laboratory evaluations to confirm the presence of TB bacilli in clinical samples. In the R4KA cohort, all presumed TB cases had at least one TB symptom at baseline with cough, fever and loss or failure to gain weight being the most common TB symptoms. On TB treatment, all symptoms resolved rapidly indicating good response to treatment. In contrast a proportion of children who remained symptomatic at month 6 was relatively higher in the unlikely TB group (5.5% cough, 4.2% fever, 3.4% failure to gain weight) than in children who were diagnosed with TB and treated. This underscores the non-specificity of symptoms for the diagnosis of TB disease in children [59] and reflect our way of inclusion criteria whereby cough alone or fever alone were used as inclusion cri-

teria. We think children who remained symptomatic perhaps had other predisposing disease such as asthma which tend to present with chronic and/or recurrent cough. Combining cough or fever with other parameters such as weight loss or positive immunological evidence for TB infection (such as TST) for fulfilment of eligibility criteria would have improved the specificity and reduce the number of children with other respiratory conditions enrolled into the study. This would in addition increase the proportion of microbiological confirmation like in a study by Owen et al.[57] and in the RaPaed-AIDA-TB cohort of which the results have been described in next sections.

MTB is predominantly acquired by inhalation route which often results into primary TB infection characterized by Ghon complex in the lungs. Other TB associated radiological abnormalities in children includes the non-cavitary and lymph node disease which can be seen on CXR. In a study by Ben Marais et. al, uncomplicated lymph node disease was the commonest findings on CXR, but with the least microbiological confirmation.[60] The observed low bacteriological yield in this group could be the paucibacillary nature of the disease. It is also well known that most of the viral infections tend to present with hilar lymphadenopathy, therefore, this can as well suggest the non-specificity of lymph node diseases for the diagnosis of TB diseases in children. In the R4KA cohort over 94% of enrolled children had CXR performed, with lobar consolidation being the most commonly recorded abnormalities both in children with confirmed TB (63%) and unconfirmed TB (68%) groups. Lymph node disease was only recorded in 30% of the children in the confirmed TB, and 24% in the unconfirmed TB group, different from previous study. The observed differences can be attributed by the difference in study populations but also the lack of standardised description of the radiological abnormalities on CXR.[61] The unlikely TB group had the lowest recorded abnormalities across all parameters, highlighting the

utilities of CXR in making the diagnosis of TB disease among clinicians. Generally, interpretation of CXR abnormalities in children is challenging and the diagnosis of TB based on CXR in children is largely observer dependant. The NIH consensus statements offers the alternative methodological approaches to report findings in a standardized manner and may help to overcome challenges of interpretations to ensure good comparability of future paediatric TB diagnostic studies.[62]

## 4.2 Diagnostic Sensitivity and Specificity of Ultra: Findings from the R4KA cohort

In the R4KA cohort, the overall sensitivity of Ultra was 66.3% in children with culture confirmed TB. This finding is similar to earlier publications using stored samples where the sensitivities of 64.3% [32] and 67.5% [33] were respectively observed with a first available sample in African settings. In a recent study involving hospitalized children [34], the sensitivity of Ultra was 74% when single SI sample was used, higher than in our study. However, this study enrolled hospitalized children who possibly had severe or far advanced TB diseases with higher likelihood of microbiological confirmation. Ultra, detected TB in 20 additional children who were culture negative. In these children, only two had a history of previously treated TB which could have led to a suspicion of false positive Ultra results due to detection of MTB DNA remnant of previous active TB disease. However, we think this is due to the pauci-bacillary nature of samples from children rather than false positive results. In our study, the specificity of Ultra was 95.4%, lower than in the recent meta-analysis and systemic review [35] but similar to a recent study in Uganda [54]. In our study, all children who had Ultra positive, but culture negative results had suggestive of TB symptoms and improved on TB treatment, which suggests that they may well have had TB disease, although clinical improvement can as well be cleared due to the known anti-bacterial effects of Rifampicin in non-TB respiratory disease. Generally Ultra is seen as

less specific than Xpert MTB/RIF assay therefore the possibility of some false positive results cannot be ruled out.

Using the eMRS, the Sensitivity of Ultra was 75% and that of culture was 74% suggesting that Ultra detects additional cases that otherwise would not be detected by culture and vice versa. These findings are similar to a very recent study in Uganda which enrolled children with minimal PTB disease [54] in which sensitivities of 72% was reported for Ultra and 63.8% for MGIT culture, highlighting the need to combine Ultra and culture, where it is available, in order to improve TB case detection in children.

The observed sensitivity of Ultra was higher in HIV negative children (67.6%) than in HIV positive children (57.1%). Possibly this is due to the paucibacillary samples from the HIV positive children with a bacterial load below the limit of detection of Ultra. This observation is in contrast to previous studies whereby the sensitivities of Ultra was higher in the HIV positive group [32,33] but these had overall smaller sample sizes and therefore less precise estimates for sensitivity.

Studies have demonstrated that the sensitivity of Ultra improves with the number of samples tested. In earlier evaluation studies of Ultra in children, the sensitivity of 75% [32] and 72% [33] were demonstrated when more than one sample was used. Similarly, in a very recent study, the sensitivity of Ultra increased to 87.5% when two nasopharyngeal samples were combined with one IS [34]. In our study, the majority of children had only one sample collected (only one sample per child was collected in Mali and The Gambia, and two in Tanzania). This may explain the slightly higher sensitivity of Ultra in Tanzania compared to the other two countries, although the differences were not statistically significant. We think our findings reflects more of the real-life scenario in many limited resource countries where often only one sputum sample is collected, given the high cost of the test,

labour-intensive and unpleasant sample collection methods in children. As was highlighted by Simon et al.,[50] it demonstrates the difficult clinical dilemma for microbiological confirmation of TB in children and thus a need for a more cost-effective approach such as the use of pooled samples that has the potential of increasing the yield from a single test.[34]

In a recent meta-analysis and systemic review,[35] the sensitivity of the Xpert assay was highest in the GA samples, followed by sputum samples, and lowest in the NPA samples. Current evidence on the diagnostic accuracy of Ultra is predominantly from SI or NPA samples; data on the sensitivity of ultra on GA samples is still lacking. In our study we observed that the sensitivity of Ultra was lower in GA samples (48.0%) than in the SI sample (68.0%), and highest in the spontaneous sputum samples (74.0%). Spontaneous sputum samples were obtained in older children who were able to expectorate which can explain the higher sensitivity in this group. The SI and GA samples were both obtained in younger children; the difference in sensitivities can be explained by the differences in the composition of the 2 groups such as: GA samples were collected predominantly in children from Mali site with large proportion of HIV positive children, and overall, GA was only performed in a small number of children (15%) as compared to those who had SI sample collected.

The strength of this study is its large sample size and large number of microbiologically confirmed TB. It is a prospective evaluation study of Ultra using fresh samples rather than previously frozen respiratory samples. The available evidence of Ultra diagnostic performance in children to date is mainly from South Africa and from East African countries.[32,34,54,63] In our study we extended the coverage to two West African Counties which

are known to have a high prevalence of *Mycobacterium africanum* [64]. We have demonstrated that Ultra perform equally good in different settings, as our findings are generally comparable to what have been previously reported.

The study has some limitations. We were not able to do head-to-head comparisons of Ultra against Xpert MTB/RIF as was phased out, following the endorsement of Ultra by WHO for TB diagnosis in children. All studies of Ultra that have been conducted to date have demonstrated that Ultra performs better than Xpert for TB diagnosis in children, and the reported sensitivities and specificities of Ultra is relatively similar to our findings. Furthermore, the semi-quantitative readout of the Ultra results was not reported, therefore further analysis of trace calls results especially in 20 children with positive Ultra but culture negative results could not be performed. However, the semiquantitative readouts categories of Ultra results have been described in the next section using data from the RaPaed-AIDA-TB cohort. Semiquantitative read out, “trace” indicate a very low level of TB bacilli in clinical samples and often it is false positive especially in those with a history of being previously treated for TB diseases.[29,36] In this cohort, all children with discordant results responded well to TB treatment, which supports the diagnosis of TB, although good response to be treatment could as well be due to medical attention and/or antibiotic activity of rifampicin that covers not only MTB. In high TB burden setting, the current WHO recommendation supports the initiation of TB therapy based on trace calls results in persons with HIV, children, and extrapulmonary TB as not treating them is a very high risk approach with possibly serious consequences.[36]

## 4.3 Diagnostic performance characteristics and semi-quantitative readouts of Ultra results: Findings from the RaPaed-AIDA-TB cohort

Through the RaPaed-AIDA-TB cohort, we assessed the diagnostic performance characteristics of Ultra in 786 children with presumed PTB at four sites in four African countries and at one site in India. Specifically, we have assessed the semiquantitative readouts of the Ultra results in relation to culture results and have demonstrated the benefit of serial testing by calculating the incremental yield from a second sample. Through this cohort, we have achieved a higher rate of microbiological confirmation (25.0%) compared to the R4KA cohort with a microbiological confirmation of 14.1%. The RaPaed-AIDA-TB study was designed to achieve a high rate of microbiologically confirmation to derive solid estimates for index test evaluation, and at least two respiratory samples were collected. The eligibility criteria allowed the enrolment of prior confirmed TB cases who were referred for study assessment following initial microbiological confirmation of active TB disease in a non-study screening. Additionally, cough alone was used as an inclusion criterion only if it was persistent for two weeks or more, but, in the case of any cough or persistent fever (which is known to be nonspecific) had to be in combination of either weight loss or a positive TST to improve the specificity. These factors may explain higher rate of microbiological confirmation in this cohort, thus, the data presented may not necessarily represent the general population.

Of all children with microbiological confirmation, Ultra, detected additional 85 (36.3%) TB cases which were culture negative. This is consistent trend to the finding from the R4KA cohort where Ultra detected additional 20 cases out of 77 confirmed TB cases. This is also similar to findings from the recent sub study involving Uganda children in the SHINE trial, where 17 (47%) out of the 36 children who were Ultra positive, were detected by Ultra alone.[54] In that study 16 out of the 17 children that were Ultra positive but culture negative had trace semiquantitative category suggestive of very low bacillary load, and one had “very low” semiquantitative category. Interpretation of the semi-quantitative

readout, “trace” results remain unclear. In our study, in those children who had TB detected by Ultra alone, 73% had “trace call” results. This is similar to other paediatric TB studies which also found a high frequencies of “trace” calls and other low semi-quantitative results in culture-negative samples, underlining the association of low bacillary load in paediatric samples.[34] In the previous analytical study which used spiked samples, it was demonstrated that Ultra can be positive in as low as 2.5 CFU/ml which is lower than that of culture (>10 CFU/ml).[29] Therefore it may not be surprising of this huge number of Ultra positive but culture negatives samples. As pointed out earlier, the recent WHO guidelines recommends that trace calls should be regarded as true microbiologically confirmed TB because of the difficulty of confirming TB disease in children.[37] This recommendation is supported by a recent study which showed that TB symptoms, extra pulmonary TB, and no previously treated TB are independently associated with culture positive TB in adults and children with trace calls results [65] although the possibility of false positive results may not be excluded.

In the RaPaed-AIDA-TB cohort we assessed the incremental microbiological yield when more than one sample was tested. Overall increment yield from a second sample was 4.3% when a second respiratory was tested. The benefit of serial testing was more pronounced in SI samples (4.8%) and less in the GA samples (1.8%). The difference in increment yield can explained by the difference in study population. GA was predominantly performed at the Indian site which had small sample size and had higher proportion of children with microbiologically confirmed TB (68%). The remaining 4 sites performed predominantly SI, and microbiological confirmation ranged from 18.2% in Mozambique to 39.5% in South Africa, therefore more likely had more precise estimate. SI and GA are the most commonly used recovery method for obtaining respiratory samples in children, but they are both very unpleasant to the child and to the healthcare worker who perform

the procedure.[12] Coupled with the higher cost of the test it is unlikely that collection of two SI or GA samples will be operationally feasible in routine pragmatic setting.

## 4.4 Outlook of novel candidate tests for evaluation: The RaPaed-AIDA-TB protocol

Analysis of the R4KA and the RaPaed-AIDA-TB cohorts shows that, with the current approach, a substantial number of children with TB may remain undetected after Ultra and/or culture tests on respiratory samples. In addition, respiratory samples are not easy to obtain especially in young children, pointing out to the need of sensitive and specific non-sputum-based test for the diagnosis of TB in children. In response to this urgent needs, WHO have recently issued a recommendations on the use of stool samples for the diagnosis of TB in children.[37] In the RaPaed-AIDA-TB project, at least eight new diagnostic test that has the potential to meet the WHO TPPs, are being evaluated for the diagnosis of TB in children. Most of these novel techniques use non sputum samples and have different testing approaches, including evaluation of host response, antigen detection, cellular immunoresponse assays, and nucleic acid amplification tests. Furthermore, the RaPaed-AIDA-TB project, has created a large repository of samples to facilitate future test evaluation of emerging new diagnostics for TB in children. Some of the new candidate tests for evaluation includes:

*T-cell activation marker assay (TAM-TB) for TB detection in children*

T-cell activation marker assay (TAM-TB) is a novel immunodiagnostic test that is capable of distinguishing between active and latent TB infection by MTB.[66–68] Specifically TAM TB can discriminate active TB disease from latent infection by assessing the TB specific T-cells using flow cytometry. In a previous study involving Tanzanian children, TAM-TB as-

say showed a sensitivity of 83.3% and a specificity of 96.8%.[69] TAM-TB has been developed into a standardized kit which is now under evaluation in the RaPaed-AIDA-TB project.

*Whole blood transcriptomics and proteomics*

Recently, a new fingerstick blood test has been developed by Cepheid (the Xpert-MTB-Host Response (HR)-Prototype), which generates a 'TB score' based on mRNA expression of 3 genes to discriminate between active TB and latent TB.[70] The first prospective assessment of this signature indicated that the MTB-HR prototype reaches the minimum TPPs for a point of care triage test using finger prick blood, however, its use in children remains unlear.[71] This prototype is currently being evaluated for the diagnosis of TB in children of the RaPaed-AIDA-TB cohort, using the GeneXpert platform.

*LAM assay for the detection of TB in children*

The RaPaed-AIDA-TB protocol is evaluating various Urine based LAM assays, including the FujiLAM, and Uri-TB direct (Karolinska Institutet), and the AlereLAM is being used as a comparator test.

*Xpert MTBRIF Ultra® on stool sample*

Recently, the WHO issued the recommendation on the use of stool as sample using Ultra or Xpert assays for initial TB diagnosis and rifampicin resistance testing in children with presumed TB.[37] One of the assay kits currently undergoing evaluation is the Stool Processing kit (SPK), at present available as a prototype and is being evaluated in the RaPaed-AIDA-TB project. The SPK is used to break down stool to allow analysis on the GeneXpert® platform, giving both a qualitative (MTB detected yes/no) and a semi-quantitative readout category in case MTB is detected.

# 5. Conclusion

A drawback of many paediatric TB diagnostic studies is that there were not enough confirmed TB cases for subgroup analysis, but the two studies presented here show that with careful design and execution this can be overcome. We have shown that, despite the improvement of diagnostic accuracy of Ultra as compared to Xpert as was previously seen, its sensitivity remains sub-optimal for the detection of TB in children. On contrary, in R4KA cohort, Ultra detected an additional 20 cases which otherwise could not have been detected by culture alone. Similarly, in the RaPaed_AIDA-TB cohort, a third of those children with MTB detection were confirmed by Ultra alone suggesting that culture is an imperfect reference standard. These findings support the reports from previous studies of Ultra with the potential to increase the reliability and reduce the delay of TB diagnosis in children in high TB burden settings, although there may be a drawback if Ultra specificity seems insufficient.

Findings from the RaPaed-AIDA-TB cohorts further underlines the importance of applying multiple samples to increase microbiological yield for TB detection in children. We have demonstrated that for many children believed to have active TB, microbiological detection was not achieved, despite the standardized and thorough workup, highlighting that currently available tests lack sensitivity and remain imperfect.

Results of the new candidate test for evaluation in the RaPaed-AIDA-TB study have not been presented in this thesis report however, we anticipate that this study will generate important diagnostic accuracy data on promising new tests for childhood TB and contribute to a better understanding of childhood TB in general.

# References


1. WHO. Global Tuberculosis Report. 2021.
2. World Health Organization. Roadmap towards ending TB in children and adolescents. 2018.
3. Oliwa JN, Karumbi JM, Marais BJ, Madhi SA, Graham SM. Tuberculosis as a cause or comorbidity of childhood pneumonia in tuberculosis-endemic areas: a systematic review. Lancet Respir Med. 2015 Mar;3(3):235–43.
4. Gunasekera KS, Walters E, van der Zalm MM, Palmer M, Warren JL, Hesseling AC, et al. Development of a Treatment-decision Algorithm for Human Immunodeficiency Virus-uninfected Children Evaluated for Pulmonary Tuberculosis. Clin Infect Dis. 2021;73(4):e904–12.
5. Marcy O, Borand L, Ung V, Msellati P, Tejiokem M, Huu KT, et al. A Treatment-Decision Score for HIV-Infected Children With Suspected Tuberculosis. Pediatrics. 2019;144(3).
6. World Health Organization. High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. 2014;(April):1–96.
7. Denkinger CM, Schumacher SG, Gilpin C, Korobitsyn A, Wells WA, Pai M, et al. Guidance for the Evaluation of Tuberculosis Diagnostics That Meet the World Health Organization (WHO) Target Product Profiles: An Introduction to WHO Process and Study Design Principles. JID. 2019 Oct 8;220(Supplement_3):S91–8.
8. Schumacher SG, Wells WA, Nicol MP, Steingart KR, Theron G, Dorman SE, et al. Guidance for Studies Evaluating the Accuracy of Sputum-Based Tests to Diagnose Tuberculosis. JID. 2019 Oct 8;220(Supplement_3):S99–107.
9. Drain PK, Gardiner J, Hannah H, Broger T, Dheda K, Fielding K, et al. Guidance for Studies Evaluating the Accuracy of Biomarker-Based Nonsputum Tests to Diagnose Tuberculosis. JID. 2019 Oct 8;220(Supplement_3):S108–15.
10. Nathavitharana RR, Yoon C, Macpherson P, Dowdy DW, Cattamanchi A, Somoskovi A, et al. Guidance for Studies Evaluating the Accuracy of Tuberculosis Triage Tests. JID. 2019 Oct 8;220(Supplement_3):S116–25.
11. Marais BJ, Pai M. Recent advances in the diagnosis of childhood tuberculosis. Arch Dis Child. 2007 May;92(5):446–52.
12. 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(9454):130–4.
13. Azzopardi P, Graham S. What are the most useful clinical indicators of tuberculosis in childhood ? Int Child Heal Rev Collab. 2008;1–8.
14. Graham SM. The Use of Diagnostic Systems for Tuberculosis in Children. Indian J Pediatr. 2010 Dec 17;78(3):334–9.
15. Eamranond P, Jaramillo E. Tuberculosis in children: reassessing the need for improved diagnosis in global control strategies. Int J Tuberc Lung Dis. 2001 Jul;5(7):594–603.
16. Brändli O. The clinical presentation of tuberculosis. Respiration. 1998 Jan;65(2):97–105.

17. Zar HJ, Connell TG, Nicol M. Diagnosis of pulmonary tuberculosis in children: new advances. Expert Rev Anti Infect Ther. 2010 Mar;8(3):277–88.

18. WHO epexpert group meeting report. The Use of Molecular Line Probe Assay for the Detection of Resistance To Second-Line Anti-Tuberculosis Drugs. World Heal Organ. 2013;(February):1–52.

19. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extra- pulmonary TB in adults and children. Policy update. World Heal Organ. 2013;

20. Rachow A, Clowes P, Saathoff E, Mtafya B, Michael E, Ntinginya EN, et al. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: a prospective cohort study. Clin Infect Dis. 2012 May;54(10):1388–96.

21. Nicol MP, Whitelaw A, Stevens W. Using Xpert MTB / RIF. 2013;187–92.

22. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010;48(1):229–37.

23. Thi N, Nhu Q, Thi D, Ha M, Anh ND, Dang D, et al. Evaluation of Xpert MTB / RIF and MODS assay for the diagnosis of pediatric tuberculosis. BMC Infect Dis. 2013;13(1):1.

24. Zar HJ, Workman L, Isaacs W, Munro J, Black F, Eley B, et al. Rapid Molecular Diagnosis of Pulmonary Tuberculosis in Children Using Nasopharyngeal Specimens. 2012;55:1088–95.

25. Chisti MJ, Graham SM, Duke T, Ahmed T, Ashraf H, Syed A, et al. A Prospective Study of the Prevalence of Tuberculosis and Bacteraemia in Bangladeshi Children with Severe Malnutrition and Pneumonia Including an Evaluation of Xpert MTB / RIF Assay. 2014;9(4).

26. Togun TO, Egere U, Sillah AK, Ayorinde A, Mendy F, Tientcheu L, et al. Contribution of Xpert W MTB / RIF to the diagnosis of pulmonary tuberculosis among TB-exposed children in The Gambia. 2015;19(March):1091–7.

27. Anne K Detjen, Andrew R DiNardo, Jacinta Leyden, Karen R Steingart, Dick Menzies, Ian Schiller, Nandini Dendukuri, and Anna M Mandalakas, MDAnne K Detjen, Andrew R DiNardo, Jacinta Leyden, Karen R Steingart, Dick Menzies, Ian Schiller, Nandini Dendukuri and AMM. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med. 2016;3(6):451–61.

28. KR S, Schiller I, DJ H, Pai M, CC B, Dendukuri N. Xpert ® MTB / RIF assay for pulmonary tuberculosis and rifampicin resistance in adults (Review). Cochrane Database Syst Rev. 2014;(1).

29. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, et al. The New Xpert MTB/RIF Ultra: Improving Detection of Mycobacterium tuberculosis and Resistance to Rifampin in an Assay Suitable for Point-of-Care Testing. MBio. 2017;8(4):e00812-17.

30. David Alland, Mazhgan Rowneki, Laura Smith, Jamie Ryan, Mitchell Chancellor, Ann Marie Simmon, David Persing, Robert Kwiatkowski, Martin Jones SC. Xpert

MTB/RIF Ultra: A New Near-Patient TB Test With Sensitivity Equal to Culture. In: Conference on Retroviruses and Opportunistic Infections. 2015. p. Absract # 91.

31. Timothy Rodwell, Samuel G. Schumacher, Pamela Nabeta, Catharina C. Boehme, Jerrold J. Ellner, David Alland, Susan E. Dorman CMD. A multicenter diagnostic accuracy study of the Xpert Ultra for tuberculosis diagnosis. In: Conference on Retroviruses and Opportunistic Infections. 2017. p. Abstract # 76LB.
32. Sabi I, Rachow A, Mapamba D, Clowes P, Ntinginya NE, Sasamalo M, et al. Xpert MTB/RIF Ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study. J Infect. 2018 Oct 1;77(4):321–7.
33. Nicol MP. Accuracy of Xpert Mtb/Rif Ultra for the Diagnosis of Pulmonary Tuberculosis in Children. Pediatr Infect Dis J. 2018;37(10):3–5.
34. Zar HJ, Workman LJ, Prins M, Bateman LJ, Mbhele SP, Whitman CB, et al. Tuberculosis diagnosis in children using Xpert Ultra on different respiratory specimens. Am J Respir Crit Care Med. 2019;200(12):1531–8.
35. Aw K, L GF, Takwoingi Y, Eisenhut M, Ak D, Kr S, et al. Xpert MTB/RIF and Xpert MTB/RIF Ultra assays for active tuberculosis and rifampicin resistance in children (Review). Cochrane Database Syst Rev. 2020;
36. WHO. WHO Meeting Report of a Technical Expert Consultation : Non-inferiority analysis of Xpert MTB / RIF Ultra compared to Xpert MTB / RIF. WHO. 2017.
37. Rapid communication on updated guidance on the management of tuberculosis in children and adolescents. Geneva World Heal Organ. 2021;
38. Branigan D. Tuberculosis Diagnostics Pipeline Report. 2021.
39. Penn-Nicholson A, Gomathi SN, Ugarte-Gil C, Meaza A, Lavu E, Patel P, et al. A prospective multicentre diagnostic accuracy study for the Truenat tuberculosis assays. Eur Respir J. 2021;58(5).
40. Jeyashree K, Shanmugasundaram D, Rade K, Gangakhedkar RR, Murhekar M V. Impact and operational feasibility of TrueNat™ MTB/Rif under India's RNTCP. Public Heal action. 2020 Sep 21;10(3):87–91.
41. Gomathi NS, Singh M, Myneedu VP, Chauhan DS, Tripathy S, Sarin R, et al. Validation of an indigenous assay for rapid molecular detection of rifampicin resistance in presumptive multidrug-resistant pulmonary tuberculosis patients. Indian J Med Res. 2020 Nov;152(5):482–9.
42. Shah M, Hanrahan C, Wang ZY, Dendukuri N, Lawn SD, Denkinger CM, et al. Lateral flow urine lipoarabinomannan assay for detecting active tuberculosis in HIV-positive adults. Cochrane Database Syst Rev. 2016;2016(5).
43. World Health Organization. The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV: policy guidance. 2015.
44. Peter JG, Zijenah LS, Chanda D, Clowes P, Lesosky M, Gina P, et al. Effect on mortality of point-of-care, urine-based lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-positive hospital inpatients: a pragmatic, parallel-group, multicountry, open-label, randomised controlled trial. Lancet. 2016 Mar;387(10024):1187–97.
45. Broger T, Sossen B, du Toit E, Kerkhoff AD, Schutz C, Ivanova Reipold E, et al.

Novel lipoarabinomannan point-of-care tuberculosis test for people with HIV: a diagnostic accuracy study. Lancet Infect Dis. 2019;19(8):852–61.

46. Nkereuwem E, Togun T, Gomez MP, Székely R, Macé A, Jobe D, et al. Comparing accuracy of lipoarabinomannan urine tests for diagnosis of pulmonary tuberculosis in children from four African countries: a cross-sectional study. Lancet Infect Dis. 2020 Dec 11;

47. Rachow A, Clowes P, Saathoff E, Mtafya B, Michael E, Ntinginya EN, et al. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: A prospective cohort study. Clin Infect Dis. 2012;54(10):1388–96.

48. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. The bacteriologic yield in children with intrathoracic tuberculosis. Clin Infect Dis. 2006;42(8):e69-71.

49. Graham SM, Cuevas LE, Jean-Philippe P, Browning R, Casenghi M, Detjen AK, et al. Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children: An Update. Clin Infect Dis. 2015;61.

50. Simon Schaaf H, Marais BJ. The role of Xpert MTB/RIF ultra in diagnosing pulmonary tuberculosis in children. Am J Respir Crit Care Med. 2019;200(12):1464–5.

51. 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 Jul;36(1):58–62.

52. Grønningen E, Nanyaro M, Sviland L, Ngadaya E, Muller W, Torres L, et al. MPT64 antigen detection test improves diagnosis of pediatric extrapulmonary tuberculosis in Mbeya, Tanzania. Sci Rep. 2021 Dec 2;11(1):17540.

53. Declation of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. 2008.

54. Ssengooba W, de Dieu Iragena J, Nakiyingi L, Mujumbi S, Wobudeya E, Mboizi R, et al. Accuracy of Xpert Ultra in Diagnosis of Pulmonary Tuberculosis among Children in Uganda: a Substudy from the SHINE Trial. J Clin Microbiol. 2020;58(9):1–8.

55. Sabi I, Kabyemera R, Mshana SE, Kidenya BR, Kasanga G, Gerwing-Adima LE, et al. Pulmonary TB bacteriologically confirmed by induced sputum among children at Bugando Medical Centre, Tanzania. Int J Tuberc Lung Dis. 2016;20(2):228–34.

56. Nantongo JM, Wobudeya E, Mupere E, Joloba M, Ssengooba W, Kisembo HN, et al. High incidence of pulmonary tuberculosis in children admitted with severe pneumonia in Uganda. BMC Pediatr. 2013;13:16.

57. Owens S, Abdel-Rahman IE, Balyejusa S, Musoke P, Cooke RPD, Parry CM, et al. Nasopharyngeal aspiration for diagnosis of pulmonary tuberculosis. Arch Dis Child. 2007 Aug;92(8):693–6.

58. Sabi I, Kabyemera R, Mshana SE, Kidenya BR, Kasanga G, Gerwing-Adima LE, et al. Pulmonary TB bacteriologically confirmed by induced sputum among children at Bugando Medical Centre, Tanzania. Int J Tuberc Lung Dis. 2016 Feb 1;20(2):228–34.

59. Govender I, Karat AS, Olivier S, Baisley K, Beckwith P, Dayi N, et al. Prevalence

of Mycobacterium tuberculosis in sputum and reported symptoms among clinic attendees compared to a community survey in rural South Africa. Clin Infect Dis. 2021 Dec 3;

60. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. The Bacteriologic Yield in Children with Intrathoracic Tuberculosis. Clin Infect Dis. 2006 Apr 15;42(8):e69–71.

61. Marais BJ, Gie RP, Schaaf HS, Starke JR, Hesseling AC, Donald PR, et al. A proposed radiological classification of childhood intra-thoracic tuberculosis. Pediatr Radiol. 2004 Aug 5;34(11):886–94.

62. Cuevas LE, Jean-philippe P, Graham SM, Cuevas LE, Jean-philippe P, Browning R, et al. Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children : An Update Clinical Case De fi nitions for Classi fi cation of Intrathoracic Tuberculosis in Children : An Update. 2015;(SEPTEMBER).

63. Nicol MP, Workman L, Isaacs W, Munro J, Black F, Eley B, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: A descriptive study. Lancet Infect Dis. 2011;11(11):819–24.

64. De Jong BC, Antonio M, Gagneux S. Mycobacterium africanum-Review of an Important Cause of Human Tuberculosis in West Africa.

65. Dowling WB, Whitelaw A, Nel P. Tracing TB: Are there predictors for active TB disease in patients with Xpert Ultra trace results? Int J Infect Dis. 2022 Jan;114:115–23.

66. Portevin D, Moukambi F, Clowes P, Bauer A, Chachage M, Ntinginya NE, et al. Assessment of the novel T-cell activation marker-tuberculosis assay for diagnosis of active tuberculosis in children: a prospective proof-of-concept study. Lancet Infect Dis. 2014 Oct;14(10):931–8.

67. Ahmed MIM, Ntinginya NE, Kibiki G, Mtafya BA, Semvua H, Mpagama S, et al. Phenotypic Changes on Mycobacterium Tuberculosis-Specific CD4 T Cells as Surrogate Markers for Tuberculosis Treatment Efficacy. Front Immunol. 2018 Sep 28;9.

68. Ahmed MIM, Ntinginya NE, Kibiki G, Mtafya BA, Semvua H, Mpagama S, et al. Phenotypic Changes on Mycobacterium Tuberculosis-Specific CD4 T Cells as Surrogate Markers for Tuberculosis Treatment Efficacy. Front Immunol. 2018 Sep 28;9.

69. Portevin D, Moukambi F, Clowes P, Bauer A, Chachage M, Ntinginya NE, et al. Assessment of the novel T-cell activation marker-tuberculosis assay for diagnosis of active tuberculosis in children: a prospective proof-of-concept study. Lancet Infect Dis. 2014 Oct;14(10):931–8.

70. Sweeney TE, Braviak L, Tato CM, Khatri P. Genome-wide expression for diagnosis of pulmonary tuberculosis: a multicohort analysis. Lancet Respir Med. 2016 Mar;4(3):213–24.

71. Sutherland JS, van der Spuy G, Gindeh A, Thuong NTT, Namuganga A, Owolabi O, et al. Diagnostic Accuracy of the Cepheid 3-gene Host Response Fingerstick Blood Test in a Prospective, Multi-site Study: Interim Results. Clin Infect Dis. 2021 Sep 22;

# Appendix

## Manuscript that I published during Ph.D. study

1. **Sabi I**, Olomi W, Nkereuwem E, Togun T, Gomez MP, Sylla M, et al. Diagnosis of paediatric TB using Xpert ® MTB/RIF Ultra on fresh respiratory samples. Int J Tuberc Lung Dis. 2022 Sep 1;26(9):862–8.

2. Gidi, N. W, Suraya A, Mutayoba B, Espinoza B, Meggi B, **Sabi I**, Noller J.M.G, Schmieding K, Tukhanova N, Manhart M, Heiber A. 'Proceedings from the CIHLMU occupational safety and health symposium 2019 "Protecting workers' health: global challenges and opportunities in work-related respiratory diseases"', BMC Proceedings. BMC Proceedings, 14(S14), p. 14. doi: 10.1186/s12919-020-00197-x.

3. **Sabi I**, Rachow A, Mapamba D, Clowes P, Ntinginya NE, Sasamalo M, et al. Xpert MTB/RIF Ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study. J Infect [Internet]. 2018 Jul 20; Available from: http://www.ncbi.nlm.nih.gov/pubmed/30036606

# Statement on Pre-release and Contribution

This PhD study was partly nested within the Reach4Kids Africa (R4KA) study which recruited participants in four African Countries namely Tanzania, The Gambia, Mali, and Nigeria. Findings from primary study has partly been published elsewhere.[46] The manuscript for my PhD study was submitted for publication in the International Journal of Tuberculosis and Lung Diseases (IJTLD) of which the peer review process has been completed and was accepted for publication. **Now the manuscript is undergoing editorial revisions by the journal, and it will be published in one of the forthcoming issues of the IJTLD**. I was the principal investigator (PI) in Tanzania for the primary R4KA study which generated data for the PhD sub study. I was responsible for the oversight and overall conduct of the study in Tanzania, I participated in the conceptualization of the primary study design, writing the protocol, and data collection. I was responsible for the design of the PhD sub study and performed data analysis with the support of a statistician.

The second part of my PhD study involved data from the RaPaed-AIDA-TB project of which I was the Co-principal investigator for Tanzanian site. I was part of the expert group that designed the inclusion and exclusion criteria and data analysis pathway for the study and participated in data analysis and publication. The results of this study are presented as output of the consortium work, mainly to put the R4KA and study design work into context. Currently two manuscripts are under initial write up of which I am the co-author.

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# List of publications


1. **Sabi I**, Olomi W, Nkereuwem E, Togun T, Gomez MP, Sylla M, et al. Diagnosis of paediatric TB using Xpert ® MTB/RIF Ultra on fresh respiratory samples. Int J Tuberc Lung Dis. 2022 Sep 1;26(9):862–8.

2. Olomi, W., Biraro, I. A., Kilonzo, K., te Brake, L., Kibirige, D., Chamba, N., Ntinginya, N. E., **Sabi, I**., Critchley, J., Sharples, K., Hill, P. C., & van Crevel, R. (2021). Tuberculosis preventive therapy for people with diabetes mellitus. Clinical Infectious Diseases. https://doi.org/10.1093/cid/ciab755.

3. Tweed CD, Wills GH, Crook AM, Amukoye E, Balanag V, Ban AYL, et al. A partially randomised trial of pretomanid, moxifloxacin and pyrazinamide for pulmonary TB. Int J Tuberc Lung Dis. 2021 Apr 1;25(4):305–14.

4. Nkereuwem E, Togun T, Gomez MP, Székely R, Macé A, Jobe D, et al. Comparing accuracy of lipoarabinomannan urine tests for diagnosis of pulmonary tuberculosis in children from four African countries: a cross-sectional study. Lancet Infect Dis. 2020 Dec 11;

5. Gidi, N. W, Suraya A, Mutayoba B, Espinoza B, Meggi B, **Sabi I**, Noller J.M.G, Schmieding K, Tukhanova N, Manhart M, Heiber A. 'Proceedings from the CIHLMU occupational safety and health symposium 2019 "Protecting workers' health: global challenges and opportunities in work-related respiratory diseases"', BMC Proceedings. BMC Proceedings, 14(S14), p. 14. doi: 10.1186/s12919-020-00197-x.

6. Mtafya B, Sabiiti W, **Sabi I**, John J, Sichone E, Ntinginya NE, et al. Molecular Bacterial Load Assay Concurs with Culture on NaOH-Induced Loss of Mycobacterium tuberculosis Viability. J Clin Microbiol. 2019;57(7).

7. David D. Nassoro, Mkhoi L. Mkhoi, **Issa Sabi**, Alfred J. Meremo, Paul S. Lawala, and Issakwisa Habakkuk Mwakyula. 'Adrenal Insufficiency: A Forgotten Diagnosis in HIV/AIDS Patients in Developing Countries', 2019, pp. 1–9. doi: 10.1155/2019/2342857.

8. **Sabi I**, Rachow A, Mapamba D, Clowes P, Ntinginya NE, Sasamalo M, et al. Xpert MTB/RIF Ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study. J Infect [Internet]. 2018 Jul 20; Available from: http://www.ncbi.nlm.nih.gov/pubmed/30036606

9. Meremo AJ, Masalu MB, **Sabi I**, Ngilangwa DP, Kapinga J T, R et al. Prevalence and Risk Factors Associated With Chronic Kidney Disease Among Patients Presenting at a Haemodialysis Unit in Dodoma, Tanzania. East African Heal Res J [Internet]. 2018;2(1). Available from: https://doi.org/10.24248/EAHRJ-D-16-00367

10. Mwasubila YK, **Sabi I**, Kabyemera R, Ntinginya N, Sauve R KB. Tuberculosis Contact Screening and Isoniazid Preventive Therapy Among Children Under 5 in the Mbeya and Songwe. East African Heal Res J. 2018;2(1):29–32.

11. **Sabi I**, Mahiga H, Mgaya J, Geisenberger O, Kastner S, Olomi W, et al. Accuracy and operational characteristics of Xpert HIV point-of-care testing at birth and different time-points until week 6 in HIV-exposed neonates in Tanzania. Clin Infect Dis [Internet]. 2018 Jun 29; Available from: http://www.ncbi.nlm.nih.gov/pubmed/29961841

12. Meremo AJ, Mwashambwa MY, Masalu MB, Kapinga J. Prevalence and predictors of anaemia among patients presenting with kidney diseases at the University of Dodoma Hospital in central Tanzania. Tanzan J Health Res. 2017;19(3):1–7.

13. Meremo AJ, Ngilangwa DP, Mwashambwa MY, Masalu MB, Kapinga J, Tagalile R, et al. Challenges and outcomes of haemodialysis among patients presenting with kidney diseases in Dodoma, Tanzania. BMC Nephrol [Internet]. 2017 Dec 4;18(1):212. Available from: http://bmcnephrol.biomedcentral.com/articles/10.1186/s12882-017-0634-2

14. Bacha JM, Ngo K, Clowes P, Draper HR, Ntinginya EN, DiNardo A, et al. Why being an expert – despite xpert –remains crucial for children in high TB burden settings. BMC Infect Dis. 2017 Dec 6;17(1):123.

*15.* Mangu CD, Manyama CK, Sudi L, **Sabi I**, Msila H, Nyanda E, Chaula Godlove, Maboko Leonard. Emerging viral infectious disease threat : Why Tanzania is not in a safe zone. Tanzan J Health Res. 2016;18(3):1–14.

*16.* **I. Sabi**, R. Kabyemera, S. E. Mshana, B. R. Kidenya, G. Kasanga, L. E. Gerwing-Adima, A. Meremo, P. Clowes, A. Rachow, R. N. Peck. Pulmonary TB bacteriologically confirmed by induced sputum among children at Bugando Medical Centre, Tanzania. Int J Tuberc Lung Dis 20(2): February 2016, pp. 228-234(7).