Clinical Study Protocol

Protocol Title: **Rapid and Accurate Diagnosis of Paediatric (RaPaed) TB** - An **AIDA (A**ssessment of **I**nnovative **D**iagnostics and **A**lgorithms for Early and Sensitive Detection of Acute **TB)** platform study

Short Title: **RaPaed-AIDA-TB**

Protocol Code: **LMU-IMPH-AIDA-02**

Protocol Version: 2.0

Amendment 1

Protocol Date: 23rd February 2018

Amendment 1 Date: 06th September 2018

Sponsor	Division of Infectious Diseases and Tropical Medicine Medical Center of the University of Munich (LMU) Leopoldstrasse 5, 80802 Munich, Germany
Sponsor Responsible Person	Prof Dr med Michael Hoelscher Division of Infectious Diseases and Tropical Medicine Medical Center of the University of Munich (LMU) Leopoldstrasse 5, 80802 Munich, Germany
Sponsor Medical Experts	Dr med Norbert Heinrich Dr med Laura Olbrich Division of Infectious Diseases and Tropical Medicine Medical Center of the University of Munich (LMU) Leopoldstrasse 5, 80802 Munich, Germany Phone: +49 89 4400 59803 / Email: aida@lrz.uni-muenchen.de
Sponsor Central Lab	Division of Infectious Diseases and Tropical Medicine Medical Center of the University of Munich (LMU) Leopoldstrasse 5, 80802 Munich, Germany Phone: +49 89 4400 59801 / Email: aida@lrz.uni-muenchen.de
Study Statistician	Dr Elmar Saathoff Division of Infectious Diseases and Tropical Medicine Medical Center of the University of Munich (LMU) Leopoldstrasse 5, 80802 Munich, Germany Phone: +49 89 4400 59812 / Email: saathoff@lrz.uni-muenchen.de
Clinical Study Partners	
Principal Investigators (PI)	Prof Dr Heather Joy Zar University of Cape Town Lung Institute (UCTLI) Red Cross War Memorial Children's Hospital MRC Unit on Child and Adolescent Health Cape Town, South Africa Phone: +27 21 6585324 / Email: heather.zar@uct.ac.za
	Dr Nyanda Elias Ntinginya NIMR - Mbeya Medical Research Centre P. O. Box 2410, Mbeya, Tanzania Phone: +255 25 2503364 / Mobile: +255 766 243836 Email: nelias@nimr-mmrc.org
	Dr Nilesh Bhatt Instituto Nacional de Saúde (INS) Avenida Eduardo Mondlane, Nº 1008, Maputo, Mozambique Phone: +258 82 28893130 / Mobile: +258 82 28893130 Email: nbhatt.mz@gmail.com
	Dr Marriott Nliwasa University of Malawi College of Medicine Private Bag 360, Chichiri, Blantyre, Malawi Phone: +265 88 8681948 / Mobile: +265 999 981439 Email: mnliwasa@gmail.com
	Prof Dr Valsan Philip Verghese Paediatrics Unit-III & Pediatric Infectious Diseases Christian Medical College Hospital Vellore-632004, India Phone: +91 416 228 2619 Email: valsan@cmcvellore.ac.in

Laboratories	Prof Dr Gunilla Kallenius Department of Clinical Science and Education Karolinska Institute 17177 Stockholm, Sweden Mobile: +46 70 7289905 / Email: Gunilla.Kallenius@ki.se
	Prof Dr Stefan Niemann Borstel Research Center – Department of Molecular and Experimental Mycobacteriology Parkallee 1, 23845 Borstel, Germany Phone: +49 4537 1885890 Email: stniemann@yahoo.de; sniemann@fz-borstel.de
	Prof Dr Gerhard Walzl Dr Novel Chegou Immunology Research Group of the Division of Molecular Biology and Human Genetics, Stellenbosch University Tygerberg Hospital, Cape Town, 7505, South Africa Email: gwalzl@sun.ac.za
	Beckman Coulter Inc. Dr Sankarnarayanan Pattabhiraman 50B, II Phase, Peenya Industrial Area, Bangalore-560058, India Phone: +91 9566261276 / Email: spattabhiraman@beckman.com
	Cepheid Dr Malin Nygren Cirkusgränd 4, 17236, Sundbyberg, Sweden Phone: +46 8 68437000 / Email: malin.nygren@cepheid.se
	Otsuka Novel Products GmbH Norbert Hittel Erika-Mann-Str. 21, 80636 Munich, Germany Mobile: +49 173 6740016 / Email: NHittel@otsuka-group.com
Other institutions involved in the study	Prof Dr Stephen Graham Department of Paediatrics , University of Melbourne The Royal Children's Hospital Melbourne 50 Flemington Road, Parkville 3052, Melbourne, Australia Phone: +61 3 9345 4788 / Email: steve.graham@rch.org.au
Study biobank	Foundation for Innovative New Diagnostics (FIND) Dr med Claudia Denkinger Biorepository, 878 Main Street, Buffalo, NY 14202, USA Mobile: +41 79 4469419 / Email: claudia.denkinger@finddx.org

Signature Page

Protocol Title: **Rapid and Accurate Diagnosis of Paediatric (RaPaed) TB - An AIDA (Assessment of Innovative Diagnostics and Algorithms for Early and Sensitive Detection of Acute TB) platform study**

Protocol Number: **LMU-IMPH-AIDA-02**

Sponsor

Herewith we approve the protocol LMU-IMPH-AIDA-02 Version 2.0, dated 06.09.2018 and confirm that it contains all information necessary to conduct the study according to the ethical principles laid down in the Declaration of Helsinki, Good Clinical Practice, and all applicable local regulations.

Sponsor Responsible Person
Prof Dr med Michael Hoelscher
Division of Infectious Diseases and Tropical Medicine
Medical Center of the University of Munich (LMU)

10.9.2018
Date

Sponsor Medical Expert
Dr. N. Heinrich

11.09.2018
Date

Clinical Study Partner

Herewith I confirm that I have read and understood the protocol LMU-IMPH-AIDA-02 Version 2.0, dated 06.09.2018, and agree to conduct this clinical trial accordingly, including all statements regarding compliance with the ethical principles laid down in the Declaration of Helsinki, Good Clinical Practice, and all applicable local regulations.

I will provide copies of the protocol and all information to the site personnel under my supervision required to conduct the clinical study duly. I will discuss this material with them and ensure they are fully informed on all study requirements.

Print name Site Principal Investigator	Date

PROTOCOL SYNOPSIS

Protocol Title	Rapid and Accurate Diagnosis of Paediatric (RaPaed) TB - An AIDA (Assessment of Innovative Diagnostics and Algorithms for Early and Sensitive Detection of Acute TB) platform study
Protocol Code	LMU-IMPH-AIDA-02
Short Title	RaPaed-AIDA-TB
Sponsor	Division of Infectious Diseases and Tropical Medicine Medical Center of the University of Munich (LMU)
Study Centres	Multi-centre study
Objectives	**Stage 1:** Primary Objective: • to evaluate sensitivity and specificity of new candidate tests for detecting paediatric tuberculosis, and calculate positive and negative predictive values in the study population Secondary Objective(s): • to describe the above mentioned diagnostic performance indicators of algorithms composed of two or more tests in the study population. • to describe negative and positive predictive values of tests and algorithms in study subpopulations. These will include but not be limited to: • a population excluding cases diagnosed at adjacent health facilities, which is thought to resemble the target population a new test will be used in to compare time to diagnosis between different diagnostic approaches **Stage 2: Objectives of Stage 1 apply to Stage 2, and additionally:** • to describe the yield of new tests when testing subpopulations of interest, which include children with pneumonia, malnutrition, or HIV coinfection. **Sub-study objectives:** • to describe the change in experimental test readout over time in children who receive TB treatment • to describe pulmonary impairment at baseline, and its change over time in a subset of children
Study Design	This study is designed as a single-gate, multi-diagnostic study in the target population of children ≤14 years suspected of having TB. Therefore, only symptomatic children will be enrolled and both standard of care and experimental diagnostics will be performed. NIH-convened consensus panel recommendations on case definitions and design of paediatric TB diagnostic studies will be followed.

	The study will compare several novel TB test candidates to the reference standard of combined microbiological and clinical disease confirmation or exclusion.
Population	It is aimed to recruit approximately 1000 children with suspicion of active TB, of which a minimum of 250 cases should be microbiologically confirmed.
Inclusion/Exclusion Criteria	**Stage 1 Inclusion Criteria:** 1) **Consent and Assent (if applicable):** signed written consent/assent, or witnessed oral consent/assent in the case of illiteracy, before undertaking any study-specific activity. **Of the following, either criterion 2), OR criterion 3), or both, have to be met:** 2) **Confirmation of TB disease:** microbiological confirmation of active TB disease by positive smear AND/OR culture AND/OR PCR (e.g. GeneXpert®); e.g. in a non-study health facility **AND/OR** 3) **Signs and Symptoms:** suspicion of active TB disease (one or more criteria): a. Chest X-Ray suggestive of TB: cavity AND/OR hilar/mediastinal lymph node enlarged AND/OR miliary pattern b. Weight loss** or failure to thrive within the previous 3 months that, in the investigator's opinion, is not solely due to inadequate feeding; or to another non-TB cause. c. Any cough combined with: • Loss of weight**; • Evidence of *Mycobacterium tuberculosis* infection: TST AND/OR IGRA positive d. Cough alone: persistent unremitting cough duration of ≥ 14 days e. Repeated episodes of fever within 14 days not responding to course of antibiotics AND positive TST or IGRA, (for malaria endemic areas: AND after malaria has been excluded by at least a negative rapid test) f. Signs & symptoms of extrapulmonary TB: • Unilateral non-painful lymph node(s) visibly enlarged ≥ 1 month; • Gibbus (especially of recent onset) • Non-painful enlarged joint

- Pleural effusion
- Pericardial effusion

g. CSF examination findings in line with TB meningitis with at least elevated protein and low glucose (in relation to serum glucose);
OR signs and symptoms in line with TB meningitis/CNS TB if lumbar puncture is contraindicated, in the view of the investigator:

At least one of the following two:

- palsy of oculomotoric nerves of recent onset
- focal neurological symptoms indicating elevated intracranial pressure OR CNS lesions, of recent onset

AND/OR at least two of the following less specific signs of TB meningitis/CNS TB (for malaria endemic areas: AND a negative malaria rapid diagnostic test*):

- Lethargy
- Convulsion
- Meningism (neck stiffness)
- Headache

Stage 2 Inclusion Criteria:

Stage 2 criteria are intended to reflect a population of children who in the future should receive TB testing in a programmatic setting.

When stage 2 is started, inclusion by stage 1 criteria will still be possible, but the either of the following will be added:

4) Malnutrition: Oedema of both feet, AND/OR weight for height less than -2 Z scores, AND/OR MUAC of less than 125 mm in children between 6 months and 5 yr. of age; which is not clearly only food related.

OR: any malnutrition that does not respond to adequate feeding therapy.

5) Acute Pneumonia: pneumonia with fast breathing and/or chest indrawing, in combination with any TB risk factor such as positive TST, relevant contact history in the last 6 months; AND/OR “severe pneumonia”, pneumonia with any general danger sign defined by IMCI, which requires referral.

	Fast breathing as defined by IMCI: children of 2-12 months of age, ≥ 50 breaths per minute; 12 months up to 5 years: ≥ 40 breaths per minute 6) Any acute respiratory infection that does not resolve after 14 days of adequate therapy. 7) Symptomatic HIV disease that requires testing for active TB following WHO guidelines. At the time of protocol writing, these were: • Loss of weight** or poor weight gain, • fever, • current cough or respiratory distress • history of close contact with an infectious TB case **Exclusion criteria (apply to stage 1 and 2):** 1) Critical condition (if study procedures seems like an undue risk to subject's life), such as hypovolemic shock or clinically relevant anaemia (tachypnoea, tachycardia) 2) Body weight is less than 2 kg 3) Children of 15 years of age or more 4) Are currently receiving anti-TB drug(s): ideally, eligible subjects should not have received any anti-TB treatment. In exceptions, up to three daily doses given since treatment start before first study blood draw are acceptable for study inclusion ** The requirement of negative MRDT may be dropped in agreement with the sponsor during study conduct* *** Documented or reported*
Evaluation Criteria	Primary Endpoint: confirmation/rule-out of TB disease; with the following degrees of certainty: - Confirmed tuberculosis - Unconfirmed tuberculosis - Unlikely tuberculosis Classification into the latter two groups will follow international consensus papers and judgement of the endpoint review committee.
Study Timeline	Estimated date of first subject enrolled: Quarter 03/2018 Estimated date of last subject enrolled: Quarter 03/2020 Estimated date of last subject completed: Quarter 01/2021 Total duration of project: ~ 54 months (24 months enrolment period + 6 months follow-up)

TABLE OF CONTENTS

Schedule of Events (SoE)	**Baseline / Visit 1:**		**Visit 2:** Month 1 / Day 28 ± 7 days	**Visit 3:** Month 3 / Day 84 ± 7 days	**Visit 4:** Month 6 / Day 162 ± 14 days *(if on TB treatment or unwell at Month 3)*	**Visit 5[A]:** Month 9 / Day 280 ± 14 days *(Follow-up spirometry)*	**Visit 6[A]:** Month 12 / Day 365 ± 14 days *(Follow-up spirometry)*
	Day 1	**Days 2 and/or 3**					
Clinical Assessment							
Informed consent	X						
Questionnaire	X						
Physical examination & Symptoms	X		X	X	X	X[A]	X[A]
Chest X-Ray (other radiology if indicated)	X						
Treatment compliance			X	X	X	X	X
Spirometry[A]	X[A]			X[A]	X[A]	X[A]	X[A]
TB Reference Standard Microbiology							
Sputum/sample for microbiology[B]	X Culture (LJ[B] + MGIT) Pellet: GeneXpert® Ultra®	X Culture (LJ[B] + MGIT) Pellet: cryostorage	1 X[C] *(if sputum produced spontaneously, and initial bacteriology positive)*	1 X[C] *((if sputum produced spontaneously, and initial bacteriology positive)*	1 X[C] *(if sputum produced spontaneously, and initial bacteriology positive)*		
Separate Sputum for storage	X *(if possible during scheduled visits)*						
Children ≤ 5 yr: nasopharyngeal aspirate (GeneXpert Ultra®)	X						
Laboratory (blood)							
Routine diagnostics							
Incl. haematology/storage/ EDTA and biochemistry, if applicable	X		X	X[D]	X[D]		
Malaria testing[E]							
HIV testing[F]	X/ Cd4 if HIV positive[G]						
Tuberculin Skin Test[H]	X						
IGRA[A]	X[A]		X[D]	X[D]	X[D]		
Experimental tests							
TAM-TB[H]	X		X	X[D]	X[D]		
PaxGene® RNA tube[K]	X		X	X[D]	X[D]		
Serum for storage	X		X	X[D]	X[D]		
PBMC isolation[A]	X[A]		X[A]	X[A]	X[A]		
Total maximum volume (blood)	*According to body weight [I]*						

Schedule of Events (SoE)	Baseline / Visit 1: Day 1	Baseline / Visit 1: Days 2 and/or 3	Visit 2: Month 1 / Day 28 ± 7 days	Visit 3: Month 3 / Day 84 ± 7 days	Visit 4: Month 6 / Day 162 ± 14 days *(if on TB treatment or unwell at Month 3)*	Visit 5[A]: Month 9 / Day 280 ± 14 days *(Follow-up spirometry)*	Visit 6[A]: Month12 / Day 365 ± 14 days *(Follow-up spirometry)*
Laboratory (urine)							
Urine analysis by dipstick	X		X[D]	X[D]	X[D]		
Alere Determine™ TB LAM	1 ml		1 ml[D]	1 ml[D]	1 ml[D]		
Uri-TB direct (LAM)	15 ml		15 ml[D]	15 ml[D]	15 ml[D]		
FujiFilm (LAM)	1 ml		1 ml[D]	1 ml[D]	1 ml[D]		
Urine for storage	1 ml		1 ml[D]	1 ml[D]	1 ml[D]		
Total maximum volume (urine)[J]	**18 ml**		**18 ml[D]**	**18 ml[D]**	**18 ml[D]**		
Laboratory (stool)							
Stool (GeneXpert® Ultra®)	X						
Stool (LAM)	X		1 X[D]	1 X[D]	1 X[D]		
Stool (storage)	X						

Table 1: Schedule of Events

Schedule of Events (SoE)

Please note: sample volumes between individual tests may vary, however maximum volumes given will not be exceeded. The standard diagnostic schedule is a non-binding recommendation and based on current practice in study sites.

A. Optional visits for procedures for sub-studies in a subset of children and not in all sites.
B. Sputum/gastric aspirate or induced sputum according to subject age and centre preference. Other diagnostic samples; e.g. bronchial secretion, or fine needle aspirate biopsy according to decision of attending clinician and best medical practice.
   Standard microbiology assessments include culture in MGIT and LJ media, smear, PCR (GeneXpert® Ultra®). LJ culture may be omitted in centres with a low contamination rate if agreed with the sponsor.
   Cultures positive for AFBs should be analysed by HAIN LPA for species, and molecular drug resistance testing.
   Isolates are to be cryopreserved in glycerol.
C. Sputum culture after Visit 1 only in subjects on TB treatment who were sputum positive in any microbiological test initially, and who are able to produce sputum spontaneously.
D. Samples for assessing treatment response – only to be taken in children who are started on anti-TB treatment; and not at all sites.
E. Only in malaria endemic settings
F. Only if HIV status unknown, or documented negative more than 3 months ago. Performance not needed if previously documented positive.
G. Before day 8, only if HIV positive: Cd4 count, HIV viral load.
H. IGRA: Interferon gamma release assay only at sites selecting to perform this. TST: tuberculin skin test; is to be applied **after** blood for TAM-TB is taken, to avoid interaction between TST antigen and TAM-TB.

I. Maximum blood volumes
J. Minimum total urine volume should be not less than 10 ml, otherwise repeated collection is advised
K. Venous and Finger prick

	Body weight category	≥ 15 kg	6 to < 15kg	4 to < 6kg	3 to < 4 kg	2 to < 3 kg
Routine diagnostics	Haematology/storage/ EDTA	3 ml	2 ml	2 ml	1.5 ml	1.5 ml
	HIV testing[F], Malaria test[E]	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
	IGRA[A]	4 ml	4 ml	-	-	-
Experimental tests	Serum for storage	3 ml	2.5 ml	2 ml	1.5 ml	1,5 ml
	PaxGene® RNA tube[K]	2.5 ml	2.5 ml	1 ml	1 ml	1 ml
	TAM-TB[H]	2 ml	2 ml	2 ml	2 ml	2 ml
	PBMC isolation[A]	4 ml	4 ml	3 ml	3 ml	-
Total blood volume		**19.0 ml**	**17.5 ml**	**10.5 ml**	**9.5 ml**	**6.5 ml**

Table 2: blood volumes for sampling, depending on individual subject weight

Summary of the proposed research in lay terms

Tuberculosis (TB) is a major cause of child morbidity and mortality in the world. There are an estimated one million new paediatric cases and at least 209.000 deaths per year. The fact that only 1% of children treated for TB have a fatal outcome highlights the fact that a large proportion of cases are never diagnosed and thereby never received appropriate treatment – which is a tragedy, considering the good outcomes, even in cases of MDR TB.

The inability to correctly and timely diagnose paediatric TB is the main obstacle to control disease and prevent adverse outcomes, as the tests themselves and the sampling/testing strategies are designed for TB among adults. Children tend to have paucibacillary and/or extrapulmonary disease and collection of adequate samples from children is difficult as well as time- and resource-consuming. Addressing the need for new diagnostic approaches implies the need for non-sputum based tests and improved sensitivity, specifically among infants and young children, children with malnutrition, HIV-infection, and drug-resistant TB. At the same time, those new diagnostics/testing strategies need to be more feasible to conduct, particularly in resource-limited settings.

Recently, increased advocacy, political and funding support have led to an effort to develop new tests and testing strategies; as such, a number of potential candidates have been identified with the potential to substantially improve paediatric TB diagnosis. Furthermore, the World Health Organization has clearly stated that new and improved diagnostics for children are a top priority.

We are proposing to assess at least eight new diagnostic techniques suitable for children in this study. Most novel tests to be evaluated in this study use non-sputum samples and have different testing approaches, including evaluation of host response, antigen-detection, cellular immunoresponse assays and nucleic-acid amplification tests (see below for further details).

List of Abbreviations

AIDA TB	*Assessment of Innovative Diagnostics and Algorithms for Early and Sensitive Detection of Acute TB*
CAP	*College of American Pathologists*
CD4	*Cluster of differentiation 4*
CRF	*Case Report Form*
CRP	*C-reactive Protein*
CXR	*Chest X-Ray*
DZIF	*German Centre for Infection Research*
eCRF	*Electronic Case Report Form*
EDCTP	*European and Developing Countries Clinical Trials Partnership*
EDTA	*Ethylene diamine tetraacetate*
GCP	Good Clinical Practice
GCLP	*Good Clinical Laboratory Practice*
HIV	*Human Immunodeficiency Virus*
ICF	Patient Information and Informed Consent Form (can also include Assent Form)
ICH	*International Council for Harmonisation*
IGRA	*Interferon gamma release assay (mostly QuantiFERON)*
IS	Induced Sputum
IRB	*Institutional Review Board*
LAM	*Lipoarabinomannan*
LMU	*Ludwig-Maximilians-University*
MDI	Metred Dose Inhaler
MDR	*Multi-drug resistant*
MOP	*Manual of Procedures*
MTA	*Material transfer agreement*
N	*Number (typically refers to number of subjects)*
NTP	*National TB Programme*
PI	*Principal Investigator*
QA	*Quality Assurance*
QC	*Quality Control*
QUADAS	*Quality Assessment of Diagnostic Accuracy Studies) tool*
RIF	*Rifampicin*
SoE	*Schedule of Events*
SOP	*Standard Operating Procedure*
STARD	*Standards for Reporting Diagnostic accuracy studies*
TAM	*T-cell activation and maturation marker*
TB	*Tuberculosis*
TST	*Tuberculin Skin Test*
Ultra®	*GeneXpert MTB/RIF Ultra®*
WHO	*World Health Organization*
Xpert®	*GeneXpert® MTB/RIF®*

1. BACKGROUND INFORMATION

1.1 The burden of TB and current state of diagnosis

Tuberculosis (TB) in children is recognized as a neglected problem within the TB epidemic. Most recent estimates show that every year, children account for an estimated one million new cases (1), and an estimated 209 000 children die from TB – half of those in Africa (2). The main problem clearly is adequate and timely diagnosis – effective treatment is available, but only less than half of all child TB cases worldwide are diagnosed and treated (3).

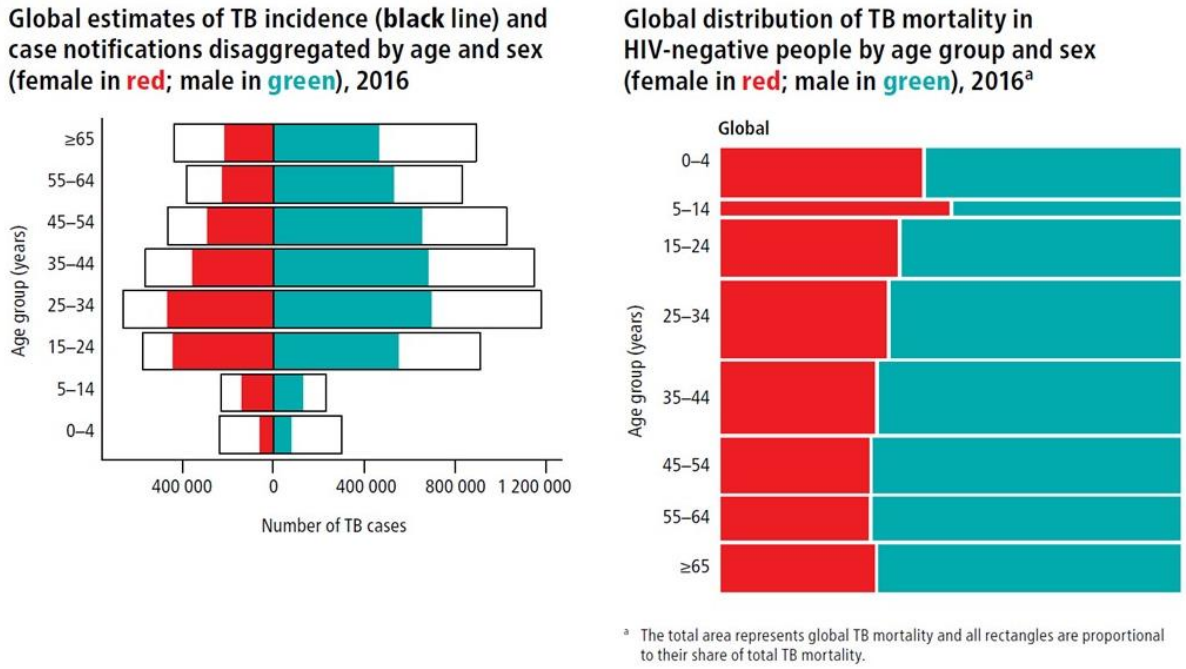

Figure 1: Global burden and mortality in TB by age group. Globally, children (aged < 15 years) accounted for 6.9% of the new TB cases that were notified in 2016, whereas childhood deaths account for 16% of the total burden. ((1))

TB is a common, often undiagnosed cause of child morbidity, but clinically it is often masked by other diseases like malnutrition or pneumonia due other pathogens, and radiological appearance is most often non-specific (4), (5). For a long time, the contribution of TB to child morbidity and mortality was thus underestimated, since the disease too often only became evident when autopsy research studies were conducted (6, 7).

The inability to correctly and timely diagnose paediatric TB is the main obstacle to control disease and prevent adverse outcomes. Those at risk for the worst outcomes are the same that pose the greatest diagnostic challenges: infants and young children, children with malnutrition and HIV-infection, and children with MDR TB.

Most current TB tests and sampling strategies are geared towards adult TB. Obtaining adequate samples for those tests from children is difficult, time- and resource-consuming, and often requires hospitalization. Moreover, paediatric samples, especially from the mentioned vulnerable groups, have small volumes and low bacterial burdens. Estimates in literature show that at best only 30-60% of true childhood TB cases can be confirmed microbiologically (8), and in most cases this is not even

attempted, especially in resource-limited settings. This is a tragedy, considering that timely treatment in children leads to good outcomes, even in the case of drug resistance.

These diagnostic difficulties, along with the perception that children would not contribute to the spread of TB since they were less infectious than adults, led to a neglect by stakeholders. It is estimated that TB probably is among the top ten causes of mortality in children under five years of age (3), (9).

Only in recent years, attention, support, and political commitment have increased. This led to the WHO roadmap for childhood TB being released in 2013, which advocates research to address the diagnostic dilemma (10). WHO has released high-priority target product profiles for tests which partly accommodate the needs of children (11). New testing strategies are desperately required – these need to have improved sensitivity, but at the same time be much more feasible than current tests; so need to work well on samples which are easy to obtain from children - one important barrier to widespread testing is the current technically challenging and time-consuming sampling of sputum or gastric aspirate which often requires hospitalization.

The STOP TB New Diagnostics Working Group in their Global Plan concluded that an algorithm of a screening and a confirmatory test is a realistic strategy to achieve these aims; as alternative to the "magic bullet" test that would fulfil all criteria (12). A screening, triage or "rule-out" test should ideally be cheap and easy enough to perform at point of care, and should have a high sensitivity to exclude disease with certainty. A confirmatory "rule-in" test would then be the next step in a smaller group of subjects; have better specificity, and could afford higher complexity. The working group specifically calls for a "biomarker test" which is independent of sputum, suitable for use in children (13).

Increased advocacy and political support have led to a number of new tests being developed; with potential to become game-changers for child TB diagnostics.

2. NEW CANDIDATE DIAGNOSTICS

2.1 Candidate TB diagnostics for evaluation and findings from clinical studies

2.1.1 Xpert® MTB/RIF Ultra® (Ultra®)

GeneXpert® MTB/RIF® (Xpert®) is widely available as a TB diagnostic test, but has a limited detection ability for TB in paucibacillary samples, corresponding to limited sensitivity in children of only 62% of that of culture, compared to 89% in adults (14, 15). Using combined nasopharyngeal aspirates and stool showed similar yield as sputum (16).

The recently developed Xpert MTB/RIF Ultra® (Ultra®) cartridge is substantially more sensitive than Xpert®. In a cohort of close to 400 children with testing on induced sputum, sensitivity was 76% (FIND, unpublished) in comparison to culture on the same sample (while only 7% were smear positive). Validation of the Ultra® on adult respiratory samples, WHO review of the data and endorsement for Ultra® have been completed in March 2017.

The Alland Lab, which developed the MTB/RIF® and Ultra® assays with FIND, have now developed an improved stool processing protocol, yielding a sensitivity of 85% in two small independent studies with the old MTB/RIF® cartridge ((17); FIND unpublished data).

We will assess a combination of nasopharyngeal aspirate and stool sampling for use with Ultra® in addition to testing other extrapulmonary specimen like fine needle lymph node aspirates for performance, to validate these promising proof-of-concept results.

Further, development of the GeneXpert® Omni resulted in a platform that allows for placement in microscopy centres (i.e. level 1 healthcare facilities). This will leverage existing infrastructure around smear microscopy and enable earlier diagnosis and treatment for TB; but is likely not to be used in this study – however, depending on circumstances, some centres may use Omni as the platform to run GeneXpert® Ultra® when Omni performance is established enough. This will be laid down in the Investigator Site File.

2.1.2 TAM-TB

The T cell activation marker assay is a novel immunodiagnostic test that - unlike conventional IGRA and TST - specifically discriminates active disease from latent infection, relying on predominance of an effector memory cell phenotype. In a previous EDCTP-funded study on children in Tanzania, TAM-TB assay showed good diagnostic performance for TB in a HIV and TB endemic setting in Africa, with a sensitivity of 83% and specificity of 97% (18); and EDCTP has published an opinion paper advocating continued development and implementation of this test (19).

The TAM-TB is being developed into a standardized assay kit, similar to conventional IGRAs, in collaboration with Beckman Coulter. This standardized kit will be tested in RaPaed-AIDA-TB and allow widespread implementation on flow cytometers used for CD4 counts.

2.1.3 Uri TB direct

The group of Prof. Gunilla Källenius (Karolinska Institutet, Sweden), who developed the first LAM, has developed a LAM test with increased sensitivity of 82% (14 of 17) in adult, HIV-negative TB patients, at a specificity of 100%; a dramatic improvement over available commercial tests (20). The test is easy to perform, a technician can handle 24-48 samples in about 2 h, requiring an ELISA reader. Currently, a point-of-care (POC) version is being developed, funded by the Gates Foundation.

2.1.4 Otsuka LAM

Otsuka Pharmaceuticals are committed to developing products for use in TB, as evidenced by their licensing of delamanid. Otsuka have developed a LAM assay in ELISA format, which is said to have a detection threshold of 100 CFU/ml in sputum. The assay is currently being adapted to stool, and proof-of-concept data show a decent sensitivity at 88% in adults. In this study, stool LAM will be tested.

2.1.5 Fujifilm urinary LAM assay

Fujifilm (in collaboration with FIND) is developing a POC test based on an innovative photographic development-like silver amplification with a sensitivity 1-2 orders of magnitude higher than current lateral flow immunoassays. This is a sample-to-answer cartridge with on-board reagents, can be manually operated, visually read, has a time-to-result of approx. 30 mins and does not include more than three simple steps. The Alere Determine™ TB LAM will be used as a comparator test.

2.1.6 Host biomarker validation (FIND)

With funding from the Gates Foundation, SomaLogic (Boulder, Colorado) using the FIND biobank, built a TB classifier with six host response serum markers on adult samples. In parallel FIND and partners systematically evaluated the real TB diagnostic potential of antibody detection in serum. In a study, the researchers combined the most promising SomaLogic host markers and FIND antibody marker, built predictive models and estimated the performance in blinded verification sets. A four markers model performed well (AUC=0.90, 72% specificity, 90% sensitivity, n=196) and reached the performance target of the WHO high-priority triage target product profile. The signature will be transferred to an appropriate POC platform and validated in adult patients. Paediatric assessment will depend on RaPaed-AIDA-TB samples.

2.1.7 Host biomarker combination (University of Stellenbosch)

The immunology research group at Stellenbosch University, in collaboration with consortium partners recently patented and published serum and plasma biosignatures, from a previous EDCTP1 funded study (the African European Tuberculosis Consortium). A seven-marker host serum protein signature diagnosed TB in adults suspected of having TB disease, recruited from five different African countries with a sensitivity of 93.8% and specificity of 73.3% in the test sample set (21). This biosignature is currently being developed into a finger-stick point-of-care screening test for TB. Another recently published signature of host plasma proteins has achieved a remarkable sensitivity for diagnosing TB in an adult study, at 100% with leave - one - out cross-validation, with a specificity of 89.3% (22). Adaptation of these signatures for child TB is currently underway in collaboration with Prof Zar, UCTLI, and will be tested prospectively in RaPaed-AIDA-TB.

2.1.8 Host RNA biomarker (LMU)

Several transcriptomic signatures for diagnosis of active TB in high burden African settings (including HIV+ and HIV- cases) have been described (21, 22) with promising sensitivity and specificity (23). Recently, a signature using only four genes to discriminate between active TB and latent infection has been published (24). An RT-PCR based test will be more feasible for use in daily clinical practice in the field, such as the 16-gene signature predicting TB progression in adolescents published by Zak et al., including members of the RaPaed consortium (23). We will aim to test this 16-gene-RT-PCR panel of Zak and colleagues to compare its performance as a diagnostic test with reference standard methods from concurrent patient samples. Additional work towards diagnostic signatures on RNA samples is intended to be performed in close collaboration with colleagues at Imperial College (Prof Michael Levin).

3. RATIONALE FOR THE STUDY

This study will serve as a platform to evaluate new diagnostics in children suspected to have TB, to establish diagnostic performance (sensitivity and specificity) and calculation of positive and negative predictive values in a real-life cohort.

All of these tests have shown promising data in previous studies, indicating a high likelihood of strongly improving the dilemma of TB diagnosis, especially in childhood TB. Due to the unique set-up of the RaPaed Tb consortium, which includes the former chair of the Stop TB Partnership's child TB subgroup and a member of WHO's Strategic and Technical Advisory Group on TB, members from NTPs, and FIND, the consortium will be well equipped to disseminate the results and facilitate their uptake into policy.

Finally, this study will comprise results of several tests in its database. This will allow simulation of diagnostic algorithms, that may be composed of screening (i.e. rule-out) tests together with confirmatory tests to maximize sensitivity and specificity.

4. STUDY OBJECTIVES

4.1 Primary Objective

Stage 1:

Primary Objective:

- to evaluate sensitivity and specificity of new candidate tests for detecting paediatric tuberculosis, and calculate positive and negative predictive values in the study population

Secondary Objective(s):

- to describe the above mentioned diagnostic performance indicators of algorithms composed of two or more tests in the study population.
- to describe negative and positive predictive values of tests and algorithms in study subpopulations. These will include but not be limited to:
- a population excluding cases diagnosed at adjacent health facilities, which is thought to resemble the target population a new test will be used in to compare time to diagnosis between different diagnostic approaches

Stage 2: Objectives of Stage 1 apply to Stage 2, and additionally:

- to describe the yield of new tests when testing subpopulations of interest, which include children with pneumonia, malnutrition, or HIV coinfection.

4.2 Specific Objectives of Sub Studies

- to describe the change in experimental test readout over time in children who receive TB treatment
- to describe pulmonary impairment at baseline, and its change over time in a subset of children

5. STUDY DESIGN, POPULATION AND SITES

5.1 Summary

This study is designed as a single-gate, multiple diagnostic study in the target population of children suspected of having TB. The term "single-gate" states that only symptomatic children will be enrolled,

and subgroups for calculating new test sensitivity and test specificity will be defined from this group, using reference standard diagnostic test results and clinical parameters of disease over time; such as response to TB treatment. Both standard of care and experimental diagnostics will be performed. NIH-convened consensus panel recommendations on case definitions and design of paediatric TB diagnostic studies will be followed (5, 25).

The study will compare several novel TB test candidates to the reference standard of combined microbiological and clinical disease confirmation or exclusion.

5.2 Measure to prevent bias: Blinding

In diseases where the reference standard for diagnostics is imperfect and does not detect 100% of cases, a case definition will necessarily include cases without definite microbiological confirmation of disease, but defined by positive response to treatment. Incorporation of candidate diagnostics into clinical decision-making for starting treatment would bias results of a diagnostic evaluation study towards an overly favourable performance of the new diagnostic (incorporation bias), and therefore is prohibited in this study. TB treatment does not only cover and cure TB, but rifampicin covers a broad spectrum of bacteria – thus, a positive treatment response is possible in non-TB cases, which could then be falsely classified as TB.

Therefore, the study subjects, the investigators and all other site staff who will be in contact with the subjects, will be kept blinded to the results of the new, investigational diagnostics throughout the study. GeneXpert® Ultra® here is seen as part of the reference standard diagnostics, and results from this test will not be blinded; with the exception of the stool processing device for Xpert® Ultra®; which is seen as experimental, and Xpert® results from this will not be available to investigators. Treatment decisions will be following guidelines and reference standard diagnostics.

Furthermore, the health professional or laboratory technician who is performing and reading the new diagnostic test will be blinded to the results of clinical examination and bacteriological investigations.

Finally, the study and CRFs will be designed and reported following STARD and QUADAS published quality standards for evaluation of new diagnostics (26, 27).

5.3 Study Endpoints

5.3.1 Primary Endpoint

The primary endpoint of a subject will be classification of active disease status, following routine diagnostics. The final classification will be laid down in the statistical analysis plan, which will be finalized and signed off before database lock; and which may still contain modifications from the classifications detailed below. The following TB disease classifications are possible (adapted from (5)):

Confirmed tuberculosis Bacteriological confirmation obtained
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:

- Symptoms suggestive of TB

- CXR consistent with TB
- Recent exposure or immunologic evidence of MTB infection (TST and/or IGRA positive)
- Positive response to TB treatment
  Requires documented positive clinical response on tuberculosis treatment - no time duration specified

  - With *M. tuberculosis* infection
    Immunological evidence of M. tuberculosis infection (TST and/or IGRA positive)
  - Without M. tuberculosis infection
    No immunological evidence of M. tuberculosis infection

Unlikely tuberculosis bacteriological confirmation NOT obtained AND criteria "unconfirmed TB" not met

- With M. tuberculosis infection
  Immunological evidence of M. tuberculosis infection (TST and/or IGRA positive)
- Without M. tuberculosis infection
  No immunological evidence of M. tuberculosis infection

An expert panel will be installed to review the subject's assessment as unconfirmed TB vs. unlikely TB when microbiological confirmation is not obtained.

5.4 Study Population and Eligibility Criteria

Patients with a suspicion of active TB are eligible for recruitment into the study, if they meet the specified inclusion criteria for each stage, and none of the exclusion criteria.

The sponsor will monitor enrolment by subject category, and decide to limit enrolment or stop enrolment into certain criteria, in case of overrepresentation of certain age groups or disease categories (e.g. predominance of children > 9 yr., or predominance of malnourished children); to achieve a population that includes children in need of future testing in a representative way. In addition, the sponsor will take a decision to advance to stage 2, and/or drop the requirement for malaria rapid test, based on good accrual of confirmed cases of TB.

5.4.1 Inclusion Criteria

Stage 1 Inclusion Criteria:

1) **Consent and Assent (if applicable):** signed written consent/assent, or witnessed oral consent/assent in the case of illiteracy, before undertaking any study-specific activity. The age threshold for child assent requirement will be laid down in each the Investigator Site File based on the local Ethics Committee requirement.

Of the following, either criterion 2), OR criterion 3), or both, have to be met:

2) **Confirmation of TB disease:** microbiological confirmation of active TB disease by positive smear AND/OR culture AND/OR PCR (e.g. GeneXpert®); e.g. in a non-study health facility

AND/OR

3) **Signs and Symptoms:** suspicion of active TB disease (one or more criteria):

   a. Chest X-Ray suggestive of TB: cavity AND/OR hilar/mediastinal lymph node enlarged AND/OR miliary pattern

   b. Weight loss** or failure to thrive within the previous 3 months that, in the investigator's opinion, is not solely due to inadequate feeding; or to another non-TB cause.

   c. Any cough combined with:

      - Loss of weight**
      - Evidence of *Mycobacterium tuberculosis* infection: TST AND/OR IGRA positive

   d. Cough alone: persistent unremitting cough duration of ≥ 14 days

   e. Repeated episodes of fever within 14 days not responding to course of antibiotics AND positive TST or IGRA, (for malaria endemic areas: AND after malaria has been excluded by at least a negative rapid test)*

   f. Signs & symptoms of extrapulmonary TB:

      - Unilateral non-painful lymph node(s) visibly enlarged ≥ 1 month;
      - Gibbus (especially of recent onset)
      - Non-painful enlarged joint
      - Pleural effusion
      - Pericardial effusion

   g. CSF examination findings in line with TB meningitis with at least elevated protein and low glucose (in relation to serum glucose); OR signs and symptoms in line with TB meningitis/CNS TB if lumbar puncture is contraindicated, in the view of the investigator:

      At least one of the following two:

      - palsy of oculomotoric nerves of recent onset
      - focal neurological symptoms indicating elevated intracranial pressure OR CNS lesions, of recent onset

      AND/OR at least two of the following less specific signs of TB meningitis/CNS TB (for malaria endemic areas: AND a negative malaria rapid diagnostic test*):

      - Lethargy
      - Convulsion
      - Meningism (neck stiffness)
      - Headache

Stage 2 Inclusion Criteria:

Stage 2 criteria are intended to reflect a population of children who in the future should receive TB testing in a programmatic setting.

When stage 2 is started, inclusion by stage 1 criteria will still be possible, but the either of the following will be added:

4) Malnutrition: Oedema of both feet, AND/OR weight for height less than -2 Z scores, AND/OR MUAC of less than 125 mm in children between 6 months and 5 yr. of age; which is not clearly only food related.

   OR: any malnutrition that does not respond to adequate feeding therapy.

5) Acute Pneumonia: pneumonia with fast breathing and/or chest indrawing, in combination with any TB risk factor such as positive TST, relevant contact history in the last 6 months; AND/OR "severe pneumonia", pneumonia with any general danger sign defined by IMCI, which requires referral.
   Fast breathing as defined by IMCI: children of 2-12 months of age, ≥ 50 breaths per minute; 12 months up to 5 years: ≥ 40 breaths per minute
6) any acute respiratory infection that does not resolve after 14 days of adequate therapy.
7) Symptomatic HIV disease that requires testing for active TB following WHO guidelines. At the time of protocol writing, these were:
   - Loss of weight** or poor weight gain
   - fever
   - current cough or respiratory distress
   - history of close contact with an infectious TB case

5.4.2 Exclusion criteria (apply to stage 1 and 2):

1) Critical condition (if study procedures seems like an undue risk to subject's life), such as hypovolemic shock or clinically relevant anaemia (tachypnoea, tachycardia)
2) Body weight is less than 2 kg
3) Children of 15 years of age or older
4) Are currently receiving anti-TB drug(s): ideally, eligible patients should not have received any anti-TB treatment. In exceptions, up to three daily doses given since treatment start before first study blood draw are acceptable for study inclusion

** The requirement of negative MRDT may be dropped in agreement with the sponsor during study conduct*
*** Documented or reported*

5.5 Currently Planned Sub-studies

This study provides the opportunity to advance the scientific understanding of TB disease and diagnosis, within a well-controlled setting generating valuable data in a large cohort of subjects, with high standard of quality control and high data quality. It is therefore ethically mandated to use this opportunity to advance the science around TB, TB treatment and other infectious diseases by making

use of this resource, in order to accelerate future developments to the benefit of TB patients and persons at risk of TB diseases and other infectious diseases.

We are currently anticipating two sub-studies to be embedded in this protocol:

- Assessment of treatment response: due to the difficulties in sampling sputum, treatment response assessment in children is even more difficult than in adults; but as in adults, there is evidence that most children with drug-sensitive disease could be cured with shorter therapy. We will therefore select a subgroup of sites, where children who are starting on TB treatment will undergo sampling for new tests at their scheduled clinic visits. New test results over treatment will be analysed and compared with classical treatment response parameters, such as weight gain, evolution of symptoms, and change in TB culture positivity in the subset of children who can produce sputum spontaneously.
- Assessment of lung function impairment, and its change over time: TB is increasingly recognized to not only be acutely life threatening, but also cause permanent damage to the respiratory and cardiocirculatory system, even if microbiological cure is achieved.
  In a subgroup of children, who are old enough to perform spirometry, and at selected sites, spirometry will be performed as indicated in the SoE. The decision to perform or not perform spirometry will be taken by the investigator based on child maturity and cooperation.
- Assessment of the possible clinical and immunologic associations between active TB, latent TB infection and a CMV-reactivation. This sub-study is planned to be performed at selected sites and aims to assess:
  - Prevalence of (active and latent) TB-CMV co-infection in the study cohort
  - Correlation/risk stratification of severity of clinical presentation with CMV viral load and outcome/survival
  - Description of CMV-specific immune response in HIV-infected, HIV-exposed, but uninfected and HIV-uninfected children with active TB and latent TB

5.6 Biobanking and future sub-studies

All samples that are not used up for the new diagnostics described above, will be cryopreserved to enhance the possible benefit from this study, by providing a sample repository that may be used for developing better diagnostics in the future; abbreviating the time to proof-of-concept results.

These samples may be used for developing new diagnostics for infectious diseases; including possible host genetic analysis. Collection of these samples will only be done if informed consent, on a form separate from the ICF for the main RaPaed-AIDA-TB study, is provided – these forms are separate to allow participation in the main study, even if parents/guardians do not want to participate in sample storage. Sample(s) will be kept until they are all used up or destroyed at FIND's discretion, which may take up to twenty years, with extension if needed. Longer storage is to be permitted if permission is granted by the applicable ethics committees.

The use of these samples will be decided on by FIND, upon applications of individual researchers or teams for their use. Results of these analyses will not be incorporated into the RaPaed-AIDA-TB study reports, and will not be disclosed to investigators before the completion of the main RaPaed-AIDA-TB study. Retention samples will be whole blood for genetic analysis, serum, sputum, urine and other

biological samples to analyse for potential correlates of TB disease classification, treatment success, bacterial load, biomarkers and other parameters registered in the main study. Storage and analysis of these samples may be performed outside of the countries hosting the trial site(s). The possibility of sample transport and analysis abroad will be included into the information provided to the patient before consenting. All use of stored samples, which is not identical with intentions and/or methods described in this protocol, shall be submitted to the relevant IRBs before any sample is used.

5.7 Capacity Building in host countries

The capacity development programme will be composed of on-site trainings in paediatric TB diagnostics, and ensure expertise at all consortium partners and adjacent health facilities to benefit recruitment.

Training in paediatric TB diagnostics for researchers and adjacent health facilities will be conducted using a short course programme on paediatric TB diagnostics by UCTLI. The programme will include general teaching about diagnosing TB in children, study specific procedures, and hands-on training in sputum induction.

5.8 Study coordination and study sites

For selection of clinical study sites, an assessment of previous diagnostic trial experience, available patient population and lab capacity was conducted by the study sponsor.

All clinical partners are highly experienced and have a proven track record of high-quality research.

The study sites (*for details see below*) have adequate clinical facilities and partner facilities for recruitment and follow up of study subjects. The Division for Infectious Diseases and Tropical Medicine, University of Munich (LMU), will be coordinating the study, fulfilling the role of study sponsor as defined by ICH GCP.

5.9 Financing

The study is conducted under the umbrella of the AIDA TB platform and is funded collaboratively by the European and Developing Countries Clinical Trials Partnership (EDCTP) and the German Centre for Infection Research (DZIF).

The CMC Vellore site is financed by the industry partner Beckman Coulter.

5.10 Participating study sites

5.10.1 Cape Town, South Africa

The *University of Cape Town Lung Institute (UCTLI)* has an excellent track record in TB diagnostic and treatment research, wide experience in the field, laboratory capacities and an excellent network for recruiting subjects. One of its partners, the Red Cross Children's Hospital (RCH), is the major paediatric tertiary hospital and referral centre for the Western Cape, the region with world's highest TB incidence. This hospital admits over 25,000 inpatients annually. Its research facility has direct access to all inpatients. Prof. Heather Zar has conducted a range of ground-breaking childhood TB diagnostic studies, which provided evidence for WHO and national South African guidelines for use of Xpert® in

children, but also on other diagnostics. She will oversee recruitment and clinical capacity development in this proposal.

More than 3,000 children have been recruited to clinical research studies during the past 5 years, including key TB diagnostic studies in children.

5.10.2 Mbeya, Tanzania

National Institute of Medical Research – Mbeya Medical Research Centre (MMRC), with Dr. Nyanda Ntinginya, has conducted high quality TB diagnostic trials in children, and was the main data collection site for the first TAM-TB assay validation. MMRC is situated on the premises of one of Tanzania' s four largest referral hospitals serving a population of about 6 million people, collaborating with the Baylor paediatric HIV clinic on the campus. The centre is closely collaborating with LMU for almost 20 years, which encompasses TB diagnostic, treatment and operational research studies, among which studies on the validation of Xpert MTB/RIF®

5.10.3 Blantyre, Malawi

The Malawi-Liverpool-Wellcome Trust (MLW) clinical programme and College of Medicine (University of Malawi) will form the administrative and laboratory centre of the study in Malawi. There is a strong history of paediatric clinical research including studies on severe pneumonia, bacterial meningitis and studies characterising paediatric respiratory infection aetiology and tuberculosis, and PK of anti-tuberculosis drugs in children. The clinical research operates within the Queen Elizabeth Central hospital (QECH) and as the only major public health facility for Blantyre this is the busiest health facility in the country. It has a specialist children's A&E department that sees over 95 000 children per year of which, on average, 25 000 cases are admitted. Since 2010 it has the only paediatric chest clinic operating in the district and directly follows up 80% of the paediatric TB cases. As well as direct attendance, QECH receives referrals from the principal health centres within Blantyre. The clinical services are backed up by well-established research laboratory at MLW, including microbiology diagnostics - both standard bacteriology and molecular diagnostics.

The TB diagnostics laboratory is set up with MLW, in collaboration with Dr Liz Corbett, at the College of Medicine in Blantyre, and is running to GCLP standards.

5.10.4 Maputo, Mozambique

Mozambique's National Institute of Health (NIS)'s mission is to conduct national surveillance, clinical trials (HIV vaccines trials and HIV/TB treatment trials), evaluation of new diagnostic technologies, and includes the national TB reference laboratory which will be used in this study, as well as a Tuberculosis Trial Unit in Maputo city established in collaboration with LMU headed by Dr. Nilesh Bhatt. A smaller evaluation study of Uri-TB-direct is currently running in collaboration with Karolinska Institute.

5.10.5 Vellore, India

The Christian Medical College (CMC), Vellore (Tamil Nadu, India) is renowned across the subcontinent for highest quality of care and research. The Paediatric Infectious Diseases Clinic has a long-standing experience in the clinical care and confirmation of disease of childhood TB. All microbiological (bacteriologic and molecular) testing for M. tuberculosis is done in the Department of Clinical

Microbiology; together these department have contributed to a multi-centre feasibility and diagnostic accuracy study of the QuantiFERON and Xpert MTB/RIF® assay in diagnosing tuberculosis in children (28).

5.11 Data collection, handling and analysis

Data acquisition and study monitoring will follow the high standards in the Division of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich (LMU) established in past TB diagnostic and therapeutic studies. Study staff will enter data from hospital records and the central experimental testing lab into a specifically developed electronic CRF; pre-programmed edit checks and data monitoring will ensure adequate recruitment, and good data quality, as stipulated in ICH GCP. Regular data review will enable early identification of issues in recruitment and study conduct in order to mitigate risks to the project.

Depending on site procedures and permission by the sponsor, clinical data may be entered directly into the eCRF without the need for any paper source data. The site procedure will be laid down in the respective investigator site file.

Laboratory data may be available directly as electronic output of laboratory devices, or from a laboratory information management system. These data will be imported into the central database; without the need for a paper source. The site procedure will be laid down in the respective investigator site file.

The sponsor will establish a monitoring plan that will contain risk-based monitoring of data entered into the eCRF.

For data analysis, an analysis plan will be finalized and signed off before study database lock. This will follow subject categorization recommended by the NIH expert panel (5), and will describe the use of an endpoint review committee for classifying certain cases of interest as “unconfirmed TB” or “unlikely TB” as described under study endpoints.

An objective of this study is the description of algorithms for diagnosis of TB. During study conduct, when the final sample size of children with confirmed TB can be estimated, the consortium will decide whether the cohort should be split into a training, and a validation sub-cohort for this task. The consortium will do so without knowledge of the new test performance.

6. STUDY ASSESSMENTS AND LABORATORY PROCEDURES

Currently established standard diagnostics in this study are described in the SoE. Additional diagnostics, e.g. fine needle aspirate of a lymph node, will be decided on by attending clinicians in the subject’s best interest, aiming for confirmation of TB disease and testing of susceptibility of an isolate, by microbiological means as is the practice in well-equipped settings.

Sample collection from TB suspects will be standardised for the study sites. Specimens collected will include a) blood, b) urine, c) sputum/gastric aspirate/nasopharyngeal aspirates, d) stool and, if necessary, e) specimen of other body fluids or other tissues. The volumes of specimens to be collected for study purpose only are shown in the SoE Table 2.

6.1 General assessment

The following demographic and background variables will be collected according to the SoE at Baseline/Visit 1 and/or during specified study visits:

- Inclusion and exclusion criteria
- Questionnaire on symptoms, comorbidities, exposure to TB and sociodemographic parameters
- Demographic data: date of birth, race/ethnicity, sex, place of birth, country of origin, etc.
- Weight in kilograms (kg) and height in meters (m)

6.1.1 Clinical examination and vital signs

The investigator will conduct a complete physical examination and any significant findings will be recorded. All information will be noted in the CRFs. The percutaneous oxygen saturation in air (%) will be measured, similarly the systolic and diastolic blood pressure (BP, mmHg) will be assessed using the same type of sphygmomanometer or device, if possible by the same observer, at each visit and after 5 minutes rest. Weight (kg) and height (m) will be recorded. The body temperature (°C) will be measured using a thermometer. Heart rate bit (bpm) and respiratory rate (rpm) will be also recorded.

6.2 Assessments of co-morbidities

Co-morbidities will be assessed using a number of methods: medical history, clinical examination (as described above), laboratory analysis (described below) and study questionnaires (CRFs).

6.2.1 General laboratory

The following laboratory values will be collected:

- Haematology: haemoglobin, haematocrit, red blood cell count, white blood cell count with differential, platelet count
- Urinalysis - dip stick analysis

Any abnormal findings will be further investigated according to the local standards. Further description of the laboratory tests will be provided below in this section as well as in the MOP, which will be distributed to the sites before study start.

6.2.2 HIV and CD4 count

All subjects will be initially screened for HIV according to the national guidelines of each respective country after providing informed consent. In addition, CD4 cell count will be performed in all HIV positive subjects. Those subjects who are newly diagnosed with HIV will be referred to the national health care system for further treatment and care.

6.2.3 Assessment of co-infections

The collected samples may be used to identify and assess co-infections, e.g. bacterial, viral and parasitic infections in addition to those described in the SoE by standard diagnostic techniques including cultures, PCR, antigen tests or microscopy. These assessments will serve to obtain more

information related to clinical patient presentation, and performance of standard and candidate diagnostic tests in this study. Should host genome sequencing techniques in addition to RNA sequencing for transcriptional signatures be envisioned, prior agreement of study ethics committees to a study amendment is necessary.

These assessments will be facultative and will only be conducted off remaining samples if lab capacity and budget permits.

6.3 Sample collection methods

Sample collection will be performed according to the laboratory manual provided for this study, and according to site SOPs.

6.3.1 Sputum/respiratory samples

From children who can voluntarily expectorate, consecutive sputum samples are collected in sterilized, well lockable labelled sputum container with scaling. The samples should be processed on the same day but can be kept at 2-8°C for a maximum of 24 hours until the start of further procedures.

From younger children, sputum samples/gastric aspirates and nasopharyngeal aspirates or the like will be collected using sputum induction as a technique to obtain respiratory specimen. Sputum induction will be carried out in an isolated and well-ventilated room. In brief, children will inhale with hypertonic saline (up to 5.8%) until they start coughing productively. Then, they will receive oro- or nasopharyngeal suctioning using a sterile catheter with appropriate diameter, and a mucus trap to collect the induced sputum sample.

This is a procedure that has been well established by colleagues at the Red Cross Memorial Hospital (29), and has found to be safe, with occasional bronchoconstriction and nose bleed being the only consequences. In children with respiratory impairment, no worsening of respiratory parameters was noted during or after the procedure. The procedure is much less time-consuming and easier to handle than gastric aspirate harvesting; which is the traditional standard tool for sample collection from children and requires inserting nasogastric tube, and gastric lavage in a fasting state. Bacteriological yields of induced sputum are similar to gastric aspirate collection (29).

6.3.2 Blood

For the routine diagnostics the respective standard tubes will be used according to the SOPs of the participating sites. For the new tests, specimen will be immediately transferred to designated tubes, described in the laboratory manual.

Taken together with routine diagnostics, the maximum volume will not exceed 3ml/kg within 24h in accordance to WHO recommendations for clinical studies in children (30). If available, a plaster with anaesthetic properties can be applied to reduce the brief pain the procedure might cause.

6.3.3 Urine and stool

Children will pass urine and stool into designated containers. Adhesive urine collection bags will be used in younger children and in non-compliant children. Urine will be aliquoted following a separate aliquotation scheme, which will be described in the laboratory manual.

6.3.4 Other specimens

Aspiration of other body fluid e.g. pleural fluid, ascites or cerebrospinal fluid (CSF) and Fine Needle Aspiration Biopsies or tissue from biopsies will be carried out following internationally accepted guidelines and SOPs if considered to be in the subjects' best interest to determine likelihood of TB, TB drug susceptibility, treatment response, other infections or comorbidities, for example.

These other specimen will be analysed for TB using reference standard diagnostics (culture, microscopy, GeneXpert® or other PCRs).

6.4 TB specific laboratory procedures and sub-study assessments

All laboratory procedures (apart from specific assays planned in the sub-studies) will be performed at each study site and at the designated site laboratory under the supervision of the named study PIs. For each procedure SOPs/MOPs will be in place and the respective staff will be trained.

6.4.1 General TB laboratory

TB laboratory procedures will be detailed in the RaPaed TB laboratory manual, which will be available before study start. These procedures are only briefly described here.

6.4.1.1 Xpert® MTB/RIF Ultra® assay or similar nucleic acid amplification test (NAAT)

Xpert® MTB/RIF Ultra® or similar nucleic acid amplification test (NAAT) rapid test will be performed to confirm the presence of MTB complex organisms and confirm rifampicin (RIF) susceptibility. Sputum, induced sputum, or nasopharyngeal aspirate samples will be directly evaluated with the assay according to the manufacturer´s instructions.

Stool will be processed for Ultra® as per the instructions in the experimental processing device. The information on the presence of RIF resistance is delivered in parallel with the TB result.

Ultra® is endorsed for use in children by WHO and will therefore be rated as reference standard test; only in combination with the experimental stool processing device will Ultra® results not be disclosed to the investigators.

6.4.1.2 Sputum smear microscopy

Decontaminated and concentrated sputum samples will be used for smear microscopy after staining and scored using the WHO/IUATLD score. The detailed procedure is described in SOPs.

6.4.1.3 Culture on solid Lowenstein Jensen (LJ) medium

Decontaminated and concentrated sputum samples will be used for culturing of mycobacteria. Positive solid culture is confirmed by Ziehl-Neelsen (ZN) staining, and species identification of all positive baseline samples will be done by HAIN MTBDR*plus*, or similar TB-specific PCR.

6.4.1.4 Liquid culture

Decontaminated and concentrated sputum samples will be used for TB culture. Positive liquid culture is confirmed by Ziehl-Neelsen (ZN) staining, and species identification of all positive baseline samples will be done by HAIN MTBDR*plus*, or similar TB-specific PCR. The duration of incubation until positive result is recorded. The detailed procedure is described in site SOPs and the laboratory manual.

6.4.1.5 Molecular speciation test

The test is based on DNA strip technology. It will be conducted at least once per subject at baseline. The test will be performed according to manufacturer's instructions.

6.4.1.6 Use/storage of decontaminated sputum pellet

The decontaminated pellet from one baseline sputum sample will be used for GeneXpert® Ultra® PCR. Pellets from all other sputum samples, and an eventual storage sputum sample from baseline, will be stored cryopreserved, ideally at -80°, and biobanked for future assessment of future innovative diagnostics on these specimens.

6.4.1.7 Storage of isolates from positive cultures

All isolates from positive cultures will be cryopreserved in glycerol or similarly suitable media to preserve viability; for later analysis of isolate whole genome sequence.

6.4.1.8 Characterization of MTB strains

Mycobacterial DNA extraction will be performed centrally on stored samples and will be further analysed using classical molecular MTB typing methods (genotyping) as well as by next generation sequencing (genome sequencing). This will allow for a detailed classification of circulating MTB lineages in our paediatric study population. This will allow alignment of sequences with rapid molecular test results.

6.4.2 Assessment and laboratory procedures related to the evaluation of new diagnostics

A number of different assays will be performed to study the performance of new tests and possible biomarkers to TB in detail and in different compartments of the body (sputum, blood, urine using stored subject specimens collected as outlined in the SoE). Although we are aiming for conducting new assays at, or near the clinical sites, we acknowledge that some are not ready in time for recruitment start and may have to be conducted at a central laboratory location.

6.4.2.1 T-cell activation marker assay

Activation marker on tuberculosis specific T-cells will be assessed using flow cytometry. As described before, the current assay is capable of distinguishing between active and latent infections caused by *Mycobacterium tuberculosis,* demonstrated by a past EDCTP-sponsored clinical study.

6.4.2.2 QuantiFERON-TB Gold plus assay

This marketed assay may be used as indicated in the SoE. In addition to the Interferon-gamma concentration as a classical readout, cytokine signatures may be analysed from supernatant of incubated tubes by multiplex assays.

The assay contains for tubes with different stimulatory agents. In comparison to the QuantiFERON-TB Gold assay, the predecessor, this new assay now contains a fourth tube with a stimulator more directed to Cd8, rather than Cd4 positive T cells. There is currently insufficient data to describe the added role of this tube in increasing sensitivity for detection of active TB; and there is a possibility that this tube may confer information on TB treatment response.

We will analyse the "classical" 3-tube readout for sensitivity and specificity for active TB; and the incremental diagnostic performance by adding the fourth tube.

6.4.2.3 Whole blood transcriptomics and proteomics

Whole blood PAXgene® will be stored for subsequent transcriptomic analysis. In addition, proteomic analysis of sputum pellets, serum and/or whole blood may be performed for selected samples.

6.4.2.4 Whole blood multiplex cytokine arrays

Antigen-stimulated whole blood supernatants and plasma samples from selected subjects may be used for multiplex cytokine arrays, such as QuantiFERON-TB Gold plus, or assays that may become available in the future.

6.4.2.5 LAM assay

Lipoarabinomannan (LAM) will be detected in stool and urine samples using different assays, including but not limited to Uri-TB direct (Karolinska Institutet), Otsuka LAM or Fujifilm LAM assays. The data will be analysed according to diagnostic accuracy, and/or the assays capacity to monitor TB treatment success.

6.4.2.6 Evaluation of additional diagnostics and biomarkers

Stored specimens will be used for the evaluation of future emerging TB diagnostics, genetic markers and biomarkers, or new diagnostics for other infectious diseases. For any additional investigations which are not mentioned in this protocol, prior agreement to a study amendment by the relevant ethic committees is necessary.

6.4.3 Sample storage and shipment

Blood, sputum, stool and urine specimens will be obtained from each subject for analysis and storage as outlined in the SoE. They will include, but are not limited to: native urine, native sputum, bacterial isolates or extracted DNA from positive liquid or solid culture, sputum pellet and supernatant, and blood (PAXgene®, plasma, serum). The sample collection and storage procedures will be described in the laboratory manual. Samples collected in this study will be stored for up to 20 years after the project end, an extension of this duration may be granted from the relevant ethics committees.

Study subjects can request that their samples to be destroyed at any time point during or after the study, should they decide to withdraw from the study.

The necessary Material Transfer Agreements (MTA) will be signed prior to sample transport.

7. ENROLMENT STRATEGY

7.1 Recruitment procedures

Recruitment will take place at study and collaborating health care facilities, through collaboration with NTP, in the communities and may be enhanced by individual and community awareness through advertisement, posters, radio announcements, community sensitization (talks, meetings and performances), etc. depending on local legal requirements and approval by ethics committees at each site.

Children with suspected TB who are attending the outpatient departments of the participating clinics/hospitals or have been referred to the collaborating hospital, and are eligible in line with the in- and exclusion criteria will be recruited.

For this, children may be transferred from non-study to study health facilities for inclusion into the study.

7.2 Patient Informed Consent

Children (patients) with a suspicion of tuberculosis and their guardians will be informed about the study, the visit schedule and sampling requirements and the anticipated benefits and the potential risks associated with the protocol procedures, and they will be invited for inclusion in the study. General medical questions about eligibility for enrolment may be asked before the informed consent procedure. Once fully informed and in agreement, the guardians will be required to sign an informed consent form (ICF) for the patient to participate in the study, and children from a certain age on, which is to be determined by local guidelines taking into account children's' maturity and ability to understand the proposed study, will receive an assent form to declare their agreement. The age limit will be laid down in the assent form.

The principal investigator (PI) or a suitably qualified person designated by the PI will be responsible for informing the guardians and the patients. The language used will be as non-technical as possible and the guardians and patients will not be unduly influenced to participate in the study.

Guardians and patients will be informed that blood analysis will include testing for HIV. They will be encouraged to receive the HIV result, but may choose not to be informed of the result. Guardians and patients will be informed that they will be free to withdraw from the study at any time, and that withdrawal will have no negative effects on them receiving standard care afterwards.

Written (or witnessed oral) informed consent must be obtained from every guardian and patient, before any procedures are being done specifically for the study.

For illiterate patients/guardians or patients for whom no information sheet is available in a language they can understand, study information is given in the presence of an impartial, literate witness or an interpreter, who will read the information sheet to the patient/legal guardian or will witness the

complete reading of the information sheet to the patient/legal guardian. The patient/legal guardian will give consent by thumb printing or signing the ICF and the witness/interpreter states that free, informed consent has been given by his/her signature on the ICF.

The signed original ICF will be kept with the patient's medical records/the Investigator Site File at the site. A second original/copy of the signed informed consent form will be given to the patient's legally acceptable representative.

The site will document the name and position of those persons at the site who are responsible for obtaining informed consent, that are approved by the PI for this task, and no other staff will perform this.

The global ICF template for this study will be provided to the investigator by the sponsor and must be approved by the ethics committees (ECs) responsible for the study and the sites. If any modifications to the form are proposed by the site or their EC, the consent form must be submitted to the sponsor for approval prior to final submission to the ethics committee for approval. The ICF will be revised in cooperation between the sites and the sponsor whenever new important information becomes available and will undergo EC review. Once EC approved, all active patients must then sign additionally the revised ICF.

7.3 Incentives and expenses

Subjects will be reimbursed with a flat compensation for each clinic visit, for travel and loss of income due to study participation. The amount will be determined following each sites ethics committee's requirement. Should a guardian claim higher expenses, these can be reimbursed at the discretion of the investigator if adequate documentation of these expenses is provided.

Preferentially, this sum will be transferred to a subject's mobile phone account to incentivize the subject for maintaining the same phone number of time, reducing the risk of loss to follow up which might otherwise be problematic in a potentially volatile patient population.

7.4 Vulnerable patients

The main focus of this study is to identify new diagnostic tools and possible algorithms especially for the use in paediatric care and with the aim of these diagnostics being aligned to age-specific disease characteristics and sampling limitations, therefore it is unavoidable to perform the study in this vulnerable population. This group will have a specific group benefit from study participation through better diagnostic means available to paediatrics if this study succeeds. As study procedures will mostly be part of the standard scheduled visits and care, the additional burden to subjects will be minimised.

7.5 Risks and benefits

The risks for the subjects comprise a physical and physiological aspect. Venous puncture for blood drawing, puncture of pleural effusions or ascites, biopsy of lymph nodes or other organs, or lumbar puncture involves the physical risk of infection and local injury, which will be minimized by strict adherence to best medical practice, SOPs and national guidelines.

In young children, induction of sputum might be performed, which is generally safe and not associated with serious risks. In the past, some nose bleed was observed, and in very few instances, children had

to receive medication for cramps of their airways, but no serious problems were reported from this procedure.

However, the procedures of those diagnostic measures are necessary to confirm the diagnosis and initiate the adequate treatment and are therefore part of the routine care, and will not be performed solely for study purposes.

The benefits of this study can be divided into benefits to the individual subjects and benefits to the community. Direct benefit for the subject will result from the use of intensified diagnostic measures, well-documented treatment and close observation during follow up of the subject affected and will be directly experienced by the subject. It is intended that direct benefits for the community will be achieved through improvement in the scientific understanding of the diagnosis of TB, leading to better TB diagnostics in future.

7.6 Subject Withdrawal

A subject may decide to withdraw from the study at any time and for any reason with no negative effects on their standard care afterwards. The investigator may also withdraw a subject for any of the following reasons:

- Protocol deviation
- Sponsor decision
- If, for any reason, the investigator concludes that continued participation in the study would not be in the subject's best interest

The investigator will also withdraw a subject upon request of the sponsor or if the study is terminated as a whole.

When a subject withdraws from the study, the reason(s) for withdrawal shall be recorded by the investigator on the relevant page of the CRF. Subjects who withdraw from the study should undergo all end-of-study assessments as far as possible. Subjects who do not return for final assessments will be contacted by site personnel in an attempt to have them comply with the protocol.

8. STUDY PROCEDURES AND ACTIVITIES

There is no screening visit for this study. However, sites should keep records of patients considered for this study and record the reasons for non-participation. Summaries of these reasons will be collected during the study for descriptive analysis.

The study consists of the following periods/visits:

- Baseline / Visit 1
- Study visits 2-4: month 1, month 3, month 6; visits 5 and 6 (sub-study spirometry)

All evaluations for each study visit must be conducted according to the SoE - Table 1. It must be documented that the evaluations were completed or, if not done, the reason must be given. Study visits must be conducted within the timeframes defined by the SoE. Each visit has a clear time window in order to allow for holidays, scheduling problems or any other reasons. Study visits are expected to be completed in one day, however, if for some reason this is not possible, subjects may return on another day within the defined study visit windows.

8.1 Study Visits

8.1.1 Baseline / Visit 1 (Days 1, 2 and/**or** 3)

After informed consent has been obtained, study-specific diagnostic procedures will be performed and documented. Data from standard diagnostic procedures will be recorded in the electronic Case Report Forms (eCRF).

- Collection of demographic data by questionnaire (age, gender, TB history etc.)
- Collection of complete medical history, including previous and concomitant medications information
- Completion of physical examination, including body weight and height
- Performance of chest X-Ray/other radiology *(if indicated)*
- Collection of sputum (or, *if applicable,* gastric aspirates) on 2 consecutive days for microbiological testing (routine) plus additional samples for storage
- Collection of a nasopharyngeal aspirate sample in children < 5 yrs for Xpert® Ultra®
- Collection of blood samples for routine haematology and clinical chemistry *(if applicable)*, including HIV-test and CD4 count (before day 8, if not performed recently and documentation is available for study purpose),
- Perform a tuberculin skin test (TST), if not done within past two weeks at the study clinic or an institution with acceptable quality of testing reagent, storage and interpretation
- Collection of blood samples for new tests, such as TAM-TB, samples for host RNA-analysis, PBMC isolation, and plasma for analysis of potential biomarkers, QuantiFERON-TB, etc.
- Collection of urine (for dipstick and new tests)
- Spirometry (optional)

Visit 1 may occur on 2-3 days according to the preference of the centre. The procedures will be performed either on Day 1 and/or Day 2 and/or Day 3, depending on study setup. The total number of samples taken will not exceed the number specified in the SoE.

8.1.2 Visit 2 (Month 1; +/- 7 days)

The following will be performed and documented:

- Completion of physical examination, including weight
- Documentation of symptoms follow-up
- Documentation of radiologic findings *(if performed)*
- Documentation of treatment
- Collection of microbiological samples (*sputum, if produced spontaneously and initial bacteriology positive*)
- Collection of other samples, such as stool for biomarker and antigen testing, and urine (for dipstick and new tests)
- Collection of blood samples for new tests, such as TAM-TB, samples for host RNA-analysis, PBMC isolation, and plasma for analysis of potential biomarkers etc.
- Spirometry (optional)

8.1.3 Visit 3 (Month 3, +/- 7 days)

The following will be performed and documented:

- Completion of physical examination, including weight
- Documentation of symptoms follow-up
- Documentation of radiologic findings *(if performed)*
- Documentation of treatment
- Collection of microbiological samples (*sputum, if produced spontaneously and initial bacteriology positive*)
- Collection of other samples, such as stool for biomarker and antigen testing and urine (for dipstick and new tests)
- Collection of blood samples for new tests, such as TAM-TB, samples for host RNA-analysis, PBMC isolation, and plasma for analysis of potential biomarkers etc.
- Spirometry (optional)

8.1.4 Visit 4 (Month 6, +/- 14 days):

The following will be performed, if subjects are started on anti-TB treatment OR are unwell at visit 3:

- Completion of physical examination, including weight
- Documentation of symptoms follow-up
- Documentation of radiologic findings *(if performed)*
- Documentation of treatment
- Collection of microbiological samples (*sputum, if produced spontaneously and initial bacteriology positive*)
- Collection of other samples, such as stool for biomarker and antigen testing and urine (for dipstick and new tests)
- Collection of blood samples for new tests, such as TAM-TB, samples for host RNA-analysis, PBMC isolation, and plasma for analysis of potential biomarkers etc.
- Spirometry (optional)

8.1.5 Visit 5 (Month 9 /Day 220, +/- 14 days: optional, if the child had spirometry examination before)

- Completion of physical examination, including weight
- Documentation of symptoms follow-up
- Spirometry (optional)

8.1.6 Visit 6 (Month 12, Day 365 +/- 14 days: optional, if the child had spirometry examination before)

- Completion of physical examination, including weight
- Documentation of symptoms follow-up
- Spirometry (optional)

9. STATISTICAL CONSIDERATIONS

9.1 Sample size determination

Recruitment will be over a two-year period with an assumed cohort size of at least 1000 paediatric TB suspects. This approach will allow to realistically enrol at least 250 with confirmed TB. A sample of 250 subjects will allow detection of a sensitivity increase from 62% (Xpert® MTB/RIF®) to 82%, with more than 90% power at the 95% confidence level.

Importantly, this sample of 250 subjects with confirmed disease will allow meaningful subgroup analyses within and between age groups, HIV and nutrition status. For example, assuming HIV coinfection in approximately 40% of the children, and new test sensitivity of 82% in HIV-negative children, the minimum detectable difference to a lower sensitivity in HIV-positive children would be -17.7% (absolute sensitivity 64.8%), with a power of 80% at the 95% confidence level.

Comparing age groups, at assumed sensitivity of 82% in the age group of 9-14 years (30% of children); the minimum detectable difference in sensitivity to the age group of 0-4 (40% of children) years would be -22% (i.e. 60% sensitivity in this group).

Such differences are clinically very meaningful and should be detected; we therefore aim to include 250 or possibly more children with confirmed disease in this cohort.

In past TB diagnostic studies, the number of subjects classified as “unlikely TB” or “not TB” that is used to determine new test specificity was usually higher than the number of confirmed cases, so new test specificity is expected to be determined with a higher precision than sensitivity.

9.2 Categorization of subjects for analysis

Baseline characteristics, such as demographic and analytical data, will be summarized using descriptive statistical methods. Continuous data will be summarized using the mean, the median, standard deviation, the range (minimum and maximum value). Categorical values will be summarized using frequency counts and percentages.

Disease classification/clinical case definition will follow recently updated consensus statements (5) and will be used for both intrathoracic and extrapulmonary TB:

i) microbiological testing
ii) clinical signs/symptoms suggestive of TB
iii) radiologic findings
iv) TB contact history
v) *Mycobacterium tuberculosis* infection (assessed via immunoresponse assays)
vi) Treatment response

Final details of statistical analysis will be laid down in the analysis plan, which will be signed off before database lock.

9.3 Analysis plan and validation analyses

Analysis of data collected in this study will be described in the statistical analysis plan. This document will be completed and signed off before database lock. The analysis plan will also detail timing and content of validation analyses that may be conducted to validate cut-offs for single new tests.

For certain tests, there may be a need in the course of the study to split up the patient population into a verification dataset to confirm and/or – if necessary – change positive/negative cut-offs, and a validation dataset to verify performance of an assay at those previously determined cut-offs, if those cut-offs cannot be reliably determined otherwise (e.g. in concurrently ongoing studies).

As this study does not include experimental interventions regarding patient management, these potential subset analyses will not affect statistical considerations, study conduct or patient safety, and thus not introduce bias.

10. DATA MANAGEMENT

10.1 Data Collection

The investigator agrees to maintain accurate source data and electronic Case Report Forms (eCRFs). For each subject enrolled, an eCRF will be completed, even if the subject discontinues at any time point during the study. All information from a performed visit must be entered in the CRF in a timely manner (e.g. within 2 weeks of data generation).

The web based Electronic Data Capture (EDC) software “OpenClinica®” is used in this study as Clinical Data Management System (CDMS). The subject data will be entered by study site personnel directly into eCRFs. Database access and permissions are granted by data management to study personnel individually depending on the specific role in the study.

No paper CRFs (pCRF) are necessary however templates of data collection worksheets are provided which resemble the eCRFs and specify which data must be captured. When discrepant data is entered in the database validation checks are triggered, subsequent queries must be resolved by the side in due time (e.g. within 1 week).

Laboratory data can be provided electronically to data management and will be merged with extracts of the clinical database into the analysis datasets.

10.2 Source Documents

The investigator agrees to maintain accurate source documents as part of the case histories and permits direct access for domestic and foreign regulatory authorities, sponsors, monitors and auditors, and archive them in accordance with local regulations (minimum of 20 years for the study).

Patient data may be recorded directly into the eCRF, which will then be considered source data. This will depend on site specific procedures for maintaining separate source documents, which will be laid down in the investigator site file.

Some, or all of the information generated on a subject, may be directly entered into an electronic document, which will then become the source document.

Laboratory information may be transferred directly to the database from a laboratory information management system or directly from a laboratory device. If this information is generated electronically without paper source, the data contained in the laboratory information management system will be the source data.

11. MONITORING AND QUALITY ASSURANCE

11.1 Study Monitoring

Study monitoring will be done by sponsor assigned personnel.

Site investigators and designated study personnel will allow the monitors to inspect study documents, pertinent hospital or clinic records as well as site facilities, as required. All aspects of the study will be carefully monitored in order to ensure compliance with Good Clinical Practice and all applicable regulatory guidelines. The monitor will be responsible for verification of:

- adequacy of study personnel's qualifications as well as facilities
- the accuracy and completeness of the CRF entries, source documents and other study-related records
- informed consent procedures and subject eligibility
- maintenance of the essential documents
- all other aspects of the study relating to protection of the rights and well-being of subjects, accuracy of study data and adherence to the protocol

The study database will only be closed after data has been verified by the monitor and the sponsor, and all queries issued through data cleaning activities have been completed.

11.2 Inspection of Records

The investigator will allow the sponsor, the sponsor's representatives, regulatory agencies and ethics committees' access to all study records, if requested. The investigator will promptly notify the sponsor of any inspections scheduled by regulatory authorities or ethics committees and promptly forward copies of any inspection reports received to the sponsor.

11.3 Records Retention

The sponsor will keep essential study documents (including CRFs) other than subject's medical files

- for at least 20 years after completion or discontinuation of the study.

Subjects' medical files should be retained in accordance with applicable legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

11.4 Confidentiality of Personal Data

All patient records, lab specimen etc. will be identified in a manner to maintain subject confidentiality and will be kept in a secure storage area with limited access. Each subject will be assigned a

pseudonymous identification number after signing consent which will be used for CRF data entry. No data that could identify the subject other than this identification number (and the sex and date of birth) will appear on the CRFs.

Should there be a decision to withdraw, the child' s legal guardian has the right to decide whether data and samples from his/her child are to be irreversibly anonymized; meaning the key that links the subject identity to the pseudonymous identification number; which is kept only at the site as described above, will have to be destroyed.

The researchers who manage the human specimen data, and other investigators who have access to it, are legally and ethically obliged to protect data that are considered personal and confidential information. Information regarding the specimens will be recorded by the investigator in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects. If specific samples, e.g. TB strains, will be transported outside of the study sites to collaborating institutions and stored by their laboratories, this is to be approved by the applicable ethics committees.

Data of study subjects will only be used as defined in the Informed Consent Form and in line with applicable data privacy regulations. Accordingly, patient records may be reviewed by inspectors of regulatory authorities or ethics committees, study monitors and auditors, who ensure the quality of the study.

An individual's study data will not be released without the written agreement of the subject (or their legal guardian), except as necessary for monitoring and auditing by the sponsor or its representative, regulatory authorities or ethics committees, or in case of medical emergencies when written consent cannot be obtained, as deemed in the subject' s best interest by the investigator.

Results of any genetic tests will not be disclosed to anybody not involved with the study, in particular not to immediate relatives without prior consent of the subject.

Since the study database will be located at the sponsor, Medical Center of the University of Munich (LMU), where study data will be collected and processed. According to Article 13 of the Regulation (EU) 2016/679 (General Data Protection Regulation - GDPR) subjects should be additionally provided the following information:

1. The responsible authority for data protection:

Bavarian State Authority for Data Protection (BayLfD)

Prof. Dr. Thomas Petri

Postal: P.O. Box 22 12 19, 80502 Munich, Germany

Address: Wagmüllerstr. 1, 80538 Munich, Germany

Tel.: +49-89 212672-0; Fax: +49-89 212672-50

2. The responsible data protection officer for Medical Centre of the University of Munich (LMU):

Mr. Gerhard Meyer

Authorised Data Protection Officer

Medical Center of the University of Munich (LMU)

Pettenkoferstr. 8, 80336 Munich, Germany

email: datenschutz@med.uni-muenchen.de

The performance of the tasks of each supervisory authority shall be free of charge for the subject and, where applicable, for the data protection officer.

12. ETHICAL and ADMINISTRATIVE CONSIDERATIONS

12.1 Basic principles

This study will be performed in accordance with the study protocol, the declaration of Helsinki (last updated in Fortaleza, October 2013) and ICH-Harmonised Tripartite Guideline for GCP E6 (R2) as well as any other applicable national and other regulatory guidelines.

12.2 Involvement of Ethics Committees

The protocol and the informed consent document to be used in this study must be submitted to the sponsor's and the responsible investigators' ethics committee, and also to the sponsor's local EC for approval. Written documentation of approval of both the protocol and the informed consent must be provided to the sponsor before starting the study.

The investigator will promptly report to the EC deviations from the protocol and all unanticipated problems involving risks to human subjects or others, and will not make changes in the research without EC approval, except where necessary to eliminate apparent immediate hazards to human subjects.

12.3 Protocol Amendment Policy

Any substantial change to the protocol will be effected by means of a protocol amendment and have to be submitted to ethics committees and regulatory authorities. In general, amendments should solely be initiated/implemented with agreement of all members of the steering group, especially the clinical study sites and the sponsor. No amendment will be implemented until approved and signed by all required parties. Exceptions to this are when the investigator considers that the subject's safety is compromised.

Protocol amendments detailing minor administrative changes should be submitted by the investigator to ethics committees and regulatory authority for notification purposes as appropriate.

12.4 Falsification of data

Any proven evidence of falsification of data will be dealt with in accordance with the policy of the sponsor and appropriate action will be taken.

12.5 Publication Policy

After completion of the study, the data may be considered for presenting at a scientific conference or for publication in a scientific journal. The sponsor will be responsible for these activities and will collaborate with the investigators to determine how the manuscript is written and edited, the number and order of authors, the journal to which it will be submitted and other related issues.

The results of the study will be published independent of the outcome – positive or negative - of the study.

Under certain circumstances, i.e. when the publication of particular findings (of an epidemiological, sociological or genetics study) may present a risk to the interest of a community or population or a racially or ethnically defined group of people, it may be considered inappropriate to publish findings.

12.6 Investigators' View of the Ethical Issues and Considerations

The investigator(s) participating in this study, as listed above, have had the opportunity to review the protocol outline. Their concerns and suggestions have been included into the final protocol.

12.7 Study Registration

Before study start, the study will be registered in a WHO recognised study registry.

13. REFERENCES

1. WHO. Global Tuberculosis Report. Geneva: World Health Organization; 2016.

2. Starke JR. Mortality in childhood tuberculosis: has there been progress? Lancet Infect Dis. 2017;17(3):239-41.

3. WHO. Global Tuberculosis Report 2016. WHO. 2016.

4. 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. The Lancet Respiratory medicine. 2015.

5. 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;61Suppl 3:S179-87.

6. 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. The Lancet Respiratory medicine. 2015;3(3):235-43.

7. Bates M, Mudenda V, Mwaba P, Zumla A. Deaths due to respiratory tract infections in Africa: a review of autopsy studies. Current opinion in pulmonary medicine. 2013;19(3):229-37.

8. 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.

9. Jenkins HE. Global Burden of Childhood Tuberculosis. Pneumonia (Nathan). 2016;8.

10. World Health Organization. Roadmap for Childhood Tuberculosis. 2013.

11. World Health Organization. High-priority target product profiles for new tuberculosis diagnostics. 2014.

12. The Global Plan to End TB 2016 - 2020. Stop TB Partnership, UNOPS; 2015.

13. API. API TB Consensus Guidelines 2006: Management of pulmonary tuberculosis, extra-pulmonary tuberculosis and tuberculosis in special situations. J Assoc Physicians India. 2006;54:219-34.

14. Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, et al. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. The Lancet Respiratory medicine. 2015;3(6):451-61.

15. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014;1:CD009593.

16. Marcy O, Ung V, Goyet S, Borand L, Msellati P, Tejiokem M, et al. Performance of Xpert MTB/RIF and Alternative Specimen Collection Methods for the Diagnosis of Tuberculosis in HIV-Infected Children. Clin Infect Dis. 2016;62(9):1161-8.

17. Banada PP, Naidoo U, Deshpande S, Karim F, Flynn JL, O'Malley M, et al. A Novel Sample Processing Method for Rapid Detection of Tuberculosis in the Stool of Pediatric Patients Using the Xpert MTB/RIF Assay. PLoS One. 2016;11(3):e0151980.

18. 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.

19. Bassyouni H, Breugelmans JG, Surette M, Olesen O. Childhood tuberculosis: a unified response to a global problem. Int J Tuberc Lung Dis. 2015;19(12):54.

20. Hamasur B, Bruchfeld J, van Helden P, Kallenius G, Svenson S. A sensitive urinary lipoarabinomannan test for tuberculosis. PLoS One. 2015;10(4):e0123457.

21. Chegou NN, Sutherland JS, Malherbe S, Crampin AC, Corstjens PL, Geluk A, et al. Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. Thorax. 2016;71(9):785-94.

22. Jacobs R, Malherbe S, Loxton AG, Stanley K, van der Spuy G, Walzl G, et al. Identification of novel host biomarkers in plasma as candidates for the immunodiagnosis of tuberculosis disease and monitoring of tuberculosis treatment response. Oncotarget. 2016;7(36):57581-92.

23. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, et al. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet. 2016.

24. Froeschl G, Ntinginya NE, Sangare A, Lawala P, Mangu C, Dobler G, et al. Integrating Local, National, and International Stakeholders in Outbreak Preparedness in Developing Countries: Conclusions from a Conference in Mbeya, Tanzania. Health Secur. 2016;14(1):29-34.

25. Cuevas LE, Browning R, Bossuyt P, Casenghi M, Cotton MF, Cruz AT, et al. Evaluation of Tuberculosis Diagnostics in Children: 2. Methodological Issues for Conducting and Reporting Research Evaluations of Tuberculosis Diagnostics for Intrathoracic Tuberculosis in Children. Consensus From an Expert Panel. The Journal of infectious diseases. 2012.

26. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol. 2003;3:25.

27. 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.

28. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. The Lancet. 2011;377(9776):1495-505.

29. 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.

30. Howie SR. Blood sample volumes in child health research: review of safe limits. Bulletin of the World Health Organization. 2011;89(1):46-53.