EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB

TB-Speed stool processing

International protocol version No 5.0 – 25/05/2021

CONFIDENTIAL

RESEARCH PROTOCOL INVOLVING HUMAN PERSON

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Approved by		
Date		
Approved by		
Date		

Coordinating Investigators		
Maryline Bonnet IRD UMI233 / Inserm U1175 Université de Montpellier, 911 Avenue Agropolis 34394 Montpellier Cedex 05 **Tel**: +33 (0)4 67 41 64 91 **Email***:* maryline.bonnet@ird.fr	Olivier Marcy (IDLIC), Bordeaux Population Health – Inserm U1219 Université de Bordeaux, 146 Rue Léo Saignat, 33076 BORDEAUX Cedex **Tel:** +33 (0)5 57 57 47 23 **Email:** olivier.marcy@u-bordeaux.fr	Eric Wobudeya MU-JHU Care Ltd Upper Mulago Hill Road P.O. Box 23491 Kampala, Uganda **Tel**: +256 712 846163 **Email**: ewobudeya@mujhu.org

☐ **Monocentric study** ☐ **European multicentre study**

☐ **French multicentre study** ☒ **International multicentre study**

Sponsor:	**Contact:**
Inserm - Pôle Recherche Clinique / Clinical Research Department Biopark Bâtiment A, 8 rue de la Croix-Jarry, 75013 Paris (FRANCE)	Benjamin Hamze, Inserm Project Manager benjamin.hamze@inserm.fr Tel: +33 (0)1 82 53 34 72

This study is funded by Unitaid.

SIGNATURES PAGE

Sponsor's signature:

French National Institute for Health and Medical Research (Inserm)

Director: Hélène Espérou BIOPARK Bâtiment A 8 rue de la Croix Jarry 75013 Paris, France **Tel:** +33 153 94 80 90 / 60 46 / 60 00 **Email:**	Paris, Date: Name: Hélène Espérou Signature:	

Coordinating Investigators' signatures:

Maryline Bonnet Institut de Recherche pour le Développement (IRD) UMI233 / Inserm U1175 *Université de Montpellier,* 911 Avenue Agropolis 34394 Montpellier Cedex 05 **Tel**: +33 (0)4 67 41 64 91 **Email**: maryline.bonnet@ird.fr	Montpellier, Date: Name: Maryline Bonnet Signature:	
Olivier Marcy Bordeaux Population Health - Inserm U1219 Université de Bordeaux 146 Rue Léo Saignat 33076 Bordeaux Cedex, France **Tel:** +33 (0)5 57 57 47 23 **Email:** olivier.marcy@u-bordeaux.fr	Bordeaux, Date: Name: Olivier Marcy Signature:	
Eric Wobudeya Makerere University-Johns Hopkins University research collaboration (MU-JHU Care Ltd) Upper Mulago Hill Road P.O. Box 23491 Kampala, Uganda **Tel**: +256 712 846 163 **Email**: ewobudeya@mujhu.org		Kampala, Date: Name: Eric Wobudeya Signature:

PRINCIPAL CORRESPONDENTS

Coordinating investigators:

Maryline BONNET
IRD UMI233 / Inserm U1175
Université de Montpellier, France
911 Avenue Agropolis, BP 64501
34394 Montpellier Cedex 05, France
Tel: +33 (0)4 67 41 64 91
Email: maryline.bonnet@ird.fr

Olivier MARCY
Infectious Diseases in Resource-Limited Countries (IDLIC)
Bordeaux Population Health - Inserm U1219
Université de Bordeaux
146 Rue Léo Saignat
33076 Bordeaux Cedex, France
Tel: +33 (0)5 57 57 47 23
Email: olivier.marcy@u-bordeaux.fr

Eric WOBUDEYA
Makerere University - Johns Hopkins University Research Collaboration (MUJHU Care Ltd)
Upper Mulago Hill Road
P.O. Box 23491
Kampala, Uganda
Tel: +256 712 846 163
Email: ewobudeya@mujhu.org

Sponsor:

French National Institute for Health and Medical Research (Inserm)
101 rue de Tolbiac
75013 Paris, France
Tel: +33 (0)1 53 94 60 00

Project Manager:
Benjamin HAMZE
Clinical Research Department
BIOPARK Bâtiment A
8 Rue de la Croix-Jarry
75013 Paris, France
Tel: +33 (0)1 82 53 34 72
E-mail: benjamin.hamze@inserm.fr

International Coordinating Clinical Trials Unit (CTU)

IDLIC/Mereva Inserm U1219 Université de Bordeaux 146 rue Léo Saignat 33076 Bordeaux Cedex, France Phone: +33 557 57 17 67 *CTU Coordinator:* Olivier MARCY E-mail: olivier.marcy@u-bordeaux.fr *International study manager:* Manon LOUNNAS IRD-Mivegec / CNRS U5290 911 Avenue Agropolis, BP 64501 34394 Montpellier Cedex 05, France Tel: +33 (0)7 86 85 32 59 Email: manon.lounnas@ird.fr	*Biostatistics:* Hélène FONT E-mail: helene.font@ u-bordeaux.fr *Monitoring:* Savine Chauvet E-mail: savine.chauvet@ird.fr *System development and data management:* Sophie KARCHER E-mail: sophie.karcher@ u-bordeaux.fr Eric BALESTRE Email: eric.balestre@u-bordeaux.fr

Reference study microbiologist:

Sylvain GODREUIL
IRD-Mivegec / CNRS U5290
911 Avenue Agropolis, BP 64501
34394 Montpellier Cedex 05, France
Tel: (+33) (0)4 67 41 63 57
Email: s-godreuil@chu-montpellier.fr

Country principal investigators: (countries appear in alphabetical order)

UGANDA Juliet MWANGA-AMUMPERE Epicentre Mbarara Research Centre P.O.Box 1956, Mbarara Tel: +256 793 328 748 Email: Juliet.MWANGA@epicentre.msf.org	***ZAMBIA*** Chishala CHABALA University of Zambia School of Medicine, University Teaching Hospital Nationalist Rd P.O Box 50110, Ridgeway, Lusaka Tel: +260 977 849 537 Email: cchabala@gmail.com

Associated investigators:

Mark Nicol University of Cape Town Cape Town South Africa Tel: +27 21 406 6727 Email: mark.nicol@uct.ac.za	Sara Eyangoh Centre Pasteur Cameroun 451,rue 2005 P.O.Box 1274 Yaoundé, Cameroon Tel: +237 222 231 015 / +237 222 231 803 Email: eyangoh@pasteur-yaounde.org

Country Clinical Trials Units:

UGANDA Epicentre Mbarara Research Centre P.O.Box 1956 Mbarara	***ZAMBIA*** University of Zambia University Teaching Hospital School of Medicine Nationalist Rd P.O Box 50110, Ridgeway Lusaka

PROTOCOL WRITING COMMITTEE

- Manon Lounnas, International study manager (IRD-Mivegec, Montpellier, France)
- Maryline BONNET, Coordinating investigator (IRD UMI233 / Inserm U1175 Montpellier, France)
- Patrick Orikiriza, microbiologist, Epicentre Mbarara Research Centre, Mbarara, Uganda
- Chishala Chabala, country principal investigator, Teaching University Zambia, Lusaka, Zambia
- Olivier MARCY, Coordinating investigator (Inserm U1219, Université de Bordeaux, France)
- Eric WODUBEYA, Coordinating investigator (MUJHU, Kampala, Uganda)
- Sylvain GODREUIL, Reference project microbiologist (IRD-Mivegec, Montpellier, France)
- Juliana SMITH, epidemiologist ((IRD UMI233 / Inserm U1175 Montpellier, France)
- Hélène FONT, project Statistician (Inserm U1219, Université de Bordeaux, France)
- Mark Nicol, microbiologist (University of Cape Town, Cape Town, South Africa)
- Sara Eyangoh, microbiologist (Centre Pasteur Cameroun, Yaoundé, Cameroon)

HISTORY OF PROTOCOL VERSIONS

TB-Speed stool processing

Version No.	Version Date	Amendment Summary
1.0	17/04/2019	
1.0	02/05/2019	Correction of a typo error
1.1	23/05/2019	WHO secretariat comments - Clarifications on consent-taking case scenarios
2.0	03/07/2019	- Further description of capacity building - Clarifications on which tests are performed only for study purpose - The information sheet and informed consent form for the feasibility studies have been simplified - The informed consent forms for children from routine care in prospective and enriched cohorts have been merged into a unique informed consent form. - The information sheet for parents/guardian of children participating already in the TB-Speed HIV/SAM studies have been simplified. - The informed consent forms for children > 7 years old from routine care have been merged into a unique informed consent form.
3.0	30/09/2019	- Clarification of the capacity building component - Clarification of the specimen collected for the study purpose only - Clarification of the dissemination of study results to families - Removal of the lower age limit (6 months) from the information sheets in Appendices 6 and 7
4.0	11/06/2020	- Add of a new study site in Zambia - Update of the study schedule - Change of sponsor project manager and update of contact details of coordinating investigator - Correction of version dates - Revision of the periods of the feasibility assessment (Ch 7.6) - Update of the study summary (appendix 1) - Revision of the feasibility assessment tool (appendix 4) - Correction of dates on appendix 5, 8, 9 and 10
5.0	25/05/2021	- Update of the study schedule - Update of the study summary (appendix 1) - Homogenization of objectives and endpoint with study summary - Update of NCT and Inserm number on ICFs

LIST OF ABBREVIATIONS

ADCH	Arthur Davidson Children Hospital
ANRS	French National Agency for Research on HIV/AIDS and Hepatitis
AE	Adverse event
ART	Antiretroviral therapy
CPC	Country Project Committees
CRA	Clinical Research Assistant
CREDIM	Centre de Recherche et Développement en Informatique Médicale
CRF	Case Report Form
CXR	Chest radiography or chest X-ray
CTU	Clinical Trials Unit
DALYs	Disability Adjusted Life Years
DMP	Data Management Plan
eCRF	Electronic Case Report Form
ERC	Ethical Review Committee
FDC	Fixed-dose combinations
GCP	Good Clinical Practices
HIV	Human Immunodeficiency Virus
ICER	Incremental Cost Effectiveness Ratio
IDLIC	Infection Diseases in Low Income Countries
IDMC	Independent Data Monitoring Committee
IRD	Institut de Recherche pour le Développement (Research Institute for Development)
MTB	Mycobacterium tuberculosis
MUAC	Mid-Upper Arm Circumference
NPA	Nasopharyngeal aspirate
NTP	National Tuberculosis Program
OSC	Output Steering Committee
PCC	Project Coordination Committee
PI	Principal Investigator
QC	Quality control
RIF	Rifampicin
SAB	Scientific Advisory Board
SAE	Serious Adverse Event
SAM	Severe Acute Malnutrition
SOP	Standard Operating Procedures
TB	Tuberculosis
TeAM/SPI	Technical Assistance for Management/Soutien Pneumologique International
TMF	Trial Master File
UBx	University of Bordeaux
WHO	World Health Organization

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1. INTRODUCTION: BACKGROUND, RATIONALE AND HYPOTHESIS

The burden of childhood tuberculosis

Despite progress in reducing tuberculosis (TB) incidence and mortality in the past 20 years, TB is a top ten cause of death in children under 5 years worldwide [1] . According to the World Health Organization (WHO), there were 1.04 million new cases, representing 10% of the overall TB case load, and 253,000 TB deaths in children in 2016 [2]. Recent modelling showed that the vast majority of children dying from TB are young children below the age of 5 not accessing treatment, most likely because they are not diagnosed [1]. However, in 2016 only 6.9% of TB cases notified to WHO were children, that is, an approximate 45% notification rate.

Childhood TB therefore remains massively underreported and undiagnosed, mostly because of the challenges in confirming its diagnosis due to the paucibacillary nature of the disease and the difficulty in obtaining expectorated sputum in children [3,4]. The goal to reach zero TB deaths in children, endorsed by the international TB community, and spearheaded by the WHO, includes taking every critical opportunity for intervention to improve diagnosis and treatment, especially among those presenting with severe clinical conditions [5].

An improved molecular diagnostic tool for paediatric TB

Xpert MTB/RIF (Cepheid, USA) is an automated nucleic acid amplification test (NAAT) that simultaneously detects *Mycobacterium tuberculosis* (MTB) and genes associated with resistance to rifampicin [6]. The assay was a major breakthrough in bringing molecular tests for the diagnosis of TB closer to the community, with performances close to mycobacterial culture [7]. WHO therefore recommended Xpert MTB/RIF as the first test to be used for the diagnosis of TB among populations who may have drug-resistant and/or HIV-associated TB [8].

In 2013, WHO updated its policy to include Xpert MTB/RIF as the initial test for the diagnosis of TB in children, based on a systematic review and meta-analysis showing a pooled sensitivity of 66% (CI 95% 51-81) and a specificity of 98% (CI 95% 96-99) of Xpert MTB/RIF performed on gastric lavages when compared with culture [9,10]. WHO recommendations, detailed in the 2014 guidance on paediatric TB, stated that Xpert MTB/RIF may be used instead of smear microscopy in all children and should be used in children with HIV infection or presumptive multidrug-resistant TB [9].

The next-generation of Xpert MTB/RIF assay, Xpert MTB/RIF Ultra (Ultra), has a limit of detection of 16 colony forming units (CFU)/mL (compared to the current version which detects 130 CFU/mL), representing an approximately 8-fold improvement [11]. This lower threshold is similar to the detection level of culture and should facilitate the rapid diagnosis of paucibacillary disease as seen in childhood TB [12,13]. Retrospective analyses on frozen respiratory samples in children have shown a significantly increased sensitivity of 71% for Ultra versus 47% for Xpert MTB/RIF [14]. A recently published study reveals, however, a lower specificity of Ultra in adults, particularly in those with a previous history of TB, potentially resulting in false diagnoses and overtreatment of TB [13]. Only two studies so far are available in children with reported Ultra sensitivities of 64.5% and 75.3% and specificities of 96.9% and 98.1%, as compared to culture on one sample [15,16]. Sensitivity was increased by 10% and specificity decreased by 2% loss as compared to the Xpert MTB/RIF assay results [16]. As highlighted by these studies and a recently published review, the risk of false-positive Ultra results could be less significant in children in whom a small proportion only have previously had TB [17]. Clinical impact of Ultra is therefore likely to vary depending on the settings; a recent modelling exercise found a larger mortality benefit in patient populations with high TB prevalence, high HIV prevalence, and high case fatality ratios for untreated TB [18].

The current WHO recommendation to use Xpert MTB/RIF as the initial diagnostic test for all adults and children with signs and symptoms of TB also applies to the use of Ultra. An updated guideline including use of Ultra is planned for 2019 [19].

Stool sample as an alternative specimen collection method adapted to children

Young children are frequently unable to expectorate sputum, adding to the difficulty in bacteriological confirmation of a paucibacillary disease. There is no clear evidence and guidance on which specimen or combination of specimens should be used in order to maximize the probability of bacteriological confirmation of TB in children. WHO currently recommends repeated GA or IS in younger children and ES in older children. At the programmatic level, implementation of gastric aspirates and induced sputum can be challenging [20,21].

Our research group and other groups in Africa and Asia have shown that alternative specimen collection methods such as **stool samples are easier to be implemented in resource-limited settings and are better tolerated in young and sick children** [22–28]**.** These methods do not require a child to fast (as for gastric aspirates) and are more suitable than induced sputum in children with severe respiratory deficit [29]. Stool

testing by Xpert MTB/RIF shows results close to respiratory samples testing in terms of sensitivity but requires a specimen processing prior to Xpert testing because stools include PCR inhibitors that can result in invalid Xpert results [24–26,30,31], and debris that can lead to clotting and errors in the Xpert test run. Stool Xpert testing performance varied greatly between studies depending on the processing method used but also on the study design and population (Table 1; [32]). This lack of standardized stool preparation and testing protocol prevents a generalizability of the diagnostic accuracy of stool Xpert MTB/RIF for PTB diagnosis [32]. The sensitivity varied between 25 and 85% across studies, as shown in the Table 1 [25,26,28,30,31,33–39] presenting the different processing methods tested with their performances.

Stool processing methods for Xpert testing

Several stool processing methods have been developed and tested so far. Some methods are based on specimen concentration approaches after dilution in Phosphate Buffer Saline (PBS), keeping the sediment for Xpert testing, and other methods use a flotation approach keeping the supernatant for Xpert testing. These two types of methods usually need centrifugation and include several manipulations that require a laboratory environment and a skilled laboratory technician. Such processing methods represent a major limitation in decentralizing stool Xpert testing at Primary Health Center (PHC) level where there is often no laboratory capacity and only nurses.

Table 1: Features, sensitivities and specificities of studies evaluating stool testing by Xpert MTB/Rif

Method	Sensitivity* (%; 95% CI)	Specificity* (%;95% CI)	Reference standard	Study population	Comments	References
Concentration methods						
Saline solution + centrifugation	5/9 (55.6%; 21.2-86.3)	54/55 (98.2%; 98.2-100)	Xpert/Culture on sputum or IS	Children <14 yrs with presumptive TB, in Uganda		Orikiriza et al. 2018
PBS + centrifugation	23/72 (31.9%; 21.4-44.0)	306/307 (99.7%; 98.2-100)	Xpert and culture of respirator and non-stool non respiratory samples	Children < 13 yrs with suspected pulmonary TB in Cape Town	13.7% HIV infected	Walters et al. 2017
	5/8 (63%; 25.0-92.0)	137/139 (99%; 95.0-100)	Culture on the 1st or 2nd respiratory specimen	HIV-infected chidren < 12 yrs in Kenya		LaCourse et al. 2018
	8/17 (47.1%; 26.2-69.0)	97/98 (99%; 94.4-99.8)	Culture on the 1st and 2nd respiratory specimen	Children < 15 years with suspected PTB in South Africa	Two Xpert tests performed on a single stool sample	Nicol et al. 2013
	13/19 (68%; ND)	195/199 (98%; ND)	A positive result on ZN smear microscopy and/or culture and/or Xpert test on IS	Children 5-16 year olds		Chipinduro et al. 2017
	20/23 (88.9%; 50.7–99.4)	ND (95%; 81.8–99.1)	Culture on GA (No possibility to find the sample size)	Children from 7 months to 15 years	HIV testing was not performed on the study participants	Hasan et al. 2017
PBS + sedimentation	6/6 (100%; ND)	42/47 (89.36%; ND)	ZN microscopy	Children < 15 in Kenya		Welday et al. 2014
PBS+incubation (Banaba)	4/16 (25%; 7.3-52.4)	222/224 (99.1%; 96.8-99.9)	Culture on the 1strespiratory specimen		Testing a single stool specimen (combined sensitivity of two stools is 41.2%)	Walters et al. 2018
	17/20 (85%; 60.0–90.0)	20/20 (100%; 77.0–100)	Xpert on IS or GA			Banada et al. 2016
PBS+incubation	3/3 (100%; ND)	21/26 (80.8%; ND)	Xpert on IS or GA	Children < 15 years with presumptive TV	Testing a single stool specimen. 3/6 Xpert MTB detected in stool not detected on IS or GA	Andryoko et al, 2019
Flotation methods						
Sheather solution + centrifugation	18/29 (62.1%; 42.3-79.3)	242/243 (99.6%; 97.7-100)	Culture on the 1st respiratory specimen	HIV infected children aged < 13 years		Marcy et al. 2016
Sheather solution + sedimentation	4/7 (57%; ND)	3/3 (100%;ND)	Culture on the 1st respiratory specimen		50% of results were invalid (5/10)	Dinardo et al. 2015

* Based on one stool testing with Xpert MTB/Rif

ND= No data

In the multicenter ANRS 12229 PAANTHER 01 study, that represents the background for using Xpert on NPA and stool in the TB-Speed project, we used a sucrose flotation method for stool processing. This method is based on the addition of Sheather's sucrose solution to the stool sample to enable isolation of the bacilli from stool particles by density gradient. It requires a filtration phase and centrifugation to separate bacilli from stool particles. Advantages of this method include its good sensitivity (60%) and its use of a very affordable and easy to purchase reagent (sucrose), which would facilitate its use in limited resource countries. However, disadvantages of this method include the important number of steps required and complexity, and the need for a centrifuge. As part of the TB-Speed project, we conducted a first sucrose flotation method optimization study in the university hospital/IRD Centre in Montpellier (France) using spiked stool specimens, aiming to obtain an optimized centrifuge-free method that could be easily used by a nurse in a PHC (TB-Speed *in vitro* study).

Existing centrifuge-free processing methods for stool

- **Stool Processing Kit (SPK)**

A stool kit (Stool Processing Kit, SPK) based on a simplified method using concentration after dilution with a stool processing buffer mixed with the sample reagent included in the Ultra kit was developed with the support of FIND (Foundation for Innovative New Diagnostics), and will be evaluated in a demonstration study. The SPK includes the same stool processing buffer than that developed in Banada et al. (2016) [39] and cap filtration replacing syringe filtration (Fig. 5). The SPK was validated in South Africa and showed good concordances with the syringe filter method developed by Banada et al. (2016). The SPK is the most advanced method in term of simplification and validation for potential use at PHC level, although its sensitivity was low compared to a recent study in South Africa (between 25% and 44%; [40]). In addition, affordability of the SPK may still be a limitation.

- **Step methods**

Another group from KNCV is currently evaluating two methods (the two-step and the one-step methods) using simple dilution with Cepheid Sample Reagent (SR) included in the Xpert Ultra kit, without concentration. A first evaluation of the two-step method has been published recently; it showed that the two-step processing method for stool Xpert testing had good results as compared to Xpert on IS and GA samples Xpert testing: out of 36 children with presumptive TB, only 7% had Xpert invalid Xpert results on stools samples. All 4 TB culture positive cases on IS/GA had stool Xpert positive results (4/4) [33]. Of 25 children with TB negative TB culture from IS/GA results, 3 had positive, 20 negative, and 2 invalid stool Xpert results. Therefore, of 7 stool Xpert positive cases (20%), 4 only were IS/GA culture positive. The authors highlighted that these additional cases had possibly extrapulmonary TB, such as miliary/disseminated and gastro-intestinal TB, and shed more bacilli via their stool than respiratory samples. The two-step method has already been integrated in Indonesian NTP guidelines for childhood TB diagnosis.

The one-step method is currently under evaluation in Ethiopia (20 primary and tertiary level clinics) and Indonesia (5 tertiary level hospitals) using a cross sectional study design. We have evaluated it during the *in vitro* TB-Speed study in Montpellier. The one-step method showed good results: of 29 stools spiked with 10^3 CFU/g of TB and processed with this method, 27 were Xpert positive, and 2 gave an invalid or error Xpert result.

TB-Speed diagnostic approach and stool processing method evaluation

The TB-Speed project is a UNITAID-funded project aiming to increase childhood TB case detection through innovative TB diagnostic approaches decentralized at district hospital (DH) and PHC levels through improvement of TB case detection among vulnerable children with severe pneumonia, severe acute malnutrition, and HIV infection. It will be implemented in 7 countries (Cambodia, Cameroon, Cote d'Ivoire, Mozambique, Sierra Leone, Uganda and Zambia) from 2017 to 2022. For microbiological diagnosis, the TB-Speed diagnostic approach is using adapted and child-friendly specimen collection methods (NPA and stools), sensitive detection tests (Xpert Ultra) close to the point-of-care (GeneXpert Omni/G1). In order to facilitate stool Ultra testing in routine conditions in resource limited settings, the TB-Speed project is evaluating optimised specimen stool processing methods. This started with an in vitro evaluation of potential stool sample processing methods using spiked stool samples. A summary of the results is presented in Appendix 2.

Rationale

There is a growing interest for the use of stool samples as an alternative to respiratory samples for the diagnosis of intrathoracic TB in children unable to produce sputum. Unlike respiratory samples, stool samples require processing before molecular testing. Several groups have already evaluated different processing

methods. However, it is difficult to know which method has the best accuracy and potential for use at PHC level, due to the difference in study designs and populations. Therefore, in this study, we propose to evaluate the accuracy of different promising stool processing methods in the same population within the same study with an adapted design. Furthermore, no study has so far evaluated for stool testing the new Xpert MTB/RIF Ultra cartridge that has a lower level of detection than the previous Xpert MTB/RIF cartridge. We propose to evaluate the accuracy of Xpert MTB/RIF Ultra (Ultra) performed on stool samples collected from children with presumptive TB and processed using four different processing methods (Standard sucrose flotation method, optimized sucrose flotation method, SPK, and STEP) against bacteriological results from respiratory specimens and to perform a head-to-head comparison of the diagnostic accuracy and feasibility of these different methods in Uganda and Zambia. The selection of processing methods was based on accuracy results, degree of simplification allowing their introduction at PHC level, and finding from the TB-Speed in-vitro stool processing study. The standard sucrose flotation method is kept to assess if results obtained with the optimised sucrose-flotation method in our in-vitro study can be reproduced in-vivo.

Participating countries

The study will take place in Zambia and Uganda, both countries participating already in other TB-Speed studies. Zambia is among the 30 high TB burden countries according to the WHO classification with a TB incidence rate of 361/100 000 population, while Uganda is in the top 20 high TB/HIV burden countries with a TB incidence rate of 201/100,000population.

2. OBJECTIVES

2.1. Primary Objective

To determine the diagnostic accuracy of Xpert MTB/Rif Ultra performed on stools processed using four different sample processing methods (standard and optimized sucrose-flotation; One Step and SPK methods) in children with presumptive TB, using an intention-to-diagnose analysis.

2.2. Secondary Objectives

- Per-protocol analysis of diagnostic accuracy of Ultral on stool using TB culture reference standard
- To compare Ultra performed on stool with the four sample processing methods in terms of:
    - Diagnostic accuracy for culture confirmed TB (culture reference standard from respiratory samples)
    - Diagnostic accuracy for confirmed and unconfirmed TB (TB composite reference standard)
    - Semi-quantitative results
    - Proportion of “trace call” results
    - Proportion of invalid results
    - Proportion of rifampicin resistance indeterminate results
- Stratification of characteristics and laboratory results by age
- Proportion of children successfully providing a stool sample
- To describe levels of agreement between results of Ultra performed on stools processed using the four methods
- To assess the relative gain (increase in detection) of a second Ultra performed on a second stool sample
- To assess feasibility of the stool processing methods

3. STUDY ENDPOINTS

3.1. Primary study endpoint

Sensitivity and specificity of Ultra on stool using TB culture reference standard (LJ and MGIT) in two respiratory samples (two sputums or two gastric aspirates according the age of the child).
Positive: at least one positive MTB culture result in one of the two respiratory samples
Negative: negative culture results from 2 samples without any positive result

3.2. Secondary study endpoints

- Per-protocol analysis of sensitivities and specificities of Ultra on stool using TB culture reference standard (LJ ans MGIT) in respiratory sample, excluding invalid Ultral results and contaminated culture results from analysis.
- Head-to-head comparisons
  - Sensitivities and specificities of each sampling method using TB culture reference standard.
  - Sensitivities and specificities of each sampling method using the TB composite reference standard as defined by the Expert Committee (Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children) [42].
- Head-to-head kappa coefficients.
- Proportions of
  - Ultra "trace" results in stools out of the number of stools tested with Ultra
  - Ultra semi-quantitative results "very low"; "low"; "medium" and "high" in stool.
  - Invalid Ultra results from stool out of the number of stools tested with Ultra
  - Rifampicin resistant results on Ultra (stool and respiratory), LPA and DST
- Stratification of characteristics and laboratory results by age groups (≤2 years and > 2 years)
- Proportion of children successfully providing a stool sample
- Relative gain of the 2nd stool sample as compared to the 1st one as measured by the number of additional positive results obtained from the addition of the 2nd sample as compared to the results of the first sample only.
- Feasibility assessment by laboratory technician of their perception of ease of use, safety and suitability to low primary health care setting using a questionnaire and a standard "Ease of use score" [41]

3.3. Reference diagnosis/case definition for the study and validation by the Expert Committee

For the secondary endpoint of the evaluation of the accuracy of the Ultra test on stool against the TB composite reference standard, children will be classified into 3 categories, according to the updated Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children, detailed in Table 2: *confirmed*, *unconfirmed*, or *unlikely* tuberculosis or any subsequent published update at the time of final case review if feasible and approved by the Scientific Advisory Board (SAB). Classification will be done by an expert committee set up for the TB-Speed HIV and TB-Speed SAM studies.

Table 2. Updated Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children (adapted from Graham et al, 2015 [42]***)***

Case definition	Refined criteria
Confirmed tuberculosis	Bacteriological confirmation obtained (Mycobacterium tuberculosis confirmed by culture or Xpert MTB/RIF assay from at least 1 respiratory specimen)
Unconfirmed tuberculosis	Bacteriological confirmation NOT obtained AND at least 2 of the following: • Symptoms/signs suggestive of tuberculosis[1] • CXR consistent with tuberculosis[2] • Close tuberculosis exposure or immunologic evidence of *M. tuberculosis* infection • Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment—no time duration specified) AND no spontaneous improvement of symptoms in the absence of antituberculosis treatment
Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND criteria for "unconfirmed tuberculosis" NOT met (including spontaneous improvement of symptoms in the absence of antituberculosis treatment)

[1] Clinical signs/symptoms suggestive of tuberculosis include: (a) Persistent cough: persistent (>2 wk), unremitting cough. (b) Weight loss/failure to thrive: (b1) Unexplained weight loss: >5% reduction in weight compared with the highest weight recorded in last 3 mo OR (b2) Failure to thrive defined as (i) Clear deviation from a previous growth trajectory, and/or (ii) Documented crossing of percentile lines in the preceding 3 mo, and/or (iii) Weight-for-age z score of ≤−2 in the absence of information on previous/recent growth trajectory, and/or (iv) Weight-for-height z score of ≤−2 in the absence of information on previous/recent growth trajectory AND (b3) Not responding to nutritional rehabilitation (or antiretroviral therapy if HIV infected). (c) Persistent unexplained fever: Persistent (>1 wk) and unexplained fever (>38°C) reported by a

guardian or objectively recorded at least once. (d) Persistent, unexplained lethargy or reduced playfulness: persistent, unexplained lethargy or decrease in playfulness/activity reported by the parent/caregiver. (e) Infants 0–60 d (or neonate): additional signs and symptoms suggestive of tuberculosis include: (e1) neonatal pneumonia or (e2) unexplained hepatosplenomegaly or (e3) sepsis-like illness.

[2] CXR will be considered consistent with tuberculosis if reviewers agree on the presence and location (right/left) of ≥ 1 lesion among the following: alveolar opacity, bronchial compression, excavation, ghon focus, gibbus, miliary, nodular infiltrates, paratracheal nodes, peri-hilar nodes, pleural effusion, tracheal compression (as suggested by Graham et al. 2012).

A child with a negative culture results and Xpert Ultra positive results on respiratory samples will be classified as "negative" for the primary endpoint analysis based on culture results only and as "confirmed TB" for the secondary endpoint analysis using the standard case definitions.

CXRs will be read by 2 independent experts experienced in reviewing CXRs in children, blinded to all of the clinical information and to each other's interpretation. In case of disagreement, advice from a third expert will be sought.

An Expert Committee will be set up at national level for the purpose of case review and validation of TB diagnosis. The Expert Committee will not review all cases systematically. In agreement with the SAB, the Expert Committee will not review cases which are Ultra (bacteriologically)-confirmed TB cases, or true negative TB (Ultra-negative untreated children alive at 2 months with normal M2 follow-up CXR). The Expert Committee will review remaining cases, which are neither Ultra-confirmed TB nor true negative TB considering the following parameters: initial clinical and follow up data, microbiological data and radiological features.

The Expert Committee will classify children using the Revised Classification of Intrathoracic Tuberculosis Case Definitions for Diagnostic Evaluation Studies in Children (see Table 2 above), following Standard Operating Procedures (SOPs) specifically developed for the study. A centralized Expert Committee will review part of all cases reviewed at country level to ensure homogeneity of classification across countries.

4. STUDY DESIGN

4.1. Study type

This is a diagnostic study evaluating the diagnostic accuracy of the Ultra assay in stools with a two-stage sequential design starting as a cohort of children with presumptive TB enriched in a second stage with Ultra positive TB cases on respiratory sample. It is both an ancillary to the TB-Speed HIV and the TB-Speed SAM studies and a study enrolling children from routine not enrolled in those two studies.

This design was chosen to be able to evaluate the sensitivity and specificity of the Ultra assay in a smaller sample size that is usually required by a "classical" prospective cohort design and avoiding the bias of overestimation of the sensitivity classically associated with the case-control design. In order to quickly generate data on appropriate stool processing method, and to contribute to the planned WHO recommendations for stool Ultra testing (expected 2nd semester 2021), we will use a two-stage sequential design. Indeed, knowing that on average only 10-15% of children with presumptive TB in a community-based setting will be confirmed, in order to reach the sample size of confirmed cases for the evaluation of sensitivity, 7 to 10 times more children with presumptive TB would need to be enrolled in a prospective design. On the other hand, the number of children with presumptive TB not confirmed with TB for the estimation of the specificity would be reached much earlier. In addition, based on the previous study results, we know that the specificity of Xpert MTB/RIF assay in stool is high (99% CI:98-99; [32]), which would result in a relatively small sample size to evaluate the specificity of the Ultra in stools.

During the first stage, we will offer to join all consecutive presumptive TB cases presenting at study sites to estimate specificity with the expected precision and calculate a preliminary sensitivity estimate. During the second stage, we will keep enrolling only those from TB Speed studies and routine care who are Xpert positive on respiratory samples in order to estimate sensitivity with the expected precision.

This two-stage sequential design first estimating specificity then sensitivity has been described by Wruck *et al.* [43] as an efficient way of validating diagnostic tests when the prevalence of the disease is low. It would not be feasible to consecutively enrol all children with presumptive TB to describe an expected sensitivity of 60% with 10% precision as this would require over 900 patients, of which, approximately 800 would be culture negative. In the two-stage process described by Wruck, only reference standard positive samples from the original population are selected in stage 2. We adapted this design to the TB context as culture results will only be available after enrolment (and if the child is positive, only after they have started treatment), hence selecting only those who are Ultra positive on respiratory samples for the second cohort as a way of enriching the study population with a subpopulation that has a higher TB prevalence probability, before their true disease status

is confirmed. Other comparable diagnostic studies have either used greater resources to include larger samples sizes (Dorman [14] and Wang [44]) or have resorted to reporting imprecise estimates of sensitivity [79-84]. To our knowledge, this is a relatively unique approach to study design for accuracy studies, with few published examples [45,46].

With such design, there should be no bias on the evaluation of the specificity similarly to a classical prospective design because this evaluation will be done among consecutively enrolled children with presumptive TB only. The sensitivity estimates may not be generalizable to all culture confirmed TB children due to the sampling approach. Xpert positive children will be more likely to have higher biological loads, causing a possible inflation of the sensitivity. However, the results will provide valuable information on variations of sensitivities of the different stool processing methods within this population.

We will carry out an interim analysis after the completion of the prospective cohort in order to describe specificity and preliminary results of the sensitivity and the agreement between the processing methods. The recruitment of participants will not be put on hold during the interim analysis. We will conduct a final analysis at the end of the study to describe sensitivity as well as the secondary end points.

4.2. Methodology

Enrolment will have 2 stages. During stage 1 (prospective cohort stage), all consecutive children with presumptive TB enrolled in the TB-Speed HIV or TB-Speed SAM or seen in routine care if ineligible for those two studies will be proposed to participate in the study until we reach the number needed for the evaluation of specificity. All children with presumptive TB will have at least two respiratory samples collected (ES or GA) tested with Ultra and mycobacterial culture. The cohort will be closed once the sample size of presumptive TB children with two negative Ultra results from respiratory samples will be reached.

During the 2nd phase (enrichment phase), only children with presumptive TB who are Xpert positive on at least one respiratory sample will be proposed to participate in the study in order to complement the number of confirmed TB cases required for the evaluation of the sensitivity.

TB-Speed SAM and HIV studies

TB–Speed SAM is a multicentric prospective diagnostic cohort study aiming to assess several diagnostic tests that could result in the development of a score and algorithm for TB treatment decision in hospitalised children with severe acute malnutrition (SAM). It will enrol 720 children <5 years old with WHO-defined severe acute malnutrition in Uganda and Zambia.
TB-Speed HIV is a Prospective, multicentre management study evaluating the safety and feasibility of the recently proposed PAANTHER TB treatment decision algorithm for HIV-infected children with presumptive TB. 550 HIV-infected children aged <15 years with clinically suspected (presumptive) TB will be enrolled from Côte d'Ivoire, Uganda, Mozambique and Zambia.

Prospective cohort:

Any child with presumptive TB will be proposed to participate in the study.

If the child is enrolled in the TB Speed SAM or HIV studies, the study nurse will collect two stool samples then collect information about the clinical examination, chest X-ray, HIV-testing and the mycobacterial culture results as soon as they are available, from the data collected in the TB-Speed records since all these procedures are already performed in these studies.

For children identified from the routine practice in the two hospitals, the nurse will collect two respiratory samples (sputum or GA) in consecutive children with presumptive TB to be tested using Ultra as done in routine care, record symptoms and refer the child for clinical exam and for chest X-ray. For the purpose of the study, two stool samples will be collected to be tested with Ultra. In addition, for study purpose the two respiratory samples will be tested with Mycobacterial culture as this test is not routinely prescribed for TB diagnosis in the study sites. However, culture results will be made available to the clinicians for case management. HIV-testing will be offered for children with unknown status as done under routine practice in both sites.

Xpert positive enrichment cohort:

Any child with presumptive TB and a positive Xpert result (MTB/Rif or Ultra according local practice) from one respiratory sample (NPA, IS or GA) will be proposed to participate in the study.

If the child is enrolled in the TB-Speed SAM or HIV studies, the study nurse will then collect two stool samples as well as information about the clinical examination, chest X-ray, HIV-testing and the mycobacterial culture

results from respiratory samples as soon as they are available, from the data collected in the TB-Speed records since all these procedures are already performed in these studies.

For children identified from the routine care in the three hospitals, once enrolled, samples collected as routine practice will be tested with mycobacterial culture in addition to Xpert. If needed, in case there is no enough sample volume remaining for culture after Xpert or if only one sample was collected because the first sample is Xpert positive, an additional respiratory sample will be collected (sputum or GA) and tested with Mycobacterial culture. The nurse will also record symptoms, refer the child for clinical exam and for chest X-ray, and collect stool samples. HIV-testing will be offered for children with unknown HIV-status. The two stool samples and additional respiratory samples if necessary will be collected for the purpose of the study only. However, culture and drug susceptibility results from the respiratory samples will be made available to the clinician for case management.

4.3. Sample size

As we want to estimate two proportions, specificity and sensitivity, we need to ensure a sufficient sample size for these two estimates.

The first stage is to estimate specificity by enrolling children with presumptive TB. With an expected specificity of Ultra in stool of 90%, the minimum sample size to estimate specificity with 5% precision, is 140 children with presumptive TB and negative TB culture from two respiratory samples. Inflating this number by 15% for attrition (to a total of 160 children) and considering a 10% prevalence of culture-confirmed TB in our population, we will need to enrol a total of 177 children with presumptive TB.

The second stage is to estimate sensitivity among presumptive TB children with culture confirmed TB. With an expected sensitivity of Ultra in stool of 60%, based on a literature review, the minimum sample size to estimate sensitivity with 10% precision, is 93 children with culture-confirmed TB. Inflating this number by 10% for attrition and invalid results, we will need a total of 103 confirmed TB cases. We expect 16 culture confirmed to be enrolled during the first stage (10% of 160 children enrolled in the prospective cohort). Then we will need to enrol an additional 87 children during the 2nd stage from presumptive TB children with a respiratory sample positive with Xpert Ultra (enrichment cohort). Knowing that only 90% of these children will ultimately be culture positive, we will need to further inflate the number of children to be recruited based on Ultra result in their respiratory sample [84]. We will therefore a total of enrol 97 children with Xpert Ultra positive results during the second stage.

Given routine site data and estimated enrolment figures for the Ugandan and Zambian study site, we expect to enrol children for the first stage in approximately 12 months, and Ultra-positive children for the second stage in 11 months.

For the secondary endpoints, in order to test Kappa coefficients with a minimum acceptable value of 0.4 [47], considering the study sample size, we will be able to detect a minimum change of 0.1 in Kappa coefficient between the stool sample processing methods with 80% power.

4.4. Provisional study schedule

- Start of prospective cohort enrolment: January 2020
- End of prospective cohort enrolment: January 2021
- Interim-analysis: April 2021
- Start of enrichment cohort enrolment: January 2021
- End of enrichment cohort enrolment: December 2021
- End of follow-up: February 2022
- Analysis-report: May 2022

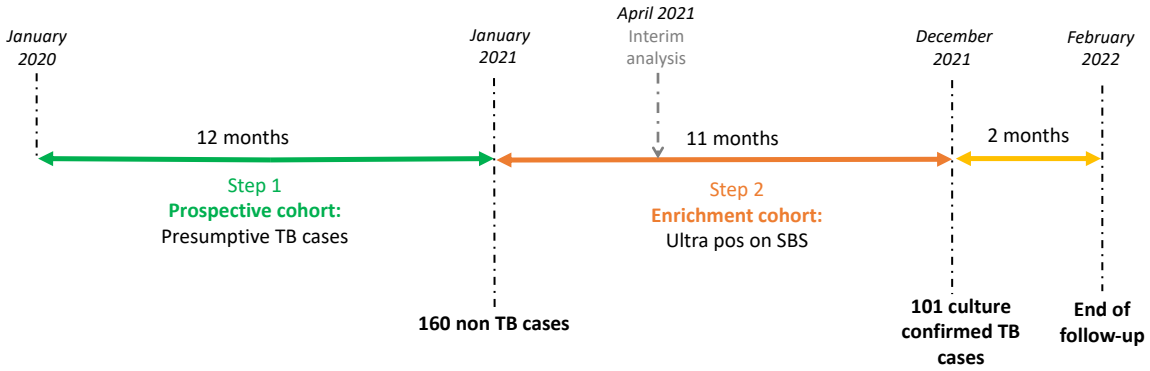

Figure 1. Study design

5. STUDY ENROLMENT

5.1. Study population

5.1.1. Prospective cohort

Inclusion criteria:
- Children < 15 years old
- Presumptive intra-thoracic TB based on at least one criterion among the following:
  - Persistent cough for more than 2 weeks
  - Persistent fever for more than 2 weeks
  - Recent failure to thrive (documented clear deviation from a previous growth trajectory in the last 3 months or Z score weight/age < 2)
  - Failure of broad-spectrum antibiotics for treatment of pneumonia
  - Suggestive CXR features

OR

  - History of contact with a TB case and any of the symptoms listed under point 2 with shorter duration (< 2 weeks) if the child is HIV infected or presents with SAM.
- Signed informed consent by parent or guardian and assent signed by children ≥ 7 years old.

Exclusion criteria:
- > 5 days of antituberculosis treatment in the last 3 months
- History of tuberculosis preventive therapy in the last 3 months
- Confirmed extrapulmonary TB only

5.1.2. Enrichment cohort

Inclusion criteria:
- Children < 15 years old
- Presumptive TB based on at least one criterion among the following:
  - Persistent cough for more than 2 weeks
  - Persistent fever for more than 2 weeks
  - Recent failure to thrive (documented clear deviation from a previous growth trajectory in the last 3 months or Z score weight/age < 2)
  - Failure of broad-spectrum antibiotics for treatment of pneumonia
  - Suggestive CXR features

OR

  - History of contact with a TB case and any of the symptoms listed under point 2 with a shorter duration (< 2 weeks) if the child is HIV infected or present a SAM.
- One positive Xpert (MTB/Rif or Ultra) result from at least one respiratory sample: NPA, sputum or GA.
- Signed informed consent by parent or guardian and assent signed by children ≥ 7 years old.

Exclusion criteria:
- > 5 days of antituberculosis treatment in the last 3 months
- History of tuberculosis preventive therapy in the last 3 months
- Confirmed extrapulmonary TB only

5.2. Study sites

Children will be recruited from the Mbarara Regional Referral Hospital in Mbarara (Uganda, South West region), the Lusaka University Teaching Hospital and the Arthur Davidson Children Hospital (Zambia).

Laboratory testing for Mbarara will be done in the Mbarara Research Centre of Epicentre. Epicentre is a non-governmental research organization created by Médecins sans Frontières in 1986 to help improve the quality of its field interventions. In 1996 Epicentre became a World Health Organization (WHO) collaborating centre for research in epidemiology and response to emerging diseases. Located within the campus of the Mbarara University of Sciences and technology (MUST) and the Mbarara Regional Referral Hospital (MRRH), Epicentre carries out clinical and operational research to assess and find better interventions to the burden of infectious diseases affecting Uganda in particular and sub-Saharan African countries in general. The mycobacteriology laboratory is quality assured by the supranational mycobacteriology laboratory of the Tropical Medical Institute in Antwerp (Belgium) and the National Health Laboratory Institute (NLHS) in South-Africa. It is part of the national reference laboratory network of Uganda.

Laboratory testing for Lusaka will be done in the TB laboratory at the University Teaching Hospital (UTH) which is the national referral hospital in Zambia, serving a population of 2 million in the capital city of Lusaka. It offers specialized health care, research, and medical training through the University of Zambia School Of Medicine. The UTH TB lab is one of three reference mycobacterial labs reference laboratories in Zambia servicing the Lusaka, Southern and Western regions. The Children's Hospital is a 352-bed hospital attending to 35,000 children annually, and housing the Children with HIV in Africa, Pharmacokinetics and Acceptability/adherence of Simple antiretroviral regimen (CHAPAS) project site established in 2001. The hospital has a dedicated HIV treatment and care center with a cohort of 2,413 patients. It also holds a Nutritional Rehabilitation Unit for children with severe acute malnutrition requiring hospitalization. The Research Clinic is now a well-established research site, which has participated in a number of multicenter clinical trials. The study site is currently led by a Principal and co-Investigator and has a staff of 15 including doctors, research nurses, data and other support staff all of whom have undergone GCP training. Progress and results from this study, that is part of the TB-Speed project, will be reviewed at national level by the Country Project Committees (CPC) that regroup national tuberculosis stakeholders including representatives from the Ministry of Health and from the National TB Program (NTP).

Laboratory testing for Ndola will be done in the laboratory of the Arthur Davidson Children Hospital (ADCH) which is the referral hospital for the Copperbelt Province. The ADCH is the only standalone paediatric hospital in Zambia, it is a 250-bed hospital attending to 19 000 children annually. The hospital has an HIV and treatment care centre and a Nutritional Rehabilitation centre for children with severe acute malnutrition. Mycobacterial cultures will be done at the Tropical Diseases Research Centre Regional National TB Reference Laboratory.

6. STUDY INTERVENTIONS

6.1. Diagnostic assay under evaluation

The Xpert® MTB/RIF Ultra (Ultra) test for use with the Cepheid GeneXpert® System is a diagnostic test based on a semi-quantitative nested real-time PCR. It allows for:
1) the detection of *Mycobacterium tuberculosis* complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either acid-fast bacilli (AFB) smear positive or negative;
2) the detection of rifampicin resistance associated mutations of the *rpoB* gene.

Ultra uses two different multi-copy amplification targets (IS*6110* and IS*1081*) and has a larger PCR reaction chamber (50 µl in Ultra compared with 25 µl in Xpert MTB/RIF). This has led to a lower limit of detection for Ultra compared with Xpert MTB/RIF (16 colony forming units per milliliter (cfu/ml) and 131 cfu/ml, respectively). Furthermore, the use of melting temperature-based analysis with Ultra instead of real-time PCR analysis used with Xpert MTB/RIF allows Ultra to better differentiate silent mutations from resistance-conferring mutations, hence improving the accuracy of RIF resistance determination. In samples characterized by a very low bacterial load, only the IS6110 and IS1081 elements can be detected by the Ultra assay (due to their presence in multiple copies in the bacterial genome), and the new semi-quantitative category, 'MTB detected trace' is used to report these results. In case of trace result, rifampicin resistance is reported as "Indeterminate".

6.2. Stool processing methods

Standard sucrose flotation

The stool processing method used in the ANRS 12229 PAANTHER 01 study is based on a sucrose (using Sheather's solution) flotation method. Briefly, it consists in processing stools by emulsification in 10 mL Sheather's solution (50% sucrose in distilled water) using wooden sticks and a vortex for 30 seconds, filtration through funnel gauze, and centrifugation at 100g for 1 minute before the supernatant (0.5 mL) can be mixed with Xpert reagent.

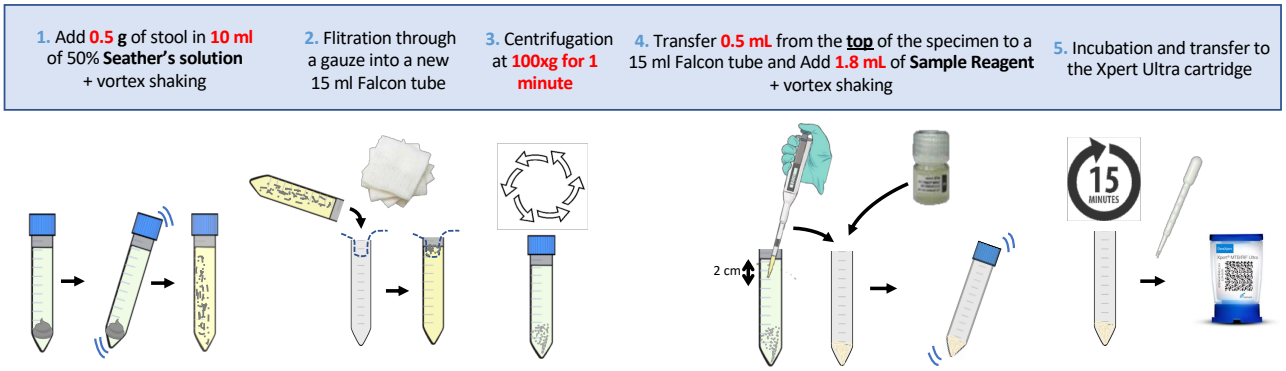

Figure 2. The sucrose flotation method used in the PAANTHER study

Optimized sucrose flotation

During the in vitro study several parameters have been tested in the laboratory of Montpellier, using spiked stools with TB. The best parameters combination was the one that includes replacing vortex shaking by manual shaking at step 1, removing the filtration step, and replacing centrifugation by 30 minutes sedimentation. Other parameters combinations had lower performances (See *in vitro* study summary – Appendix 2). The optimized sucrose flotation method can be summarized as follows:

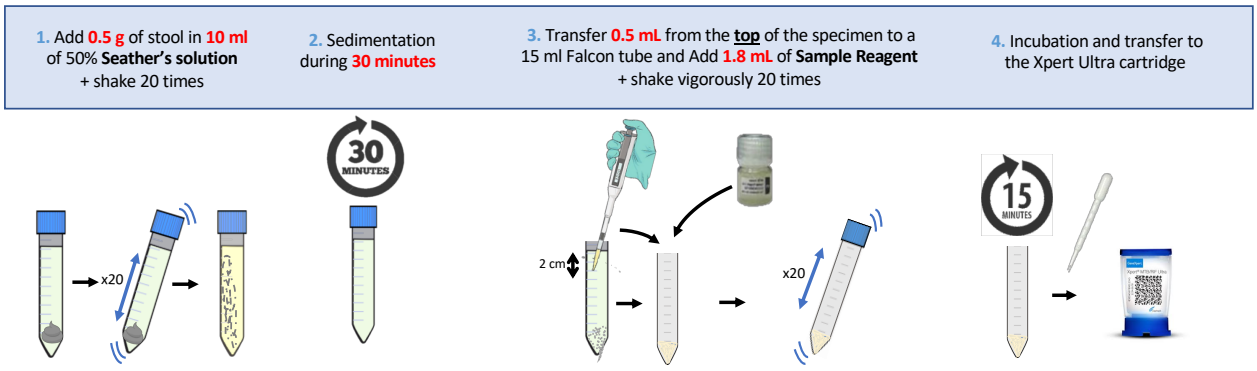

Figure 3. The sucrose flotation method optimized after the in vitro study

Stool Processing Kit (SPK)

The SPK (FIND) method includes use of a 5 mL stool processing buffer mixed with 5 mL Ultra kit sample reagent. A specific collection spoon is provided to collect the correct amount of stool and add it in the previously mixed buffer. After mixing and incubating for 30 minutes, the solution is filtered through a filter cap and added to the cartridge.

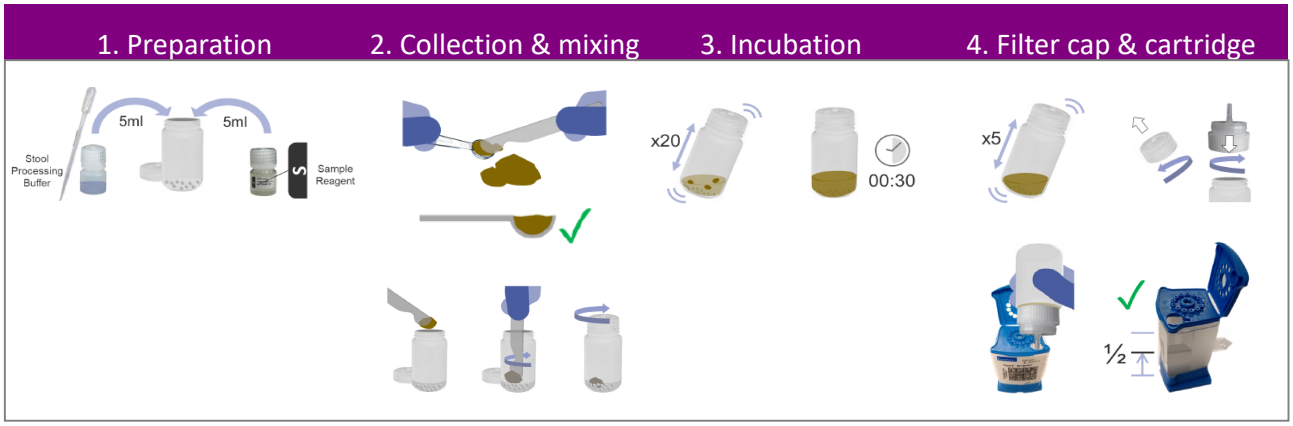

Figure 4. Stool processing kit procedure

STEP method:

The One step method developed by KNCV does not require any equipment or consumable (Fig. 6). A 0.8 g stool sample is directly added to the 8 mL sample reagent bottle provided in the Xpert Ultra kit. After mixing and incubating for 15 to 20 minutes to let solid particles gravitate, 2 mL of the upper part is transferred into the cartridge.

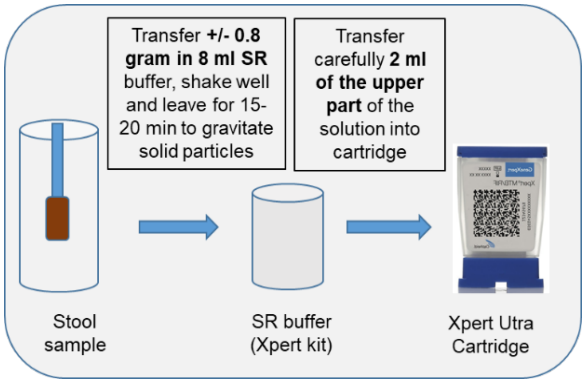

Figure 5. One step method for stool specimen preparation implemented by KNCV

6.3. Sample flow

Two stool samples over two consecutive days will be collected and transported within 24h to the CTU laboratory for processing and testing. At laboratory, samples will be homogenised and split in four equal aliquots that will be processed with the four processing methods and tested with Ultra (Figure 6).
To avoid experimenter bias, the four aliquots will be processed by the same laboratory technician at the same time.
In case of insufficient stool volume (<5g) another stool sample will be collected.

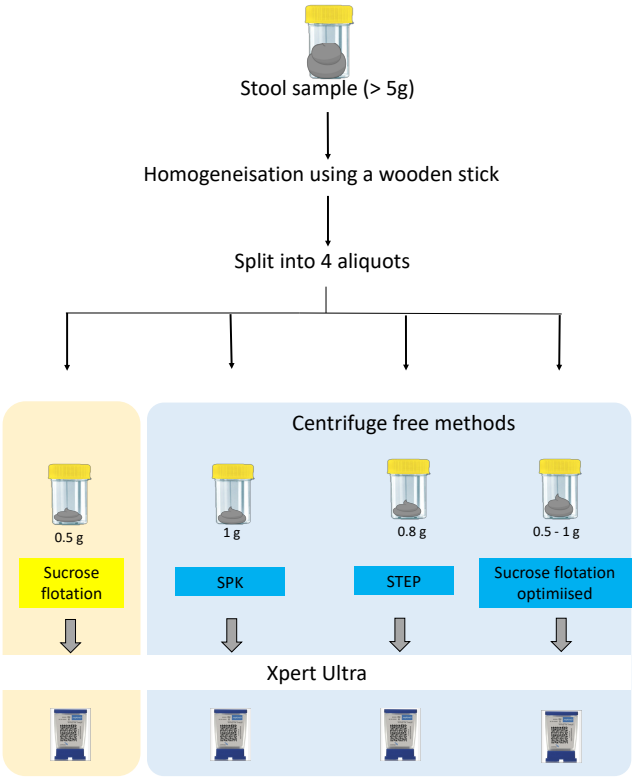

Figure 6. Sample flow for comparison of the four stool processing methods

For cases enrolled from the routine, two GAs or sputum will be collected over two consecutive days. The two respiratory samples will be tested on Ultra and sent for liquid and solid culture.

6.4. Capacity building

The TB-Speed stool processing study will take place in three laboratories of tertiary level reference university teaching hospitals that are also involved in other TB-Speed studies. These laboratories have the capacity to perform mycobacterial culture and Xpert testing, including Ultra. The MMRH in Mbarara is participating in the TB-Speed project with TB-Speed HIV study that evaluates a TB diagnostic algorithm for children with HIV-infection. Lusaka University Teaching Hospital, a national referral hospital in Zambia is participating in the TB-Speed HIV study and the TB-Speed SAM study, evaluating a TB diagnostic algorithm for children with SAM. As part of the capacity building for the TB-Speed SAM and HIV studies, these sites will be equipped with DR plates for digital CXR and laboratories will receive additional equipment if needed for sample collection and sample processing before Xpert testing. Additional sample collection material, stool processing kit and consumables, and Xpert Ultra kit will be provided.

Training will be provided onsite by the study central coordinating team to the site investigators and hospital staff (doctors, nurses, and laboratory technicians) on TB diagnosis, study procedures for staff directly involved with the follow-up of study participants and Good Clinical Practice. Based on didactic and practical presentation, trainings will include the following topics:

- Childhood TB diagnosis including sample collection and management
- Clinical follow-up (eligibility criteria, informed consent process, patient schedule, TB assessment, management in the case of AE/SAEs) for staff involved in the children enrollment after screening from routine practices. Otherwise, this training will be already provided for sites involved in the HIV and SAM TB-Speed studies.
- Study procedures
- Stool processing methods
- Data collection and use of electronic CRF
- Data management and monitoring
- Good Clinical Practice (GCP)
- Use of DR plates for digital CXR

The TB-Speed laboratory coordinator in each country will train and supervise the laboratory technicians in charge of the stool processing methods with support from the project coordinator who is also the international TB-Speed laboratory coordinator.

7. STUDY PROCEDURES

7.1. Selection process

7.1.1. Prospective cohort

Children from the TB-Speed studies:
Study nurses from the TB-Speed SAM and TB-Speed HIV studies will identify potential eligible cases based on the selection criteria of having presumptive TB (all children enrolled in the TB-Speed HIV study and part of those enrolled in the TB-Speed SAM study) and will ask for the informed consent of the parent/guardian and assent of the child older than 7 years. This will be an additional consent and assent for co-enrolment in the TB-Speed stool processing study.

Children from the routine practice:
The nurses from the ward will be informed about all the TB-Speed studies taking place in the hospital wards and will inform the TB-Speed study nurse for any potentially eligible child. If the child is eligible for the TB-Speed HIV or SAM study, he will be proposed to participate to these studies first. Then, the participation in the stool processing study will be proposed secondarily for those who accepted to be part in the TB-Speed HIV or SAM study. Children that will not be eligible for the HIV or SAM studies will only be offered participation to the stool processing study. After verification, the study nurse will ask the parent/guardian's informed consent and the child's assent if older than 7 years.

7.1.2. Enrichment cohort:

Children from the TB-Speed studies:
Study nurses from the two TB-Speed Studies will identify potential eligible cases among those who are Ultra-positive on respiratory samples based on the selection criteria and will ask for the informed consent of the parent/guardian and assent of the child older than 7 years.

Children from the routine practice:
Because respiratory sample collection is not systematically collected in children with presumptive TB in the pediatric wards, the nurses from the ward will be informed about the study and will inform the study nurse when a child presents with a positive Xpert result. After verification, the study nurse will ask for the informed consent of the parent/guardian and assent of the child older than 7 years to complete the enrolment.

7.2. Obtaining informed consent

The informed consent and assent process will be implemented by the study nurse following specific SOPs. They will ensure that parent(s)/guardian(s) and children over 7 years old have read and understood the content of the information sheets, and that they have received answers to all their questions before signing the informed consent. Written informed consent should be obtained prior to any study-specific clinical, biological or radiological exam.

Different case scenarios of consent-taking are proposed for children already participating in another TB-Speed study or recruited from the routine of cares depending if they are included in the prospective cohort or enrichment cohort:

- Prospective cohort:
    - For children participating to the TB-Speed HIV or SAM studies (admitted or not admitted), parents/guardian will be consented just after consenting for the child's participation to the TB-Speed HIV or SAM study on the same day by the same study nurse. They will be explained that only two additional stools will be collected from their child and that all the other needed information will be extracted from the other TB-Speed study data set.
    - For children not participating in a TB-Speed study and screened from the routine program activities, parents and guardian will be consented by the study nurse of the stool processing study as soon as a child (admitted or not admitted) meets eligibility criteria (presumptive TB) to participate to the study. If the child is HIV positive and is eligible for the TB-Speed HIV study he will be proposed to participate to the HIV study first.
- Enrichment cohort:
    - For children participating to the TB-Speed HIV or SAM studies (admitted or not admitted), only parents/guardian of a child with an Xpert Ultra positive respiratory sample (NPA, GA, sputum) will be consented. In these two studies, Xpert Ultra is performed on the NPA collected on the

day of enrolment (D0) and on the first sputum/GA collected on the following day (D1). Therefore, the consent-taking will be done by the TB-Speed HIV/SAM study nurse as soon as she receives a positive result, which should be within 2 days after the child's enrolment. The consent-taking will be done during scheduled study visit of HIV/SAM studies during the first 3 days after enrolment. The only additional study procedure will be collecting two additional stools.

- For children not participating in a TB-Speed study and screened from the routine program activities, parents/guardian will be consented by the study nurse of the stool processing study as soon as a child (admitted or not admitted) meets eligibility criteria (presumptive TB with one Xpert positive result from a sample collected under routine of cares) to participate to the study. Consent-taking will be done when the child comes back to the hospital to initiate TB treatment, which should be on the same day or next day of the positive Xpert result.

The purpose, the nature of constraints and the foreseeable risks and benefits of the study will be fully explained to the parent(s)/guardian(s) of eligible children. Parent(s)/guardian(s) will be informed that participation is voluntary and that they will be free to withdraw from the study at any moment without justification or without any negative consequences for the quality of care and follow-up provided to their child. In addition to oral explanations, a written information sheet will be systematically provided (see Appendix 6 and 7).

Children aged over 7 years are unable to consent but their assent should be obtained using age-appropriate information. Separate information sheets and assent forms will be used to explain the purpose, the risks and benefits of the study. Whenever possible, a written confirmation will be obtained from the child. Consent should be obtained from the parent(s)/guardian(s) before assent is sought from the child. If the child's assent is not collected, this will be recorded in the consent form with the reasons.

If the participation agreement is given by the parent(s)/guardian(s) and child if over 7 years old, the consent forms will be completed, signed and dated by the parent(s)/guardian(s), the child whenever possible and the study nurses or site investigators. Oral consent in the presence of a witness (not from the medical team) is acceptable in the case of illiteracy.

Agreement of one parent/guardian only is needed for participation but if one of the parent(s)/guardian(s) refuses the child's participation, the child will not be enrolled. Consent must represent the child's presumed will, and may be revoked at any time without detriment to the child. The explicit wish of a child to refuse participation or to be withdrawn from the study should be considered at any time. In this case, children will be referred for routine standard of care.

A copy of the signed consent(s) will be given to parent(s)/guardian(s). The original(s) consent(s) form will be retained by the site investigator in a safe place inaccessible to others, even when moving, throughout the study period and for 15 years after its end.

In the absence of national regulation, a person who usually assumes responsibility for the child's custody, care, and maintenance even though no court order exists formally appointing the person as the guardian, custodian, or adoptive parent of the child, will be considered as a guardian. Should any of the child's parents be alive but not living with the child, the usual caregiver will be considered as a guardian.

Two different information notice will be given to children identified from the TB-Speed studies and the routine practice because additional procedures will be done for the latter. For children identified from the TB-Speed studies only additional stool samples will be collected. Different consent will be used for the children identified from routine practice.

7.3. Patient schedule

Table 3: Patient schedule including specimen collection

	D0	**D1**	**M2** (+/- 3 days)
HIV and SAM TB-Speed studies			
● Eligibility criteria	x		
● Informed consent(s) and assent	x		
● Stool sample collection +Ultra	x	x	

Routine practice			
● Eligibility criteria	x		
● Informed consent(s) and assent	x		
● Clinical evaluation	x		x
● Medical history	x		x
● Chest X-ray	x (o)[1]		x
● HIV test	x (o)[1]		
● Gastric aspirate or sputum	x (o)[1]	x (o)[1]	
● Xpert MTB/RIF Ultra	x[2]		
● Mycobacterial culture	x	x	
● Stool sample collection + Ultra	x	x	

o Routine care
x Research
(1) Performed if not available in the patient medical chart
(2) Performed during the phase 1 prospective cohort only

7.4. Inclusion visit

After obtaining informed consent, the following procedures will be performed during the inclusion visit (D0) for cases selected from the routine practice:

- **Complete clinical evaluation**
  - Demographic information (month and year of birth, sex)
  - Interview of parent/guardian on medical history, symptoms, past and current medication, immunization, TB preventive Rx
  - TB assessment: family exposure, household and neighbours exposure, fever, cough for more than 2 weeks, weight loss
  - History of chronic diseases (HIV, asthma, cardiac disorders, …)
  - Physical examination: respiratory and cardiovascular, adenopathy, hepatomegaly, assessment of nutritional status
  - Vital signs: respiratory rate, heart rate, temperature, weight, height, measurement of oxygen saturation

- **Radiographic exams**
  - A CXR standard anteroposterior and lateral view in children below 5 years and standard postero-anterior in children above 5 years old will be performed and read by the clinician

- **Blood samples**
  - For HIV testing (in children with unknown HIV status, using national guidelines)

- **Gastric aspirates or sputum collection**
  - Early morning samples at the clinic, GAs or sputum will be collected over 2 consecutive days (D0 and D1).

- **Stool collection**
  - 2 stool samples, collected as soon as the child is able to produce stool on 2 consecutive days (D0 and D1). A window of 3 days will be allowed in case that the child is not able to produce stool.

7.5. Month 2 visit

The visit will include the assessment of TB disease evolution with or without TB treatment depending on the clinician's diagnosis. It will comprise:

- Clinical evaluation: complete physical examination, vital signs
- Treatment adherence assessment for children started on TB treatment
- Medical history since the last visit
- Chest radiography

The date of follow-up visit must comply with the provisional patient schedule generated from the date of inclusion of the child, but a window of 3 days will be allowed. If a child fails to attend a study follow-up visit, the clinical team on site will confidentially contact the parent(s)/guardian(s) and encourage/assist them to bring back the children for follow-up.

7.6. Feasibility assessment of stool processing methods

Each method will be assessed according their feasibility and ease-of-use. This assessment will be divided in two parts:

(1) General characteristics of the stool processing method (shelf-life, storage temperature, storage volume, cost, number of steps needed, additional material and equipment needed, health and safety, storage and disposal of waste test materials and reagents, training experience and knowledge needed, interpretation and judgement needed). Each stool processing method will be scored according a rating system laying on the criteria described above [41]. This scoring system will be administering at the end of the study.

(2) Characteristics related to the opinion of the laboratory technician (rapidity and ease of performance, quality of the instruction sheet, perceived feasibility at each step). The opinion of all the study technicians will be assessed independently and using a short self-administered questionnaire containing open and multiple-choice questions at three time points: after the enrolment of the first 30 children, after enrolment of 150 children (towards the end of the prospective phase) and at the end of the study (Appendix 4). This questionnaire will allow to capture qualitative information related to the perceived utility and feasibility. The questionnaire will be filled online and a study monitor will ensure that the questionnaire is filled independently from the other laboratory technicians involved in the study.

Written informed consent will be obtained from the laboratory technician before administering the questionnaire and scoring system.

7.7. Unscheduled visits and care in case of clinical adverse events

NA

7.8. Management in the case of selected adverse events

7.8.1. Management in the case of sample collection adverse events

GA will be performed by study nurses using standard procedures. The sample collection is routinely collected in the three hospitals participating in the study. GA may cause vomiting.

No AE are expected with the stool and sputum collection.

Management in case of AEs will be detailed in the study SOP.

7.8.2. Management in the case of treatment related-limiting adverse events

Treatments will not be provided by the study. Management of drug toxicity will be undertaken according to National guidelines and will be detailed in the clinical SOP.

7.9. Final study visit

The study is a cross-sectional study. Final visit will be the M2. Treatment decision and management will be the responsibility of the NTP.

7.10. End of the research

7.10.1. Definition

The official end of the study, except in case of premature termination, is defined by the last visit (M2) of the last child included in the study. Children will benefit from the regular care provided by National Programs of their respective countries.

The sponsor or its representative will notify the end of the study to the ethical and regulatory authorities of each participating countries within 90 days.

A premature end may be decided by the sponsor, following the advice of the Scientific Advisory Board (SAB), or the ethical and/or regulatory authorities issuing a decision to discontinue the study. If the study is ended prematurely, the sponsor or its representative will notify the ethical and regulatory authorities within 15 days, and clarify the reasons for such a premature termination. The sponsor and the investigators, in close collaboration with the country health authorities, will take appropriate decision to ensure that patients have access to the best available care and treatment according to each country conditions.

7.10.2. Withdrawal of consent

Withdrawal of the participant from the study may be at the initiative of the investigator or the parent(s)/guardian(s) of the participant (withdrawal of consent or premature exit). The parent(s)/guardian(s) may decide to withdraw the child from the study at any time if they wish to, without any consequence on the quality of subsequent follow-up and care.

When parent(s)/guardian(s) withdraw their consent for the child's participation in the study as they have the right to do at any time, no new information must be collected and recorded in the database after the date of withdrawal. Similarly, no samples must be collected after that date in the context of the study.

When parent(s)/guardian(s) who withdraw consent explicitly express the will that the child's data be removed from the database and the laboratory samples be destroyed, the study team will carry out such wishes. When parent(s)/guardian(s) who withdraw consent do not express such wishes, data and samples collected prior to the date of the withdrawal will be used for analysis.

The child's parent/guardian should be informed of the possibility to withdraw from the study. The explicit wish of a child to refuse participation or to be withdrawn from the study will be considered at any time, provided it is not considered detrimental to his/her health.

Withdrawals of consent to participate in the study must be reported to the country CTU as soon as possible. The investigator must document in the patient's medical records the date, the reason for withdrawal if possible, and any answers given in response to the child's parent(s)/guardian(s).

7.10.3. Loss to follow-up

When a child for whom parent(s)/guardian(s) have not explicitly withdrawn consent does not show up for D1 and M2 visits, the study nurse must make every effort to contact the parents(s)/guardian(s). With their prior agreement, the study nurse will contact the parent(s)/guardian(s) via telephone or any other means available and acceptable locally (home visits, home base care team). The investigator should show "due diligence" by documenting in the source documents steps taken to contact the patient.

Particular attention will be given to the descriptive analysis of patients lost to follow-up and protocol withdrawals during the study: numbers, characteristics and reasons for refusal/lost to follow-up.

8. LABORATORY AND RADIOLOGIC EVALUATIONS

8.1. Biological specimen collection

Stool, GA, sputum and blood samples are collected between D0 and D2 (see Table 3) for laboratory tests performed on-site or at the country reference laboratory.

All specimen collection methods and biological exams procedures will be detailed in specific SOPs.

- **Respiratory specimens:**

Patients will be asked to provide two samples during two consecutive days (D0 and D1). The sputum collection will be attempted in cases aged 5 years and more, able to self-expectorate. If expectorated sputum cannot be obtained, it will be replaced by GA. The collection of gastric contents will be done in the morning through a nasogastric tube in a child fasting since midnight the previous day, in supine position, before any rise in case of hospitalization and after 1 hour of supine for children seen in consultation. If it is impossible to collect at least 5 ml of gastric fluid, 10 to 20 ml of sterile water will be injected through the catheter and aspirated.
GAs will not be attempted in children presenting severe respiratory distress or Sp02 <90% under appropriate oxygen therapy, severe dehydration or features of shock. Advice will be sought from a clinician not directly involved in the study to make the determination on case by case basis for very sick children.

- **Stool**

Two samples will be collected at the hospital on two consecutive days (D0 and D1). The first one as soon as the child is able to produce stool and the 2nd one of the next day. The nurse will explain parent of children how to collect at least 2 large teaspoons of stool specimen into a clean, dry leak-proof container. Sample will be kept in a fridge until shipment to the laboratory and will be shipped in a cool box.

- **Blood samples**

Blood collection will be performed for HIV testing at inclusion only if HIV status is unknown, following routine procedure.

8.2. Laboratory assessment

8.2.1. Xpert MTB/RIF Ultra

Ultra testing will be done at the trial laboratory on standard GeneXpert platforms for the two respiratory samples and the two stools samples.

The Ultra assay will be carried out according to the manufacturers' guidelines and will be defined as positive, negative or indeterminate based on the manufacturers' recommended criteria.

Results will be interpreted as follows:

- In case of positive result for the presence of MTB (including "trace call" positive result) on any respiratory sample or stool sample processed with the sucrose flotation method, the global result will be given as "MTB detected".
- In case of negativity of Ultra performed on the two respiratory samples and the two stool samples processed with the sucrose flotation method, the global result will be given as "MTB not detected".
- In case of an invalid or error result for MTB detection, the test will be repeated if sample volumes allow for it.
- In case of rifampicin resistance detected on one or more sample, the result will be given as "MTB detected, rifampicin resistance detected". Otherwise the result will be given as "MTB detected, rifampicin resistance not detected (or indeterminate)". Children with rifampicin resistance detected by Ultra will have culture and phenotypic DST performed on leftovers from stool and respiratory samples (with additional samples taken if needed), and will be started on empirical MDR-TB treatment according to national guidelines.

Xpert Ultra results will be communicated to the study nurse by message by the laboratory technician and to the treating physician as soon as available. If positive, TB treatment will be initiated immediately.

For stools, only Ultra results from stools processed with the sucrose flotation method will be communicated to the clinician as this is the only method published and validated in a clinical study. Results from Ultra assay on stools processed with the SPK, STEP method and sucrose flotation optimized method will not be disclosed.

8.2.2. Mycobacterial culture

Culture will be done at the trial laboratory on two respiratory samples (ES or GA). Mycobacterial culture will be done in liquid medium (MGIT) by an automated method (BACTEC 960), and on solid media (Lowenstein Jensen) if available. Identification of mycobacteria will be done by the molecular method Gen-Probe, or Niacin test, or MPT64 antigen test depending on availability at the laboratory level. Detection of TB drug

resistance will be performed using first-line drug sensibility testing (DST) on liquid media (MGIT) or solid media or line probe assays (LPA).

8.2.3. HIV testing

HIV serology will be performed at inclusion if not available in the patient medical chart, according to national routine practices. HIV testing in children aged less than 18 months will be based on an HIV RNA or DNA nucleic acid amplification tests (PCR).

8.2.4. Laboratory quality control

Internal Quality Control (QC) will be routinely performed for Ultra testing, including calibration tests and procedures provided by the manufacturer. Internal QC results and logs will be available for monitoring.

Procedures for laboratory quality assurance will be detailed in study-specific SOPs.

8.3. Radiological assessment

A chest radiograph anteroposterior and lateral view in children below 5 years old will be performed using standard analogue X-ray machines at visits D0 and M2.

9. STUDY VIGILANCE

9.1. Definitions

➢ **Adverse events**

An “adverse event” (AE) is defined as any unfavourable, expected or unexpected sign (clinical or biological) occurring during the study in a human subject participating in the research, whether or not considered related to treatment or procedures or to participation in the study.

➢ **Serious adverse events**

A “serious adverse event” (SAE) (ICH-E6 step 4. 1996) refers to any untoward medical occurrence that:

- Results in death;
- Is life-threatening (meaning that the subject was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- Requires hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect.
- Is an "important medical event" (medical events, based upon appropriate medical judgment, which may jeopardize the subject or may require medical or surgical intervention to prevent one of the above characteristics/consequences). Examples: allergic bronchospasm requiring intensive treatment at an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization.

➢ **New fact**

A new fact is defined as any safety data that could significantly modify the evaluation of the benefit/risk evaluation of the research or the study product, likely to affect the safety of participants or that could modify the study product administration, the study documentation or the conduct of the study, or to suspend or interrupt or modify the protocol or similar studies.

➢ **Severity**

The severity of an AE will be graded using the “Division of AIDS table for grading the severity of adult and paediatric adverse events” (Version 2.1 – July 2017) as included in the SOPs [69].

➢ **Causality**

"Causality" refers to causal relationship between a specific AE, the study intervention and any other concomitant intervention/medication.

9.2. Expected adverse events related to the study intervention

Both GA and sputum collection are components of routine practice in the two hospitals participating in the study. They are recommended by WHO for paediatric presumptive TB sample collection. No AE is expected from sputum collection but vomiting can occur with GA.

No AEs are expected from stool sample collection.

9.3. Reporting of adverse events

There will be no systematic reporting of other AEs in the study.

9.4. Notification of serious adverse events

In this diagnostic study without investigational medicinal product, and with very low expected risk of AEs linked to the intervention, there will be no systematic notification of SAEs to the sponsor at the exception of:

- Death;
- Life-threatening AEs, excluding asymptomatic biological AEs of grade 4

For children participating in both TB-Speed HIV/SAM studies and the TB-Speed stool-processing study, only one SAE will be notified in one SAE report with the mention that the child is enrolled in both studies.

9.5. Responsibilities of the investigators

The investigators are responsible for:

- Grading the severity of AEs occurring from GA collection reported by study nurses as severe or potentially life-threatening
- Reporting SAEs, to the sponsor and to the appropriate country authorities.
- Assessing the causality of all SAE in relation to the study intervention and to concomitant intervention/medication.

The assessment on expectedness will be done by the sponsor.

SAEs, as defined above, should be reported as soon as they are known to the country CTU according to the last updated SOP. A specific "SAE report form" will be used. SAEs will be reported immediately by the country CTU to the INSERM Pharmacovigilance Department according to appropriate SOPs. If needed, queries on SAEs will be sent to the investigators by the INSERM Pharmacovigilance Department representative.

All SAEs must be reported if it they occur in a participant:

- from the date of signature of the informed consent to the study;
- during the follow-up of the participant scheduled by the study;
- until 4 weeks after the end of follow-up when it still could be related to the study intervention.

9.6. Responsibilities of the sponsor

9.6.1. SAE Recording and assessment

The sponsor shall keep detailed records of all SAEs which are reported to him by investigators.

The sponsor is responsible for the assessment of the causality of the SAE. In the absence of information on causality from the reporting investigator, the sponsor should consult the reporting investigator and encourage him to express an opinion on this aspect. The causality assessment given by the investigator should not be downgraded by the sponsor. If the sponsor disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor should be provided in the report to the National Competent Authority.

All SAE for which the investigator or the sponsor considers that a causal relationship is a reasonable possibility are considered as suspected Serious Adverse Reaction (SAR).

The expectedness of the SAR shall be determined by the sponsor. The sponsor assesses if the SAE is expected or not using information described in the protocol (section 9.2), especially concerning, acts and methods performed for the research. An unexpected adverse reaction is an adverse reaction, the nature, the outcome or severity of which is not consistent with this information.

9.6.2. New fact reporting

When a new event is likely to affect the safety of participants, the sponsor and the investigator will take appropriate urgent safety measures to protect participants against any immediate hazard.

The sponsor will inform without delay the Competent Regulatory Authorities of safety data that may be relevant in terms of subject safety, or safety issues which might alter the current benefit-risk assessment of the study.

The INSERM Pharmacovigilance department shall transmit a written report to the Competent Regulatory Authorities and concerned Ethic Committees.

10. DATA COLLECTION AND PROCESSING

10.1. Description of data collected

10.1.1. Individual patient data

Once enrolled in the study, the following data will be collected for each patient by study nurses or will be extracted from databases of the TB-Speed SAM and HIV studies for children included in these two studies.

- Individual identifiers: month and year of birth, sex
- Co-enrolment in other studies
- Medical history
- Anthropometric and clinical data: weight, height, vital signs, symptoms, and AEs
- Radiological data: digital images and interpretation
- Dates of each specimen collection
- Laboratory data: HIV testing result, Ultra results, culture results

For each new patient included in the study, an anonymised individual identification code will be attributed and used as the only patient identifier in the REDCap database.

Radiological data will also be collected as digital imaging and communications files (.dicom files) and transferred to a FTPS in a server hosted at UBx. CXR interpretation will be directly reported in the patient's CRF using standardized forms developed as part of the capacity building component of the study.

Data transfer for dicom and gxx files is detailed in Chapter 10.3.6.

10.2. Definition of source data

Source data will be available to document the existence of patients enrolled in the study and should substantiate the integrity of the data collected. It will include the original documents relating to the study, the medical treatments and medical history of the patient.

The following information should be collected from source documents, where possible:

- Patient's demographic data (month and year of birth, sex)
- Details related to the study eligibility criteria
- Date of signing informed consent form
- Medical history and physical examination details
- Laboratory results

For the purpose of the study, specific forms may be developed for source data collection.

10.3. Electronic data entry

The eCRF system, the methods to ensure restricted access to the database, and the data management procedures, including the procedures to check completeness, accuracy, quality and validity of the data, will be described in specific study SOPs in accordance with good clinical, scientific and data management principles.

10.3.1. eCRF

Real time data collection is needed for optimised monitoring of data entry. No paper CRFs will be used; patient data collected at inclusion and follow-up visits will be recorded directly into an electronic CRF (eCRF) by study nurses, mostly through single data entry.

The TB-Speed data management system will be based on the electronic data capture application REDCap (Research Electronic Data Capture; https://www.project-redcap.org/). REDCap is an online tool for eCRF development, allowing data input from anywhere in the world over a secure connection with authentication and data logging.

Design and conception of the eCRF will be done by the international study manager in close collaboration with the international data manager and international clinical research assistant.

10.3.2. Data hosting

The REDCap MySQL database server will be hosted by the international CTU at the University of Bordeaux (UBx), France. REDCap is a free, secure web application for building and managing online surveys and databases, it's geared to support online and offline data capture.

Developed by Vanderbilt University, REDcap complies with internationally recognized standards including the Health Insurance Portability and Accountability Act (HIPAA, 1996), the United States legislation providing data privacy and security provisions for safeguarding medical information, as well as part 11 of Title 21 of the Code of Federal Regulations (CFR) that establishes the United States Food and Drug Administration (FDA) regulations on electronic records and electronic signatures.

10.3.3. Data security

The eCRF will be accessible 24 hours a day by secure authentication to a restricted users group. The connection will be authenticated by a user ID, password and digital certificate enabling data encryption during transfer and storage to the central server. Access levels will be granted and managed by the international data manager (international CTU).

The server hosted at UBx will be backed up every weekend on a hard drive, and send to bands (rotation frequency of the bands will be every five weeks). The database will be backed up incrementally on a hard drive every working day.

10.3.4. Data entry

Field-based users will be able to access REDCap either through a classical Internet-connected tablet or computer, or through the REDCap mobile App application.

The mobile App also enables offline data entry through a tablet or an Android mobile phone. In such a case, the tablet or mobile phone will be brought by the CRA to the country CTU and further synchronized with the central database once connected to the Internet (Figure 2).

Tablets will be purchased locally by country CTUs. Configuration of tablets will be managed by the international data manager.

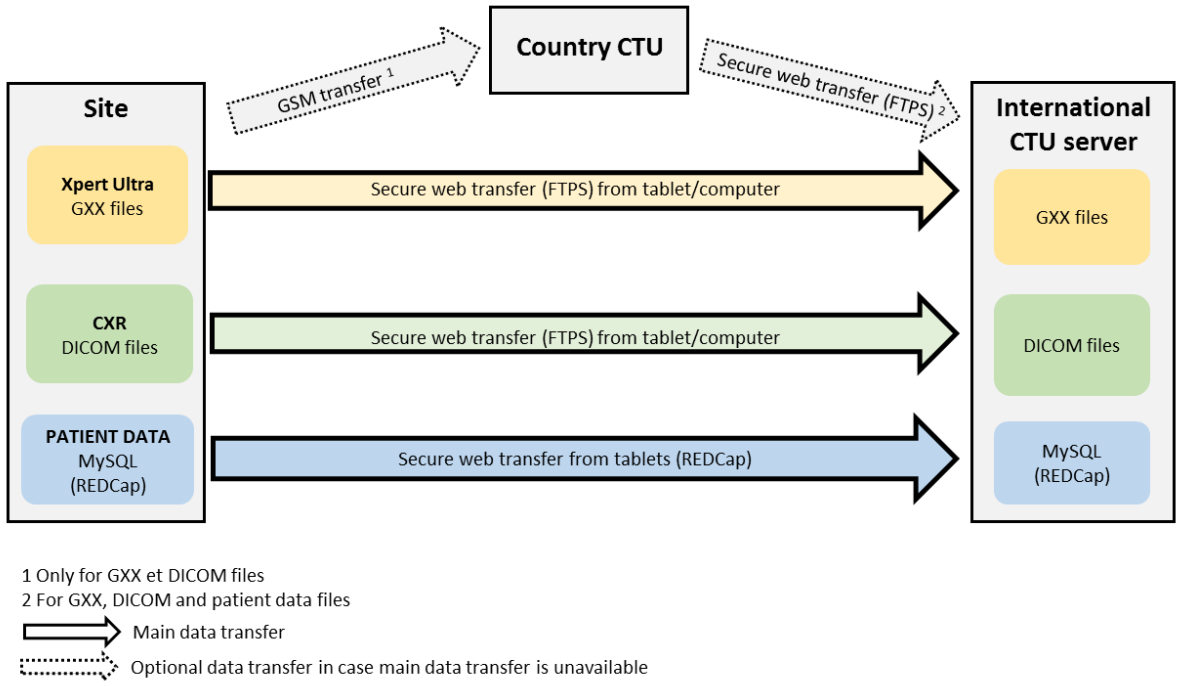

Figure 7. Secure data flow

Project Managers from country CTUs will be in charge of training relevant study staff for data collection and for issuing electronic data queries for quality control. The investigator is responsible for ensuring that all sections in the eCRF are completed correctly and that entries can be verified against source data. If the investigator authorizes other staff members to make entries into the eCRF, the names, positions, and signatures will be documented in writing. The eCRF will be completed during/after each study visit. Any person entering data in the eCRF will be trained beforehand and appointed to do this task.

10.3.5. Data coding

The Anatomical Therapeutic Chemical (ATC) system will be used for drug classification and coding. As part of safety monitoring, AEs will be coded using the Medical Dictionary for Drug Regulatory Affairs (MedDRA, version 17.1). Coding will be performed by country CTUs based at TB-Speed consortium members institutions.

10.3.6. Data transfer

Individual patient data will be transferred from tablets to the server located at UBx using a secure file transfer protocol (ftps) with individual authentication and data logging.

CXRs (dicom files) and Ultra test result files exported from GeneXpert (.gxx files) will be transferred to the international CTU central server using a secure web transfer (ftps). In case implementing sites experience web access issues, transfer of CXRs and Ultra result files will be done 1) by GSM with a tablet (equipped with a SIM card) from the implementing sites to the country CTU, and 2) from the country CTU to the international CTU central server via a secure web transfer (ftps). The study ID will be the only identifier in the electronic study database.

10.4. Description of the data verification, validation and processing (data management)

A data management SOP will be established and validated by the study coordination team at UBx. Verification of data completeness and consistency will be performed for all key data as well as a list of additional data defined in the DM SOP.

A data management system (DMS) will be developed at UBx within the REDCap database to enable generation of standardized lists of data management queries at country level. Queries will be programmed for data completeness, integrity and consistency. They will be run on at least a monthly basis at the country level. Data management checks will be implemented at the central level on a monthly basis. Furthermore, centralized correction queries will be sent by the international coordinating CTU to the country CTU. The investigator, co-investigators, head of laboratory must allow access to relevant hospital, laboratory or clinical records, to confirm their consistency with the CRF entries. All research staff working in the study, including study nurses, national CTU team (PMs, CRAs), PIs, international coordination team (CRA, Trial Manager, Coordinating

Investigators) will sign a confidentiality agreement with regards to access to individual patient data and medical records.

Central statistical monitoring will also be implemented by the international coordinating CTU to look at variables for which distributions differ from the rest of the observed data at the country, site or patient level. Its purpose is to highlight systematic (non-random) faults in data collection and study implementation procedures and to guide targeted monitoring. Variables subject to statistical monitoring are specified in the monitoring plan. Comparison of distributions is made by statistical tests or models.

Before database freezing, a final data review will be conducted by the international data manager and remaining issues will be adjudicated.

10.5. Length of data retention, archiving conditions and management

In addition to the source medical records, radiological data will be collected as digital imaging and communications files (.dicom files). CXR interpretation will be directly reported in the patient's CRF using standardized forms developed as part of the capacity building component of the TB-Speed study.

In the same way, Ultra test result files (.gxx files) will be extracted directly from the GeneXpert software and mycobacterial source data will be obtained from the laboratories registers and logs.

Data transfer for dicom and gxx files is detailed in Chapter 10.3.6.

All data will be stored in a server hosted by the CREDIM (*Centre de Recherche et Développement en Informatique Médicale*) at UBx.

The server is located in a secure computer room. The network is protected by uninterrupted power supply firewalls and up-to-date virus and malware scanning software. Data backups are performed regularly. Reading, entry, modification or deletion of data will be granted via the standard authentication and access-control features.

Medical records will be stored in the clinical sites as per standard practices. Electronic data and files will be maintained on password-protected computers. Essential study documents will be retained at the coordinating centre for 15 years.

No displacement or destruction of data will be done without the agreement of the sponsor. At the end of the regulatory archiving period, the sponsor will be consulted for destruction.

10.6. Study documents archiving conditions and management

Essential documents and study records will be kept secured of a minimum of 15 years after study completion, under the responsibility of each country investigator, the international CTU, and the sponsor.

Study documents will be made available online to investigators on a secured website. The international coordinating CTU will be responsible for routinely updating global documentation on the study website. Country CTUs will be responsible for routinely updating national documentation on the study website.

Investigators will ensure that study records are not disposed of or removed from the study sites or the country CTU without prior notification and approval from the sponsor or his representative.

Each investigator will keep a hard copy of original documents whenever those are manually signed or generated. This includes, at site level, medical records (source documents) and study ID assignment log which are subjected to professional secrecy and confidentiality, and task delegation lists.

Data, documents, reports and SOPs should be available to be audited or inspected at any time.

11. STATISTICAL DATA ANALYSIS

11.1. Statistical analysis manager

The statistical analysis manager will be the study statistician, based at UBx.

The statistical analysis plan will be written by the study statistician and validated by the coordinating investigators.

11.2. Description of the statistical analysis plan

11.2.1. Analysis of the primary endpoint

Sensitivity of the 4 Ultra on stool using TB culture reference standard (LJ and MGIT) in respiratory sample: point estimate and 95% CIs.

Specificity of the 4 Ultra on stool using TB culture reference standard (LJ and MGIT) in respiratory sample: point estimate and 95% CIs.

We will use as TB culture reference standard:

- Positive: at least one positive MTB culture result in one of the two respiratory samples
- Negative: negative culture results from 2 samples without any positive result

An intention-to-diagnose analysis will be performed at:

- Sample level (denominator: total number of samples collected): 1 stool vs the TB culture reference standard
- Patient level (denominator total number of cases with at least one stool sample collected): at least 1 of the two stools vs the TB culture reference standard.

A patient will be classified as stool positive, when they have at least one positive stool Ultra result (for example the first stool may not have any positive Ultra results, but the second stool could have 1 positive Ultra result, in which case the patient would be considered stool positive).

Invalid Ultra results, contaminated and NTM culture results will be considered negative.

11.2.2. Analysis of secondary endpoints

- Per-protocol analysis of sensitivities and specificities of Ultra on stool using TB culture reference standard (LJ ans MGIT) in respiratory sample, excluding invalid Ultra results, contaminated and NTM culture results from analysis. Regarding contamination, since two culture methods will be used per respiratory sample (LJ and MGIT), to be excluded both culture results per sample will need to be contaminated.
- Head to head comparison of the sensitivities and specificities between the different stool processing methods using culture reference standard and McNemar test for matched data:
  - Sucrose flotation classic method vs Sucrose Flotation optimised method
  - Sucrose flotation classic method vs SPK method
  - Sucrose flotation classic method vs STEP method
  - Sucrose flotation optimised method vs SPK method
  - Sucrose flotation optimised method vs STEP method
  - SPK method vs STEP method
- Head to head comparison of the sensitivities and specificities between the different stool processing methods using the TB composite reference standard as defined by Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children, and McNemar test for matched data:
  - Sucrose flotation classic method vs Sucrose Flotation optimised method
  - Sucrose flotation classic method vs SPK method
  - Sucrose flotation classic method vs STEP method
  - Sucrose flotation optimised method vs SPK method
  - Sucrose flotation optimised method vs STEP method
  - SPK method vs STEP method
- Description of results for rifampicin resistance using stool Ultra, respiratory Ultra, LPA and DST
- Agreement of the different stool sample processing methods for Ultra detection head to head using Kappa analysis.
- Comparison of the proportion of rifampicin resistance indeterminate Ultra test results between different stool processing methods using McNemar test for matched data.
- Comparison of the proportion of invalid Ultra test results between different stool processing methods using McNemar test for matched data.
- Comparison of the proportion of Ultra results “trace” between the methods using McNemar test for matched data.
- Proportion of very low, low, medium and high Xpert Ultra results by different methods

- Change in sensitivity and specificity at patient level by using 2 stools vs using just the 1st stool specimen for each processing method.
- Stratification of children's characteristics and laboratory results by age groups (<2, 2 to 5, 5 to 10, and >10 years))
- Proportion of children successfully providing a stool sample
- Relative gain of Ultra MTB detection by using ratio of TB detected using 2 stools vs using just the 1st stool specimen for each processing method.
- Description of the results of the ease of use questionnaire for feasibility by technician and time of assessment (beginning, half way and end of the study).

11.2.3. Intermediate analyses

One intermediate analysis on the primary endpoint and some of the secondary endpoints (agreement of Ultra results between different models) will be performed after results from prospective cohort are available and presented to the study SAB for review.

12. COMMUNICATION AND PUBLICATION POLICY

12.1. Findings publication procedure

All data collected during this research are the property of the study sponsor and cannot be communicated, under any circumstances, to a third party without the written consent of the sponsor.

The results will be published after final analysis in the form of scientific articles in peer-reviewed journals, or presented at national and international conferences. Any publication or communication (oral or written) is decided by mutual agreement between the coordinating investigators, the SAB and the sponsor, and will respect the international recommendations: " Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals" (http://www.icmje.org/recommendations).

All publications must follow the rules contained in the publication charter defined by the TB-Speed project as part of the project communication plan. The mention of the origin of the funding, the authorizations of the competent authorities, and the consent of the participants must appear in the acknowledgments according to the model suggested below:

" / Ethics statement / * / This study is part of clinical trial **** CXX-XX ** sponsored by Inserm. It was granted approval by local Ethics Committee or "Committee for the Protection of Persons" on --- **** DATE ** ---, and registered in a public trials registry (**** CT XXXX **)./ Funded by the Unitaid /. All study participants gave their informed written consent to participation, in line with ethical guidelines.*

12.2. Procedure for writing up the final report

The international CTU will establish the final report of the study as well as summary report within a year after the end date of the study, i.e. the last visit of the last patient. The report and its summary are established according to ICH recommendations (International Conference for Harmonisation – ICH Topic E3 – Structure and Content of Clinical Study Reports CPMP/ICH/137/95. Accessible at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E3/E3_Guideline.pdf). The report will be approved by the SAB of the TB-Speed project.

Within one year after the end of the study, the sponsor or its representative will release to the ethical and regulatory authority of each country involved in the study the final study report and/or summary including the results of the study and the scientific publications or communications related to these results.

12.3. Procedure for informing the health care provider and study participants of the overall research findings

The final study results will be presented to the investigators, national authorities, study site health care providers and families taking part in the study of each participating country. A series of documents (written detailed report, and short summary) will be released to help investigators, national authorities, health care providers and families to understand the results of the study. Families taking part in the study will be invited to attend a meeting during which the results will be presented and explained orally in lay language.

12.4. Procedure for informing the participants of their health data during and after the research

Parent(s)/guardian(s) are informed of their right during or after the research, to be given information concerning their child's health held by the investigator or, where appropriate, the qualified person who represents it.

During the study, any clinically significant abnormality detected in the examination or test results will be communicated the parent(s)/guardian(s)) and the physician selected by them unless they have objected.

12.5. Press communication procedure

A press release in collaboration with the funders and the sponsor will be developed by the TB-Speed communication group to inform the press about the study results.

13. STUDY OVERSIGHT

13.1. Output Steering Committee

The study (Output) Steering Committee (OSC) is the operational team that will undertake the day-to-day decisions related to study implementation in each country, based on the model applied in all clinical studies currently managed by the IDLIC team at UBx.

The OSC will consist of the coordinating investigators, country principal investigator and co-principal investigator, country project manager, the international study manager, the laboratory coordinator, the international study CRA and data manager, and any relevant participants invited to discuss specific issues.

The OSC will be in charge of the reporting and formulation of proposals for the Executive Committee regarding work-plan and budget reallocation and execution of the decisions taken by the PCC.

Members of OSC will interact once a month. Every 6 months, the OSC meeting will be opened to Protocol Writing Committee external members for a review of study progresses and results (not analysed by study arm).

13.2. Scientific Advisory Board

The TB-Speed Scientific Advisory Board (SAB) is an expert consultative committee providing scientific advice to the project management teams. It gives input on the relevance and scientific validity of the project design and implementation, monitors progress and ensure scientific and ethical integrity of the project.

➢ **Role**

The SAB members will bring their individual expertise to review and advice on the following:

- The relevance of the project objectives within the context of the paediatric TB research landscape;
- The appropriateness of designs and methods of the proposed studies (outputs) to the research questions;
- The scientific strength, safety and feasibility to meet the stated objectives of the project;
- The complementarity of the project with other ongoing or planned external trials;
- The continued relevance of the project in light of new scientific and/or clinical developments;
- The final Research Protocols, including informed consent forms, prior to their submission to relevant ethics committees;
- The project progress upon receiving of progress reports, including interim and final statistical analyses;
- Any important scientific decisions or changes made during the course of the project (e.g., major protocol amendment) or based on the reports from IDMC;
- Any publication ahead of submission to international peer-reviewed scientific journals;
- Confidential scientific reports transferred to WHO for consideration and inclusion of outcomes into development and update of WHO normative guidance.

➢ **Composition and appointment**

SAB members are initially appointed on an invitation basis from a list of nominees developed by the TB Speed Executive Committee.

The SAB is led by a Chairperson who is independent from the project consortium and includes independent external experts, as well as members of the protocol development teams. The committee will consist of at least 10 but no more than 12 members bringing their individual knowledge, experience and expertise. The experts will include at least two paediatric TB experts, one expert in operational research on TB diagnostic, one mycobacteriologist, one paediatric HIV expert, one paediatric pulmonologist, one health economist, one representative of NTP from a high TB burden country), and one representative from the community.

Members of the SAB will be required to meet at least once a year. Additional bi-annual meetings may be considered as needed for the project.

14. CONFIDENTIALITY

14.1. Procedure for respecting the confidentiality of participants

Each case or control will be assigned a unique study identification code. Every effort will be made to have this code as the only patient identifier on any document, record, report or laboratory specimen related to the study. This will be the only identifier in the electronic study database, including gxx and dicom files, as well as for samples in the biobank.

The study ID assignment log (only in paper form) will be kept shut-away on site under the responsibility of the investigator. Direct personal identifiers (including names, dates, demographic and contact information) will only be made available to those whose job within the operational activities of the study makes having such information absolutely essential, at the discretion of the investigator.

All documents (such as the signed consent forms) containing patients' names will be kept in a locked cabinet under the responsibility of the site's principal investigator.

14.2. Procedure for keeping the necessary study data confidential

Individual medical information obtained as result of this study will be confidential. Study team members are subject to the obligation of professional secrecy. Individual patient data will be made available upon request to the study investigators, physicians in charge of patients' care, representatives of the sponsor, and representatives of the ethical and regulatory health authorities in case of external audit or inspection. Disclosure to other third parties is strictly prohibited. Parent(s)/guardian(s)'s consent for this is obtained as part of the consent process.

The data recorded during this study will be subjected to computer processing on behalf of the Sponsor. The sponsor will declare the database to the French CNIL, in compliance with the provisions of the French Law No. 78-17, dated January 6, 1978, and amended by Law No. 2004-80, dated August 8, 2004.

15. PROTECTING RESEARCH PARTICIPANTS

15.1. Ethical justification of the protocol

15.1.1. Risks

Children with presumptive TB have a high risk of death, especially if they have comorbidities like HIV infection or malnutrition, which is independent to the study intervention.

Risks due to para-clinical investigations are well known and will be explained to the participants. The potential risks of respiratory sample collection and blood draw will be limited by insuring they are performed by trained nurses with appropriate supplies and standardized procedures detailed in the study SOP. The risk due to chest radiography is exposure to radiations, however, two chest-X-rays done within 2 months represent a low exposure to radiations.

Blood draw from a vein may induce discomfort at the site of puncture, possible bruising and swelling around the puncture site, rarely an infection, and, uncommonly, faintness from the procedure.

No new drugs will be tested during this study but parent/guardian will nevertheless be informed of possible TB drugs and antiretroviral side effects and other possible concomitant AEs (paradoxical reactions and IRIS).

Risks specific to study participation include the potential for breach of confidentiality. To minimize this risk, as well as stigma and emotional risks associated with TB and HIV diagnosis, testing will be confidential and performed with pre and post-test counselling. HIV-infected children identified through the study will be referred to ARV treatment programs.

15.1.2. Benefits

This study is providing the following opportunity for eligible children:

- an improved diagnosis of TB, especially by optimizing bacteriological specimen collection and processing for young children with the systematic use of two gastric aspirates and one stool;

This factor will positively impact the local NTPs by improving case detection rates as well as TB outcomes. It is also hoped that lessons learned from this study will help to improve management of TB suspicion for children from other high TB burden countries.

15.2. Regulatory provisions

The investigators undertake to conduct the research in compliance with the protocol and in accordance with:

- the French regulations in force, including provisions relating to research involving the human person provided for in Articles L 1121-1 *et seq.* of the Public Health Code, the Bioethics Laws, the Data Protection Act;
- Participating countries' laws and regulations relating to biomedical research on human participants;
- The Declaration of Helsinki (approved by the World Health Association on June 1964, lastly amended at the 64th WMA General Assembly, Fortaleza, October 2013)
- The Good Clinical Practice (ICH Harmonized Tripartite Guidelines for Good Clinical Practice E6 step 4 - 1996) and Good Clinical Laboratory Practice (GCLP. World Health Organization on behalf of the Special Programme for Research and Training in Tropical Diseases, 2009).
- The 2017 revision of the ANRS Ethics Charter for research in Developing Countries.

This trial will be registered at the ClinicalTrials.gov registry and The Pan African Clinical Trials Registry (PACTR).

15.3. Ethical approvals

Before carrying out the research, the protocol, the information sheet, the consent form and any other relevant documents will be submitted to the approbation of each implementing country's National Ethics Committee (Uganda National Council for Science and Technology in Uganda and National Research Ethics Authority (NREA) in Zambia), to relevant Institutional Review Boards (Research Ethics Committee of the MUST in Uganda and University of Zambia Biomedical Research Ethics Committee), to the WHO Ethical Review Board, and to the Inserm Ethics Evaluation Committee.

The study will be implemented in each country only once the ethical clearance document of the Ministry of Health or relevant Health Authority is received. The research can only start when Inserm has been informed of the favourable opinion delivered by the different ERCs concerning the submitted protocol. This notice will include the title and protocol number assigned by the proponent, the documents reviewed, as well as the date of review and the list of ERC members who participated.

Once approved and authorized, the final version of the protocol will be signed by the coordinating investigators and the sponsor. All principal investigators will sign the protocol as a commitment to conduct the study according to the protocol, the declaration of Helsinki, the Good Clinical Practice and adhere to the procedures described in the SOPs.

The proponent will inform the different ERCs of any subsequent amendments and any serious or unexpected AEs and developments that occur during the course of the research that would likely affect the safety of those who are suitable for the research.

15.4. Additional approvals

The study will be approved by health authorities of the implementing countries, namely the National Tuberculosis Programs as implementing partners, and if relevant the Ministry of Health at a broader level.

In Zambia, approval to conduct health research projects requires to obtain an administrative clearance issued by the Ministry of Public Health.

15.5. Data protection

The data recorded during this study will be subject to computer processing on behalf of the Sponsor. The protocol will be submitted for approval to the French data protection authority (CNIL). It will also be conducted following the African Union Convention on Cyber Security and Personal Data Protection adopted on 27 June 2014.

15.6. Insurance

Inserm, which is sponsoring this study, accepts the legal responsibility in the name of the investigator for any direct or indirect harm caused to patients by the methods used in this research.

Inserm has taken out a civil liability insurance for the entire duration of the study under number XXXX [insert here the national insurance certificate number], in accordance with the French legal provisions and regulations on research.
The certificate of insurance relating to this protocol constitutes Appendix 11.

15.7. Participants amenities

Study investigators will ensure that each subject receives the reimbursement of transportation fees to the hospital and receive free of charge medical exams, tests, and medications related to the study.

If not covered by the national health system, hospital stays will be free of charge, covered by the TB-Speed project, whenever prescribed or approved by the country study investigators, within the limits of the available budget. Patients will not incur additional costs by participating in the study.

16. QUALITY ASSURANCE AND MONITORING

16.1. Description of the quality assurance system

The role of quality assurance is to ensure the safety of individuals who are amenable to research involving the human person and to ensure the credibility of data derived from such research and their recognition by the medical and scientific community.

Research monitoring will be conducted according to the Good Clinical Practices (ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996) to guarantee the quality of the research and safeguard the health and the rights of the patient. The site's principal investigator is above all the guarantor of the quality of the study progress.

The monitoring plan is established by the international coordinating CTU with the sponsor and the coordinating investigators before the start of the study. Key data for verification against source data shall be detailed in the monitoring plan. Procedures for monitoring will be detailed in study-specific SOPs developed by the Mereva team at the international CTU.

16.2. Monitoring (quality control of the study)

16.2.1. General organization

Country CTUs are in charge of the monitoring of study process and data collected in the country. The international coordinating CTU, based at the UBx IDLIC/Mereva team, coordinates and supervises monitoring performed by country CTUs and performs targeted monitoring.

16.2.2. Monitoring by the country CTU

. During these visits, the country CTU will be in charge of the following, according to the monitoring plan:

- Check adherence to the protocol, SOPs and Good Clinical Practice, including patient's screening, eligibility criteria, informed consent;
- establish and maintain the investigator's TMF up-to-date;
- check the completeness and the accuracy of patient key data on the CRF (source data verification) not for all patients (percentage defined in monitoring plan);
- verify that confidentiality of data is fully respected;
- verify SAEs reporting, documentation and follow-up, and send the forms to the sponsor's pharmacovigilance and to the international coordinating CTU;
- follow-up with study sites staff centralized correction requests sent by the national and international coordinating CTU.

After each visit a report will be written by the country CRA.

The country laboratory coordinator will regularly visit each laboratory during all study period. During these visits, the laboratory coordinator will be in charge of the following according to the laboratory procedure:

- check the quality management of samples
- ensure the quality controls and quality management for laboratory assessments are implemented.

Furthermore, the country CRA and the laboratory coordinator will also hold regular meetings with the study staff at each sites to discuss any process and country CTUs deem problematic, as well as practical and logistic issues in study implementation and patient or sample management.

16.2.3. Monitoring by the international coordinating CTU

The Opening process will be done according to the opening site procedure provided by the international coordinating CTU. Only upon completion of equipment, training, ethical and regulatory approvals (including civil liability insurance) will a site be authorized to start enrolling patients.

A member of the international coordinating CTU will visit each study site at least once during the study period. The purpose of these visits will be to review with the country CTU advances and issues with the local monitoring and data management process, as well as perform a targeted/random monitoring of a limited number of files. In the context of the Covid pandemic, due to travel restrictions, remote site monitoring visits might be conducted by the international CTU. Where needed, pseudonymized source documents will be forwarded by the country CRA through a ftps for monitoring purpose only; source documents will be destroyed afterwards.

The following aspects will be reviewed according to the monitoring plan:

- Informed consent for a subset of patients (percentage define in monitoring plan)
- Compliance with the study protocol, SOPs and Good Clinical Practices, including eligibility criteria and reporting of SAEs
- Consistency with the source documents for key data for a subset of patients (percentage define in monitoring plan)
- Management of samples
- Laboratory quality controls

Each visit will be recorded in a written monitoring report, sent to the coordinating investigator, the clinical and country project managers, the country principal investigators and the sponsor.

The country CTU will also be monitored on specific aspects such as the availability and maintenance of an updated TMF.

A closing visit will be carried out at the end of the study by the national CTU according to the site closing procedure provided by the international CTU.

16.2.4. Direct access to source data

Participating investigators should agree to allow study-related monitoring, including audits, ethics committee review and regulatory inspections by providing direct access to source data/documents as required. Patients' agreement for this is obtained as part of the informed consent process.

16.3. Audits/inspections

All documents and data relating to the research should be made available at any time to the sponsor as well as ethical and regulatory health authorities in case of external audit or inspection. Those should be carried out in the respect of the professional secrecy and without being able to be opposed the medical confidentiality. Disclosure to other third parties is strictly prohibited.

17. SUBSTANTIAL AMENDEMENTS TO THE PROTOCOL

Any change or addition to this protocol requires a written protocol amendment to be approved by each country's National Ethics Committee, the WHO Ethical Review Board, and signed by the coordinating investigators, the principal investigators and the Inserm before implementation.

These requirements for approval should in no way prevent any immediate action from being taken by the investigators or by the sponsor in the interests of preserving the safety of all study participants. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented by him/her for safety

reasons, the Inserm should be notified and each country's National Ethics Committee should be informed within 10 working days.

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19. APPENDICES

19.1. APPENDIX 1: study summary

Clinical trial ID number:

Title: Evaluation of four stool processing methods combined with Xpert MTB/Rif Ultra for diagnosis of intrathoracic paediatric TB

Short title: TB-Speed Stool processing

Coordinating investigators: Dr Olivier Marcy, Dr Maryline Bonnet, Dr Eric Wobudeya

Participating countries: Uganda, Zambia

Primary Objective: To determine the diagnostic accuracy of Xpert MTB/Rif Ultra performed on stools processed using four different sample processing methods (standard and optimized sucrose-flotation; One Step and SPK methods) in children with presumptive TB, using an intention-to-diagnose analysis.

Secondary Objectives:

- Per-protocol analysis of diagnostic accuracy of Ultral on stool using TB culture reference standard
- To compare Ultra performed on stool with the four sample processing methods in terms of:
  - Diagnostic accuracy for culture confirmed TB (culture reference standard from respiratory samples)
  - Diagnostic accuracy for confirmed and unconfirmed TB (TB composite reference standard
  - Semi-quantitative results
  - Proportion of “trace call” results
  - Proportion of invalid results
  - Proportion of Indeterminate results for rifampicin resistance
- To describe levels of agreement between results of Ultra performed on stools processed using the four methods
- Stratification of characteristics and laboratory results by age
- Proportion of children successfully providing a stool sample
- To assess the relative gain (increase in detection) of a second Ultra performed on second stool sample
- To assess feasibility of the stool processing methods

Primary endpoint:

Sensitivity and specificity of Ultra on stool using TB culture reference standard (LJ and MGIT) in two respiratory samples (sputum, gastric aspirate).
Positive: at least one positive MTB culture result in one of the two samples
Negative: negative culture results from 2 samples without any positive result
An intention-to-diagnose analysis will be used for the primary end point. Invalid Ultra results and contaminated culture results will be classified as negative.

Secondary endpoints:

- Per-protocol analysis of sensitivities and specificities of Ultra on stool using TB culture reference standard (LJ ans MGIT) in respiratory sample, excluding invalid Ultral results and contaminated culture results from analysis.
- Head-to-head comparisons
  - Sensitivities and specificities of each sampling method using culture reference standard.
  - Sensitivities and specificities of each sampling method using the TB composite reference standard as defined by the Expert Committee.
- Head-to-head kappa coefficients.
- Proportions of
  - Ultra “trace” results in stools out of the number of stools tested with Ultra
  - Ultra semi-quantitative results “very low”; “low”; “medium” and “high” in stool.
  - Invalid Ultra results from stool out of the number of stools tested with Ultra
  - Rifampicin resistant results on Ultra (stool and respiratory), LPA and DST
- Stratification of characteristics and laboratory results by age groups ($\leq$2 years and > 2 years)
- Proportion of children successfully providing a stool sample
- Relative gain of the 2nd stool sample as compared to the 1st one as measured by the number of positive results obtained from the addition of the 2nd sample as compared to the results of the first sample only.
- Feasibility assessment by laboratory technician: Perception of easiness and safety based on individual questionnaire at beginning, half way and end of the study.

Study design:
Multicentric, two-stage prospective diagnostic cohort study to evaluate the diagnostic accuracy of Xpert MTB/RIF Ultra performed on stool samples collected from children with presumptive TB and processed using four different processing methods (Standard sucrose flotation method, optimized sucrose flotation method, SPK, and STEP).
Implementing sites: Children will be recruited from the Mbarara Regional Referral Hospital in Mbarara (Uganda, South West region), Lusaka University Teaching Hospital (Zambia) and the Arthur Davidson Children Hospital, Ndola (Zambia).
Methodology:
- During stage 1 (prospective cohort) all consecutive eligible presumptive TB children will be enrolled. During stage 2 (enrichment cohort) only eligible children with an Xpert positive result on a respiratory sample will be enrolled.
- The diagnostic strategy will include an initial clinical, radiographic and bacteriological evaluation. For the purpose of the study, additional diagnostic methods will be evaluated including Ultra as well as alternative sample collection methods (stool samples). Children will be retrospectively classified as confirmed, unconfirmed, or unlikely TB, using the updated version of the Clinical Case Definition for Classification of Intrathoracic Tuberculosis [42].
- An interim analysis will be conducted to describe the specificity of each stool processing method as well as preliminary results of the sensitivity and the agreement between the processing methods.
- A final analysis will be conducted at the end of the study to describe sensitivity as well as the secondary end points.
- Follow-up: children will be followed up for 2 months upon enrolment, regardless of their TB diagnosis with protocol visits at day 1 and month 2.

Sample size: 274 children (below 15 years old)

Inclusion criteria (prospective cohort):
- Children < 15 years of age
- Presumptive intra-thoracic TB based on at least one criterion among the following:
    - Persistent cough for more than 2 weeks
    - Persistent fever for more than 2 weeks
    - Recent failure to thrive (documented clear deviation from a previous growth trajectory in the last 3 months or Z score weight/age < 2)
    - Failure of broad-spectrum antibiotics for treatment of pneumonia
    - Suggestive CXR features

    OR

    - History of contact with a TB case and any of the symptoms listed under point 2 with a shorter duration (< 2 weeks) if the child is HIV infected or present a SAM.
- Signed informed consent by parent or guardian and assent signed by children $\geq$ 7 years old.

Inclusion criteria (enrichment cohort):
- Children < 15 years of age
- Presumptive intra-thoracic TB based on at least one criterion among the following:
    - Persistent cough for more than 2 weeks
    - Persistent fever for more than 2 weeks
    - Recent failure to thrive (documented clear deviation from a previous growth trajectory in the last 3 months or Z score weight/age < 2)
    - Failure of broad-spectrum antibiotics for treatment of pneumonia
    - Suggestive CXR features

    OR

    - History of contact with a TB case and any of the symptoms listed under point 2 with a shorter duration (< 2 weeks) if the child is HIV infected or present a SAM.
- One positive Xpert (MTB/Rif or Ultra) result from at least one respiratory sample: sputum or GA .
- Signed informed consent by parent or guardian and assent signed by children > 7 years old.

Non-inclusion criteria: Antituberculosis treatment > 5 days in the last 3 months, History of tuberculosis preventive treatment in the last 3 months, confirmed extrapulmonary TB only.

Trial agenda:
- First enrolment: January 2020
- End of prospective cohort enrolment: January 2021
- Interim-analysis: April 2021
- Start of enrichment cohort enrolment: January 2021
- End of enrichment cohort enrolment: December 2021
- End of follow-up: February 2022
- Analysis-report: May 2022

Statistical analysis (primary endpoint):
Sensitivity of Ultra on stool using TB culture reference standard (LJ and MGIT) in respiratory sample: point estimate and 95% CIs.
Specificity of Ultra on stool using TB culture reference standard (LJ and MGIT) in respiratory sample: point estimate and 95% CIs.
An intention-to-diagnose analysis will be performed at sample level (denominator: total number of samples collected) and patients level (denominator total number of cases with at least one stool sample collected). Invalid Ultra results and contaminated culture results will be classified as negative.

Expected results:
Evaluation of the diagnostic accuracy and feasibility of each of the four stool processing methods.

19.2. APPENDIX 2: in-vitro study summary

Development of a simple sample processing method for diagnosis of intrathoracic pediatric tuberculosis using Xpert Ultra testing of stool- results of an *in vitro* study.

Manon Lounnas, Abibatou Diack, Mark Nicol, Sara Eyangoh, Eric Wobudeya, Olivier Marcy, Sylvain Godreuil and Maryline Bonnet

Background
Stool samples are promising alternatives to respiratory samples for molecular diagnosis of childhood tuberculosis (TB) but they require intensive laboratory processing before molecular testing to remove PCR inhibitors and debris. We aimed to develop a centrifuge-free stool processing method for use at peripheral level in resource-limited settings.

Method
In an *in vitro* laboratory study, we tested alternative parameters to optimize a sucrose-flotation method using centrifugation, filtration and vortex shaking that previously showed good sensitivity for childhood TB diagnosis when combined with Xpert: modified amount of stool, manual shaking, no filtration, sedimentation vs centrifugation, modified dilution ratio. Each alternative parameter was compared head-to-head with the reference method using Xpert MTB/RIF Ultra (Ultra) on stool samples spiked with 10^3 *M. tuberculosis* colony forming units (CFU)/g. We selected the best index methods using a drop-the-loser rule: after 15 tests, methods with invalid/errors > 20% (maximum threshold) were dropped; after 30 tests methods with invalid/errors < 20% and a sensitivity difference with the reference method <10% were kept. For final selection, we tested the best parameters combinations at 10^2 CFU/g. We also assessed methods in terms of quantitative Ultra results (cycle thresholds (CTs)) for MTB detection.

Results
Out of 13 different combinations three were tested at 10^2 CFU/g (Table). The best combination used 0.5g stool, manual shaking, no filtration, 30-minutes sedimentation, and a 1:5 dilution ratio. It had 10% invalid/error results and a sensitivity of 70% (95% CI 50.4-84.5) and 53% (38.8-78.1) vs 63% (43.9-79.4) and 58% (40.7-79.1) as compared to the reference method, at 10^3 and 10^2 CFU/g, respectively.

Discussion
We identified an optimized centrifuge and vortex-free stool sample processing method with potential to facilitate stool Xpert Ultra testing in high burden and resource-limited settings. It should be further evaluated among children with presumptive TB.

Table:

Methods tested at 10^2 CFU/g	**- Manual shaking - Sed 30' - 0.5 g - No filtration - dilution ratio 1:5**	**- Manual shaking - Sed 30' - 1 g - No filtration - ratio 1:5**	**- Manual shaking - Sed 30' - 0.5 g - Gauze filtration - ratio 1:5**
Invalid Index vs reference method	10% vs 6%	11% vs 0%	10% vs 6%
Errors Index vs reference method	0% vs 0%	0% vs 0%	0% vs 0%
Sensitivity* Index vs reference method	53% vs 58%	11% vs 22%	30% vs 58%
Mean CTs IS6110-1080** Index vs reference method	25.9 vs 27.4	27.2 vs 29.1	26.4 vs 27.4
Mean CTs SPC*** Index vs reference method	28.6 vs 29.1	29.1 vs 29.2	29.1 vs 29.1

** Sensitivity = Proportion of TB detected among all samples; invalid results and errors are counted as negative results.*
*** CT IS6110-1080 = Cycle thresholds of the two multicopy M. tuberculosis-specific amplification targets (IS6110 and IS1080)*
**** CT SPC = Cycle thresholds of the internal Xpert Sample Processing Control*

19.3. APPENDIX 3: Description of study sites

COUNTRY	UGANDA
SITE	Mbarara Regional Hospital
CITY	Mbarara, South Western Ankole Region
LEVEL	Regional Referral Hospital Teaching Hospital
CATCHMENT AREA (population)	4 000 000 (Region)
NB PAEDIATRIC BEDS	100
NB CHILDREN ADMITTED	4 122 in 2017
SPECIALIZED WARDS	HIV Nutrition Ward
CLINICAL RESEARCH EXPERIENCE	Yes (HIV, TB, malaria, Yellow Fever) MSF-Epicentre Research Centre in Uganda
HUMAN RESOURCES	HIV Clinic: 12 staff
TECHNICAL RESOURCES	Laboratory: mycobacteriology, Xpert TB tests, PCR, and routine laboratory analyses.

COUNTRY	ZAMBIA	
SITE	University Teaching Hospital, Children's Hospital	Arthur Davidson Children Hospital
CITY	Lusaka - capital city	Ndola, Copperbelt Province
LEVEL	national referral hospital	Provincial Referral Hospital Only standalone peadiatric hospital in Zambia
CATCHMENT AREA (population)	2 000 000 (Lusaka)	2 362 000 (Region)
NB PAEDIATRIC BEDS	352	250
NB CHILDREN ADMITTED	35 000 /year	19 000 /year
SPECIALIZED WARDS	HIV Treatment and Care Centre Nutritional Rehabilitation Unit, Paediatric Intensive care unit, Haematology, Infectious diseases, Cardiac Research Clinic	HIV Treatment and Care Centre Nutritional Rehabilitation Centre TB ward
CLINICAL RESEARCH EXPERIENCE	Yes (HIV) GCP training: all staff from the Research Clinic	Yes
HUMAN RESOURCES	Research Clinic: PI, co-PI, and 15 staff (doctors, research nurses, data managers).	Research staff
TECHNICAL RESOURCES	Laboratory: mycobacteriology, Xpert TB tests, PCR, and routine laboratory analyses.	Laboratory: mycobacteriology, Xpert TB test, microbiology, routine blood tests

19.4. APPENDIX 4: Feasibility assessment tools

Head-to-head comparison of three centrifuge free stool processing methods

Acceptability and feasibility of stool processing methods

Self-reported questionnaire for laboratory technicians

ID: ___ ___ ___ ___ ___ ___ ___

Date: |___|___|___|___| / |___|___| / |___|___|

Thank you very much for accepting to participate in this sub-study assessing the ease-of-use of the three stool processing methods in a head-to-head comparison study.

The questions you will answer will help us understand what you think about the stool processing methods. We are interested in knowing about your experience and perception towards these procedures, and what you think about their possible implementation as routine practices in the future.

The questionnaire will take you approximately **20 minutes to complete**.

Each questionnaire is **anonymous**. A unique identification number is used for each participant. All your answers will be kept **confidential** and only aggregated data used to inform stakeholders on the acceptability and feasibility of stool processing methods.

Please remember that there are no wrong or right answers.

When you have completed the questionnaire, please send a scanned copy by e-mail to manon.lounnas@ird.fr.

PART 1. Who are you?

1. Job position and education

a) What is your position?

□ Head of the laboratory
□Laboratory technician
□ Laboratory Technologist
□ other: please specify……..

a) What is your education level?

b) How many years have you been in this position?

□ <1 year
□ 1-3 years
□ >3 years

c) How many years of experience do you have handling stool samples <u>prior</u> to starting this study?

□ no experience
□ <1 year
□ 1-3 years
□ >3 years

d) How many years of experience do you have working in the TB laboratory?

□ no experience
□ <1 year
□ 1-3 years
□ >3 years

e) Have you ever worked or participated in a research project before?

□ No □ Yes

f) When did you start working within this study?

|___|___|___|___| / |___|___|
Year *Month*

g) When were you trained for each of the methods?

SOS: |___|___|___|___| / |___|___|
Year *Month*
SPK: |___|___|___|___| / |___|___|
Year *Month*
OSF: |___|___|___|___| / |___|___|
Year *Month*

PART 2. Overall experience handling stool as sample for TB diagnosis

Do you agree or disagree with the following statements, irrespective of the method?

a) I am comfortable handling stool samples

□ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

Please explain: ……

b) I feel that there is no additional biosafety risk in handling stool samples compared to sputum

□ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

Please explain: ……

What do you think about a possible routine implementation of stool as a specimen for diagnosing TB in children?

a) It would be a good thing

□ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

b) It won't improve TB diagnosis in children

□ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

c) It should be implemented for all children

□ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

d) It isn't realistic in my laboratory

□ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

PART 3 - What do you think about the three centrifuge-free stool processing methods?

1. How would you qualify your overall experience performing the three stool processing methods?

	Poor										*Excellent*
	0	1	2	3	4	5	6	7	8	9	10
Simple One Step method (SOS):	□	□	□	□	□	□	□	□	□	□	□
Stool Processing Kit (SPK):	□	□	□	□	□	□	□	□	□	□	□
Optimized Sucrose Flotation method (OSF):	□	□	□	□	□	□	□	□	□	□	□

Do you agree or disagree with the following statements regarding the possible advantages or difficulties linked to that procedure?

a) The stool processing is time consuming

SOS:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree
SPK:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree
OSF:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree

b) The stool processing is not difficult to perform

SOS: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

SPK: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

OSF: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

c) The processing instructions (SOP, OnePager) provided are clear

SOS: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

SPK: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

OSF: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

d) It requires additional equipment and consumables compared to sputum Xpert processing

SOS: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

SPK: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

OSF: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

e) There is no additional risk (biosafety) for the laboratory staff compared to Xpert sputum processing

SOS: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

SPK: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

OSF: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

f) The risk of sample to sample contamination when performing more than one test at the time is low

SOS: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

SPK: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

OSF: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

g) The risk of environmental contamination (e.g spill on the bench) is limited

SOS: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

SPK: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

OSF: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

2. Did you find any of the stool processing steps particularly difficult?

SOS: □ Yes □ No

If yes, specify the **stool processing** step(s) and the reason(s):

SPK: □ Yes □ No

If yes, specify the **stool processing** step(s) and the reason(s):

OSF: □ Yes □ No

If yes, specify the **stool processing** step(s) and the reason(s):

3. **Did you find any of the stool processing steps unnecessary or for which the rationale was not clearly explained?**

SOS: □ Yes □ No

If yes, specify the **stool processing** step(s) and the reason(s):

SPK: □ Yes □ No

If yes, specify the **stool processing** step(s) and the reason(s):

OSF: □ Yes □ No

If yes, specify the **stool processing** step(s) and the reason(s):

4. **Would you have any suggestions to simplify or improve any stool processing method?**

SOS:

SPK:

OSF:

Which aspect(s) do you think could be problematic in the day-to-day use of each of the stool processing methods?

Tick all that apply. If nothing applies fill the bottom of the table.

	SOS	**SPK**	**OSF**

Hands-on time to prepare for processing			
Hands-on time handling the stool specimen			
Total assay time from preparation until Xpert result			
Handling more than one sample at the same time			
Throughput; No of stool specimens processed in one day assuming enough Xpert capacity			
Stool volume requirement (assuming only one test to perform)			
Stool volume measurement (use of additional supplies, precision)			
Overall number of steps			
Accurate measurement of reagent			
Time-sensitive steps			
Need for additional supplies/reagents compare to Xpert sputum testing			
Training requirements			
Storage conditions and stability of reagents (where needed)			
Waste management requirements			
If you have ticked none of the options above, why not?			
I see no barriers at all			
I see other barriers, specify for each of the method if needed	_____ _____ _____	_____ _____ _____	_____ _____ _____

5. Do you agree or disagree with the following statements regarding user/operator requirements?

a) The procedure is easy to perform for a laboratory staff

SOS: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

SPK: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

OSF: □ Totally agree □ Partially agree □ Partially disagree □ Totally disagree

b) The procedure could be performed by a NON-laboratory staff (e.g. nurse, health worker)

SOS:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree
SPK:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree
OSF:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree

c) The procedure could be implemented in a peripheral health center <u>without</u> a laboratory (assuming GeneXpert is available)

SOS:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree
SPK:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree
OSF:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree

d) The procedure could be implemented in a peripheral health center with a microscopy laboratory (assuming GeneXpert is available)

SOS:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree
SPK:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree
OSF:	□ Totally agree	□ Partially agree	□ Partially disagree	□ Totally disagree

PART 4 – Overall opinion

Considering the ease of use, advantages and disadvantages of each method, which one would you recommend?

If the performance all of the methods was equally good, and you had to use one of the methods, which would be your first choice to perform? Write the number "1" next to your preferred option. Then, enter "2" and "3" for your second and third option respectively. If you value two or more methods equally, use the same number.

SOS: ____
SPK: ____
OSF: ____

Please explain why:

Which method was easiest to perform?

Write the number "1" next to that method in the list below. Then, consider the remaining methods and continue ranking using "2" and "3" until you've ranked all of the tests. If you consider two or more methods equally easy to perform, use the same number.

SOS: ____
SPK: ____
OSF: ____

Please explain why:

Which method was most difficult to perform?

Write the number "1" next to that method in the list below. Then, consider the remaining methods and continue ranking using "2" and "3" until you've ranked all of the tests. If you consider two or more methods equally difficult to perform, use the same number.

SOS: ____
SPK: ____
OSF: ____

Please explain why:

19.5. APPENDIX 5: Information sheet and Informed consent form for the stool processing feasibility sub study

EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB

Short title of the study: **TB-Speed-Stool Processing**

Clinicaltrials.gov identifier: NCT04203628

Inserm No: C19-34

Information sheet and Informed Consent Form for Laboratory Technician

Sub-study "Acceptability and Feasibility assessment of stool processing methods"

Version n°2.0 - 03/07/2019 [Replace by local date and version]

Approved by IEC/IRB < > on DD/MM/2018

The study coordinating investigators are:

Dr Olivier MARCY, Inserm U1219, University of Bordeaux, France

Dr Maryline BONNET, IRD-Montpellier, France & Epicentre-Uganda, Uganda

Dr Eric WOBUDEYA, MUHJU Care Ltd., Uganda

The study sponsor is: French National Institute for Medical Research (Inserm)

Biopark Bâtiment A, 8 rue de la Croix-Jarry, 75013 Paris, France

The study funder is: Unitaid

INFORMATION SHEET FOR LABORATORY TECHNICIANS

Dear Madam, Sir,

As part of the TB-Speed stool processing study conducted in your laboratory, you are being invited to fill a questionnaire to assess the acceptability and feasibility of four stool processing methods (sucrose flotation, the STEP method, the SPK and the sucrose flotation optimized) combined with the Xpert MTB/RIF Ultra assay.

We will also ask you about your opinion on how these methods could be improved. Responding to the questionnaire will take approximatively 20 minutes.

If you wish to participate in this sub-study, you will first sign a consent form.

Even after signing, you may change your mind and ask to stop participating at any time. We only ask you to inform the study staff about your decision as soon as possible.

Participation in the survey does not involve any direct benefits or risks to you. It may have some time inconvenience and – in the event that confidentiality is breached – others might become aware of your responses.

The knowledge that we get from doing this sub-study will be shared with you if you are willing to. We will publish and present the results of the TB-Speed Stool Processing study.

Any data or information collected will be identified by an anonymised code attributed to you at the start of the study. Your name will never be used so that you cannot be recognized.

Information collected on the questionnaire will be entered on tablets and computers that can only be accessed with a password.

The data will be transferred securely to a database located in France for storage and analysis. This data will be handled according to the European and French regulation for the protection of personal data. Only authorized study staff will have access to the database.

During the study, your data will also be stored in a database located at the TB-Speed partner institution in your country <Institution name> for analysis.

Data will be stored for 15 years. However, you have the right to access your data or to request that it be deleted from the database at any time. You also have a right of rectification, a right to refuse or to limit the use of your data.

To exercise these rights, or for any information about the use of your data, you can contact the TB-Speed national laboratory coordinator, name and contact details are written below (section contact for further information). If you met any difficulties to reach your study, you can also contact the Data Protection Officer (DPO) appointed by the sponsor by email at dpo@inserm.fr.

You can ask any questions or request additional information to the TB-Speed National Laboratory coordinator.

……

<Professional address>

<Phone>

<Email>

EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB

Short title of the study: **TB-Speed-Stool Processing**

Clinicaltrials.gov identifier: NCT04203628
Inserm No: C19-34

INFORMED CONSENT FORM FOR LABORATORY TECHNICIANS
Sub-study "Acceptability and Feasibility assessment of stool processing methods"

Version 2.0 - 03 July 2019

I, the undersigned, confirm that:

- I have read the information sheet;
- I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction.

I consent voluntarily to participate this sub-study.

Name of Laboratory technician (performing the four stool processing methods):
_____

_____
___

Date |__|__| |__|__| |__|__|__|__| Signature of participant:

Name of investigator taking consent):
_____

Date |__|__| |__|__| |__|__|__|__| Signature:

Signed in duplicate (participant and investigator). A copy of this Informed Consent Form has been provided to the participant.

19.6. APPENDIX 6: Information sheet for patients under routine care

[logos]

Information sheet for parent(s) or guardian(s)

EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB DIAGNOSIS

- TB-Speed- Stool processing -

Clinicaltrials.gov identifier: NCT04203628

Inserm No: C19-34

Version n°4.0, 30/09/2019 [Replace by local date and version]

Approved by IEC/IRB < > on DD/MM/2019

The study coordinating investigators are:

Dr Olivier MARCY, Inserm U1219, University of Bordeaux, France

Dr Maryline BONNET, IRD-Montpellier, France & Epicentre-Uganda, Uganda

Dr Eric WOBUDEYA, MUHJU Care Ltd., Uganda

The study sponsor is: French National Institute for Medical Research (Inserm)

Biopark Bâtiment A, 8 rue de la Croix-Jarry, 75013 Paris, France

The study funders are: Unitaid

INFORMATION SHEET

Dear parent(s) or guardian(s),

The child under your care, whom we will refer as **your child, is being invited to participate in the research study called TB-Speed Stool processing.**

This information sheet is a document written for you, in order to help you understand the study and decide to participate or not. You have the right to take some time to think and to discuss about this study.

There may be words that you do not understand, or information that is unclear or confusing. If you have any question, now or later, you can ask them to the study doctor or to the study nurse.

WHAT IS A HEALTH RESEARCH STUDY?

A health research study is a way to find out new information about a disease. Children do not participate in a research study if you, the parent(s) or guardian(s), do/does not want him or her to be included.

WHAT IS THE PROBLEM RAISED BY TUBERCULOSIS?

Tuberculosis (or "TB") is a very frequent disease in Asia and Africa, affecting adults and children. It is caused by a germ that most often affects the lungs. The main symptoms of TB are prolonged fever and cough (lasting for more than two weeks) as well as weight loss. Children usually get the disease at home, when they are in contact with an adult infected with TB. If it is not treated, TB can lead to death; but if children are diagnosed in time and treated, TB can be cured. A diagnosis is usually made by a combination of clinical assessment, laboratory tests done on sputum or gastric aspirates (samples obtained from a child's stomach with a tube) and a chest x-rays if the laboratory tests are negative.

WHAT IS THE PURPOSE OF THE TB-SPEED STOOL PROCESSING STUDY?

The purpose of this study is to evaluate stool as an alternative sample for diagnosing TB in children (aged below 15 years old) using a new test call Xpert Ultra. In this study, we will test different methods to prepare the stool sample before testing with Xpert in order to get the best result with this test. Four different stool preparation methods will be evaluated in the laboratory. This study will support development of diagnostic methods to help doctors decide quickly if a child needs to be treated for TB.

This study will not test the existing TB treatment, or test any new TB treatments.

HOW MANY CHILDREN WILL PARTICIPATE IN THE STUDY?

This study will be conducted in Zambia and Uganda, and will include 274 children.

WHY HAS MY CHILD BEEN PROPOSED TO PARTICIPATE IN THE STUDY?

Your child is being offered to participate in this study because he/she is aged below 15 years, has symptoms suggestive of TB (like cough, fever, weight loss, abnormal chest X-ray) or has received a positive test result for TB.

DOES MY CHILD HAVE TO PARTICIPATE?

Your child will participate **only if you, parent or guardian, agree with his/her participation**. If you accept that he/she participates to the study, you will first sign a consent form before any procedures are done.

Moreover, if your child is aged over 7 years, his participation will also require that he/she will give him/her assent to participate in the study in addition to your consent. A separate information sheet will be used to explain him/her the purpose, the risks and benefits of the study.

If you do not want your child to participate in the study, he/she will continue to receive the best possible care and treatments according to National Guidelines. This may include some of the TB tests used in this study, such as chest radiography and gastric aspirates, if the doctor suspects your child has TB.

Even after signing to participate, you may change your mind at any time and ask to stop participating in the study (withdrawal). In any case, your child's care will not be affected if you decide not to participate or change your mind about your child's participation. We only ask you to inform the study staff about your decision as soon as possible.

Information and samples collected before your decision of withdrawal may be used for the study, unless you explicitly request for the information to be removed and sample(s) to be destroyed.

WHAT WILL BE DONE TO MY CHILD IF I ACCEPT HIS/HER PARTICIPATION TO THE STUDY?

If you agree to your child's participation in the study, additional TB tests will be performed.

The following visits will be conducted:

- **Inclusion visit (Day 0)**

After you have signed the informed consent form, the study nurse will ask information about your child's health and medical history.

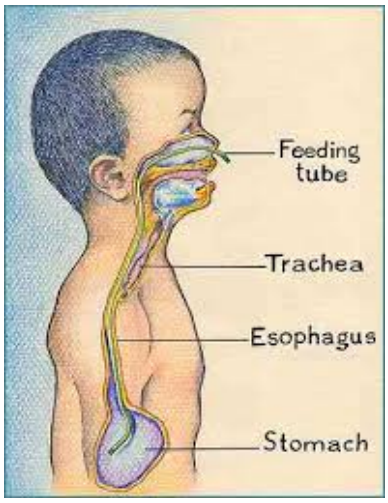
Your child will have a complete physical examination by a doctor and a chest radiography will be performed. If your child's HIV status is unknown, the nurse will also collect blood samples to check if he/she has HIV.

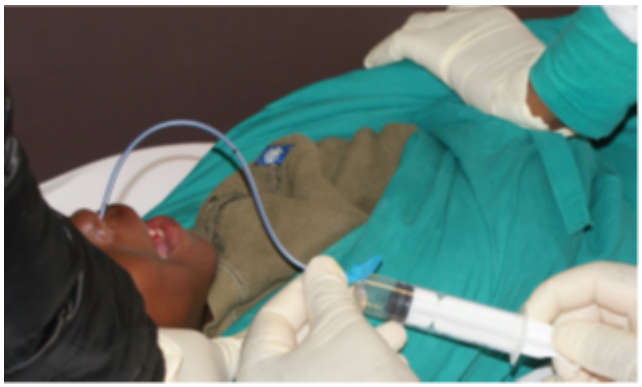
We will also collect 1 gastric aspirate (or 1 sputum sample in children who are able to expectorate) as routine care and 1 stool sample for study purpose. These samples will be used to look directly for the TB germ at the laboratory.

- **Gastric aspirates**

Gastric aspiration consists of passing a tube down through the child's nose to remove the content of the stomach to see if it contains the TB germs. It is what is usually done when a child is suspected with TB. It is done in the morning, before the child has eaten or drunk anything or after an hour resting on a bed in the consultation.

The procedure will be done by a trained nurse, and will be repeated 2 days in a row.





- **Stool sample**

The stool will be collected into a plastic jar as soon as the child will be able to produce stool. For smaller children, a stool sample will be collected straight from the potty or taken directly from the nappy for babies.

- **Follow up visit (Day 1):**

Your child will need to have another gastric aspirate (or sputum) sample taken as routine of care and another stool sample collected for study purpose the day after the inclusion visit (day 1). Once the doctor has received results of laboratory tests and the chest X-Ray, he/she will make a diagnosis and decide if your child has TB or not according to the National Tuberculosis Program criteria. If necessary, a treatment will be started immediately following National Guidelines.

We will use sample collected in routine care as much as possible but if it is not possible, additional respiratory sample might be collected for study purposes to ensure that two respiratory samples are tested with tuberculosis culture. Laboratory results from the additional respiratory samples will be given to the clinician for the clinical management of your child.

- **Follow-up visit (month 2)**

Whether your child is diagnosed with TB or not, a follow-up visit will happen at month 2 from the day he/she was enrolled in the study (as showed in the table below). The visit will include a complete clinical examination, questions about the medical history and a second chest xray will be performed.

You may stay with your child during each visit and during the procedures.

Schedule of visits:

	Inclusion Visit (day 0)	Day 1	Month 2
Clinical evaluation	X		X
Medical history	X		X
Chest radiography	X		X
Blood samples: HIV test	X		
Gastric aspirates or sputum	X	X	
Stool sample	X	X	

Tests that are not part of the research study may be requested by the doctor in accordance with national recommendations.

WHAT HAPPENS IF TB DISEASE IS DETECTED IN MY CHILD?

If the doctor suspects TB, your child will be started immediately on treatment following the recommendations of the National Tuberculosis Program.

If TB is not detected at first, but your child develops new symptoms or gets worse during the study, he/she will be re-tested for TB with a full clinical assessment, a chest X-Ray and laboratory analyses as requested by the doctor, in accordance with national guidelines.

DURATION OF THE STUDY

If you agree to your child's participation, the duration of the study will be 2 months from the day he/she is enrolled in the study.

You will be expected to attend all the scheduled of visits, and bring back your child to the hospital 1 day and 2 months from the day he/she was first enrolled in the study.

After end of the study, your child will continue to receive treatment and care as usually offered in the hospital.

WHAT ARE THE POSSIBLE BENEFITS FOR MY CHILD IF HE/SHE PARTICIPATES?

By participating in the study, your child will undergo a set of diagnostic tests for TB and receive careful monitoring from the study staff. He/she may benefit from a better and earlier diagnosis of TB. This will enable doctors to decide quickly if your child has TB and needs a treatment.

In case the doctor suspects TB and a treatment is needed, your child will receive the most appropriate medication according to National Guidelines, which may increase his/her chance of recovery.

The results of this study may help other children affected with TB. They could benefit from a quick diagnosis of TB, which will reduce the delay to start treatment and reduce their risk of dying from TB.

WHAT ARE THE POSSIBLE DISADVANTAGES FOR MY CHILD IF HE/SHE PARTICIPATES?

Risks associated with the drawing of blood include light-headedness, bleeding and/or bruising and/or minor infection at the vein puncture site. To minimize these risks, the procedures will be done only by trained nurses at the hospital. They will use sterile material to prevent infections, including new and disposable syringes for each child.

The risk due to chest radiography is exposure to radiations. However, two chest-X-rays done within 2 months represent a low exposure to radiations for your child. This examination is recommended for children presenting signs suggestive of TB.

Gastric aspirates may be unpleasant or distressing for the child. It can cause cough, sneezing, nausea, vomiting, local nose injury and nose bleeding generally minor, oesophageal erosion or perforation, wrong position of the tube (in the lung for example), and more rarely slowing of the heartbeat, or reduce the flow of oxygen in the blood (especially in children aged 3 to 6 months).

WHAT IF THERE IS A PROBLEM?

In case of any problem, you have to report it to the study doctor, whose name and contact are written at the end of the information sheet.

The promoter of the study (the French INSERM institute) is subscribing an insurance to cover any damage to children caused by the study.

WILL MY CHILD'S PARTICIPATION TO THIS STUDY BE KEPT CONFIDENTIAL?

All information collected about your child during the study will be kept **confidential.**

As soon as your child is enrolled in the study, he will be identified by a code: his/her name, day of birth, and address will be removed so that he/she cannot be recognized. This code will be used for any information and any sample collected from your child throughout the study. Only your doctor will know what your number is and will guard that information with a lock and key.

Your child's medical chart may be reviewed by authorized study staff only, or authorized persons from Health Authorities. These individuals will keep information strictly secret.

The knowledge that we get from doing this study will be shared with you if you are willing to. We will publish or present the results of the TB-Speed Stool Processing study in order that other interested people may learn from our research. Confidential information will not be shared, and the identity of your child will never be mentioned.

PROTECTION OF INFORMATION ABOUT YOUR CHILD

During study visits, information about your child's health and history of TB in your family will be collected by the study nurse and entered on tablets and computers that can only be accessed with a password.

Your child will be identified by the code attributed at the start of the study. His/her name will never be used so that he/she cannot be recognized.

Information about your child, called "data", will be transferred securely to a database located in France for storage and analysis. This data will be handled according to the European and French regulation for the protection of personal data. Only authorized medical and study staff will have access to the database.

During the study, your child's data will also be stored in a database located at the TB-Speed partner institution in your country <Institution name> for analysis.

Part of the data collected during the study could be shared with other partners, according to signed agreements under security rules mentioned below.

Data will be stored for 15 years. However, you have the right to access your child's data or to request that it be deleted from the database at any time. You also have a right of rectification, a right to refuse or to limit the use of your child's data.

To exercise these rights, or for any information about the use of your child's data, you can contact the study doctor who follows you as part of research, name and contact details are written below (section contact for further information). If you met any difficulties to reach your study doctor, you can also contact the Data Protection Officer (DPO) appointed by the sponsor by email at dpo@inserm.fr.

Security rules

Only authorized and identified persons can use or analyse your child's data.

The person in charge of the Study Data Protection at the University of Bordeaux will guarantee the security of the data, including when data is transferred outside the European Union.

EXPENSES AND COMPENSATIONS

You will not incur any costs related to this study. Study investigators will ensure that you receive reimbursement of transportation fees to the hospital and that the medical exams, tests, and medications related to the study are covered by the study.

The participation of your child in the study is voluntary and you will not receive any financial compensation.

CONTACT FOR FURTHER INFORMATION

You can ask any questions or request additional information about the study to the doctor or to the study nurse:

Dr……	Nurse……
…….……	…….
<Professional address>	<Professional address>
<Phone>	<Phone>
<Email>	<Email>

If you have questions about the rights of your child as a study participant or have complaints about this study, please contact the <Ethics Committee> that verified the study at:

<Institution name>
<Professional address>
<Phone>
<Email>

19.7. APPENDIX 7: Information sheet for patients enrolled in other TB-Speed studies

[logos]

Information sheet for parent(s) or guardian(s)

EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB DIAGNOSIS

- TB-Speed- Stool processing -

Clinicaltrials.gov identifier: NCT04203628

Inserm No: C19-34

Version n°3.0, 14/08/2019 [Replace by local date and version]

Approved by IEC/IRB < > on DD/MM/2019

The study coordinating investigators are:

Dr Olivier MARCY, Inserm U1219, University of Bordeaux, France

Dr Maryline BONNET, IRD-Montpellier, France & Epicentre-Uganda, Uganda

Dr Eric WOBUDEYA, MUHJU Care Ltd., Uganda

The study sponsor is: French National Institute for Medical Research (Inserm)
Biopark Bâtiment A, 8 rue de la Croix-Jarry, 75013 Paris, France

The study funders are: Unitaid

INFORMATION SHEET

Dear parent(s) or guardian(s),

The child under your care, whom we will refer as **your child, is being invited to participate in the research study called TB-Speed Stool processing.** Your child is being proposed to participate in this study because is **already enrolled in a TB Speed study (HIV or SAM) on TB diagnosis.**

WHAT IS THE PURPOSE OF THE TB-SPEED STOOL PROCESSING STUDY?

The purpose of this study is to test stool as an alternative sample for diagnosing TB in children (below 15 years old) using a new test call Xpert Ultra. In this study, we will test different methods to prepare the stool sample before testing with Xpert in order to get the best result with this test. Four different stool preparation methods will be evaluated in the laboratory. This study will support development of diagnostic methods to help doctors decide quickly if a child needs to be treated for TB.

This study will not test the existing TB treatment, nor test any new TB treatments

This study will be conducted in Zambia and Uganda, and will include 274 children.

WHAT WILL BE DONE TO MY CHILD IF I ACCEPT HIS/HER PARTICIPATION TO THE STUDY?

If you agree to your child's participation in the study, in addition to the tests being done for the TB-Speed SAM/ TB-Speed HIV study, he/she will have 2 stool samples collected and tested for TB on the day on Inclusion (Day 0) and on the next day (Day 1).

The stool will be collected into a plastic jar as soon as the child will be able to produce stool. For smaller children, a stool sample will be collected straight from the potty or taken directly from the nappy for babies.

Other needed information will be collected from the other study in which your child is participating.

Even after signing to participate, you may change your mind at any time and ask to stop participating in the study (withdrawal). In any case, your child's care will not be affected if you decide not to participate or change your mind about your child's participation. We only ask you to inform the study staff about your decision as soon as possible and to precise if your withdrawal decision apply to TB-Speed stool processing study or to all TB-Speed study which you have consent.

WHAT ARE THE POSSIBLE BENEFITSAND/OR DISADVANTAGES FOR MY CHILD IF HE/SHE PARTICIPATES?

There are no additional benefits or disadvantages from participating in this study.

PROTECTION OF INFORMATION ABOUT YOUR CHILD

All information collected about your child during the study will be kept **confidential.** The same rules that are used for the TB-Speed HIV and SAM studies to ensure confidentiality, data transfer and storage will be followed.

To exercise these rights, or for any information about the use of your child's data, you can contact the study doctor who follows you as part of research, name and contact details are written below (section contact for further information). If you met any difficulties to reach your study, you can also contact the Data Protection Officer (DPO) appointed by the sponsor by email at dpo@inserm.fr.

Security rules

Only authorized and identified persons can use or analyse your child's data.

The person in charge of the Study Data Protection at the University of Bordeaux will guarantee the security of the data, including when data is transferred outside the European Union.

EXPENSES AND COMPENSATIONS

The participation of your child in the study is voluntary and you will not receive any financial compensation.

CONTACT FOR FURTHER INFORMATION

You can ask any questions or request additional information about the study to the doctor or to the study nurse:

Dr……	Nurse……
…….……	…….
<Professional address>	<Professional address>
<Phone>	<Phone>
<Email>	<Email>

If you have questions about the rights of your child as a study participant or have complaints about this study, please contact the <Ethics Committee> that verified the study at:

<Institution name>
<Professional address>
<Phone>
<Email>

19.8. APPENDIX 8: Information letter and certificate of assent for children aged over 7 years- - routine care

[logos]

Information sheet for children aged over 7 years

EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB DIAGNOSIS

- TB-Speed- Stool processing -

Clinicaltrials.gov identifier: NCT04203628

Inserm No: C19-34

Version n°2.0, 03/07/2019 [Replace by local date and version]

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The study coordinating investigators are:

Dr Olivier MARCY, Inserm U1219, University of Bordeaux, France

Dr Maryline BONNET, IRD-Montpellier, France & Epicentre-Uganda, Uganda

Dr Eric WOBUDEYA, MUHJU Care Ltd., Uganda

The study sponsor is: French National Institute for Medical Research (Inserm)
Biopark Bâtiment A, 8 rue de la Croix-Jarry, 75013 Paris, France

The study funders are: Unitaid

INFORMATION SHEET

This information sheet talks about our research study, and the choice that you can make to take part in it or not. You can ask any questions you want, at any time you want.

Important things to know:

- You can decide if you want to take part in the research or not
- You can say “No” or you can say “Yes”
- No one will be upset if you say “No”
- If you say “Yes” you can always change your mind, at anytime
- Doctors will still take good care of you no matter what you decide.

What is a research study?

Research is something you do when you want to discover new knowledge about something. We use research studies to help us find out more about a disease. Research helps us to find better ways of treating sick children.

What is Tuberculosis?

Tuberculosis (often named “TB”) is a very frequent disease in Africa. It affects both adults and children. TB is caused by a germ that, most often, does harm to the lungs. The symptoms of TB are cough, fever, and weight loss. These symptoms can last for several weeks.

Usually you get the disease at home, when you live close to an adult who has TB.

To know if you have TB, the doctor should examine you and ask you if you are coughing or have fever, and make a picture of your lungs, called radiograph. He can also look for the TB germ in your sputum at the laboratory. Sometimes the doctor is not sure, because it is difficult to detect the TB germ in children. If TB is detected on time and the treatment is started quickly, you can be cured of TB.

Why are we doing this research?

We want to find ways to detect TB quickly before it makes children very sick. By doing this, we will be able to help children like you, to receive better care and treatment for TB.

Why are you asking me?

We are asking you to take part in that research study because you are a child, and because you have symptoms such as cough, fever, or weight loss for a few days or weeks, that resemble those of TB or because you have had a positive test result for TB.

What is going to happen to me if I take part?

① First of all, we explained the study to your parent(s) or guardian, and asked them if they agree that you take part in the research study. If they say yes, they have to sign a paper.

Now we explain the study to you and ask if you are willing to take part. If you say yes, you will also have to sign a paper.

② Once you and your parent(s) or guardian have agreed to take part in the study, you will see the doctor to check if you have TB.

- The doctor will first ask questions about you, your health and your family

- Then the doctor will examine you and measure your length and your weight, listen to your heart and to your lungs, and check if you have fever.

- After that, the nurse may need to take a small amount of your blood. This will be taken by a needle in your arm.

- We will take a picture of your lungs (called “X-ray” or radiograph). For that, you will need to stand a few minutes in front of a big machine taking the picture.

- The nurse will then ask you to expectorate or “spit” in a small plastic jar to look for the TB germ. If you cannot spit, the nurse will do a gastric aspirate to look for the TB germ in your stomach. A gastric aspirate consists of introducing a tube down to your stomach through your nose to aspirate a bit of the liquid in your stomach.

- Finally, the nurse will take a bit of your stool in a jar

If needed, either you will stay at the hospital for the night, or you will take an appointment with the doctor and come back on the day after.

④ Follow-up visits

Whether you have TB or not, the doctor and the nurse will take care of you during 2 months. You will have to come back 2 times to the hospital to see the doctor at 1 day, and 2 months from the day of your first visit. At month 2 the doctor will examine you again and we will make another picture of your lungs.

Your parent(s) or guardian may stay with you during each visit

What happens if doctors find out that I have TB?

If you have TB, your doctor will give you medications. You will start to take medicines as soon as possible, for six to nine months.

How long will it take?

The study will last about 2 months. After 2 months, the research will be finished, but you will continue to receive care and treatment as it was before the study.

Could bad things happen if I join this research?

Some of the tests might make you feel uncomfortable. We will try to make sure that no bad things happen.

The needle used to collect blood might hurt and you could see some drops of blood. It could get slightly red and hard around the place where the needle goes in. That should go away in a day.

The gastric aspirate may be unpleasant or make you feel upset. It could make you cough, sneeze, feel sick or vomit, and make your nose bleed a little, but this rare and generally not too strong.

What is good about taking part?

If you take part to the study, the nurse and the doctor will take care of you during 2 months. If you have TB, you will be given the best possible treatment to help you feel better and recover from TB.

Things that we find out in this study will also help other children like you to receive a good treatment if they get TB.

Do I have to do this?

You do not have to participate in this research study if you do not want to. Even if your parent(s) or guardian(s) have agreed that you participate, you can say no.

If you decide not to be in the study, you will not have any trouble. This is still your hospital; everything will stay the same as before.

Even if you say “yes” now, you can change your mind later, at any time.

Just tell your parent(s) or guardian(s) and the doctor or the nurse that you would like to stop.

Will anyone know that I am participating in the study?

We will not tell other people that you participate in this research, and we will not share any information about you with anyone who does not work on the study.

Any information about you will have a number on it instead of your name, so that you cannot be recognised. Only the doctor will know what your number is, and will keep that information secret with a lock and key.

What happens to information that researchers find out?

When we have finished the research, we will write articles in scientific journals to explain important things that we found out about TB. These articles will be in journals that researchers, doctors and nurses read.

We will never write your name in the articles; no one will know that you participated in the research study.

Whom can I talk to or ask questions to about the study?

You can ask questions about this study now or later. You can talk to me, or your parents, or someone else if you like.

Thank you very much for taking the time to read this.

***EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB DIAGNOSIS* - TB-Speed-**

Stool processing -

[Trial number]

CERTIFICATE OF ASSENT FOR CHILDREN AGED OVER 7 YEARS

International version 2.0 – 03 July 2019

If you decide to participate, and your parents agree, we will give you a copy of this form to keep, so that you can look at it later if you want to.

Please circle the answer you agree with:

Do you understand this research study?
Yes / No

Has the doctor or the nurse answered all your questions?
Yes / No

Do you understand that you can stop being in the study at any time?
Yes / No

Are you willing to take part?
Yes / No

✓ **If you do want to take part, please write your name and today's date below.**

Your name: _____

Date: ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Your signature: _____

✓ **The researcher who explained this study to you needs to sign too**.

Name of Investigator/person conducting assent:
_____

Date: ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Signature: _____

**If verbal assent only is being obtained:*

Investigator or Person Conducting Assent Discussion: Initial here if child cannot sign, to document that child received this information and gave assent verbally: _____

A copy of this Certificate of Assent has been provided to the child's parent(s) or guardian(s)

19.9. APPENDIX 9: Information letter and certificate of assent for children aged over 7 years- TB Speed co-enrolled

[logos]

Information sheet for children aged over 7 years

EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB DIAGNOSIS **- TB-Speed- Stool processing -**

Clinicaltrials.gov identifier: NCT04203628

Inserm No: C19-34

Version n°2.0, 03/07/2019 [Replace by local date and version]

Approved by IEC/IRB < > on DD/MM/2019

The study coordinating investigators are:

Dr Olivier MARCY, Inserm U1219, University of Bordeaux, France

Dr Maryline BONNET, IRD-Montpellier, France & Epicentre-Uganda, Uganda

Dr Eric WOBUDEYA, MUHJU Care Ltd., Uganda

The study sponsor is: French National Institute for Medical Research (Inserm)
Biopark Bâtiment A, 8 rue de la Croix-Jarry, 75013 Paris, France

The study funders are: Unitaid

INFORMATION SHEET

This information sheet talks about our research study, and the choice that you can make to take part in it or not. You can ask any questions you want, at any time you want.

Important things to know:

- You can decide if you want to take part in the research or not
- You can say “No” or you can say “Yes”
- No one will be upset if you say “No”
- If you say “Yes” you can always change your mind, at anytime
- Doctors will still take good care of you no matter what you decide.

Why are we doing this research?

We want to find ways to detect TB quickly before it makes children very sick. By doing this, we will be able to help children like you, to receive better care and treatment for TB.

Why are you asking me?

We are asking you to take part in that research study because you are a child, and because you have symptoms such as cough, fever, or weight loss for a few days or weeks, which resemble those of TB, or you have had a positive test result for TB and because you have accepted participate in another study named TB-Speed HIV or SAM study.

What is going to happen to me if I take part?

① Once you and your parent(s) or guardian have agreed to take part in the study, you will see the nurse. The nurse will take a bit of your stool in a jar.

② You will have to come back 1 more time to the hospital to see the nurse the day after your first visit. The nurse will take a bit of your stool in a jar again.

If needed, either you will stay at the hospital for the night, or you will take an appointment with the doctor and come back on the day after.

Your parent(s) or guardian may stay with you during each visit

Could bad things happen if I join this research?

No, for this study we will only need you to give the nurse some stool. We won't need you to do any other tests.

What is good about taking part?

Things that we find out in this study will also help other children like you to receive a good treatment if they get TB.

Do I have to do this?

You do not have to participate in this research study if you do not want to. Even if your parent(s) or guardian(s) have agreed that you participate, you can say no.

If you decide not to be in the study, you will not have any trouble. This is still your hospital; everything will stay the same as before.

Even if you say "yes" now, you can change your mind later, at any time.

Just tell your parent(s) or guardian(s) and the doctor or the nurse that you would like to stop.

Will anyone know that I am participating in the study?

We will not tell other people that you participate in this research, and we will not share any information about you with anyone who does not work on the study.

Any information about you will have a number on it instead of your name, so that you cannot be recognised. Only the doctor will know what your number is, and will keep that information secret with a lock and key.

What happens to information that researchers find out?

When we have finished the research, we will write articles in scientific journals to explain important things that we found out about TB. These articles will be in journals that researchers, doctors and nurses read.

We will never write your name in the articles; no one will know that you participated in the research study.

Whom can I talk to or ask questions to about the study?

You can ask questions about this study now or later. You can talk to me, or your parents, or someone else if you like.

Thank you very much for taking the time to read this.

EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB DIAGNOSIS

- TB-Speed- Stool processing -

[Trial number]

CERTIFICATE OF ASSENT FOR CHILDREN AGED OVER 7 YEARS

International version 2.0 – 03 July 2019

If you decide to participate, and your parents agree, we will give you a copy of this form to keep, so that you can look at it later if you want to.

Please circle the answer you agree with:

Do you understand this research study?
Yes / No

Has the doctor or the nurse answered all your questions?
Yes / No

Do you understand that you can stop being in the study at any time?
Yes / No

Are you willing to take part?
Yes / No

✓ **If you do want to take part, please write your name and today's date below.**

Your name: _____

Date: ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Your signature: _____

✓ **The researcher who explained this study to you needs to sign too**.

Name of Investigator/person conducting assent: _____

Date: ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Signature: _____

**If verbal assent only is being obtained:*

Investigator or Person Conducting Assent Discussion: Initial here if child cannot sign, to document that child received this information and gave assent verbally: _____

A copy of this Certificate of Assent has been provided to the child's parent(s) or guardian(s)

19.10. APPENDIX 10: Informed Consent form for parents

EVALUATION OF FOUR STOOL PROCESSING METHODS COMBINED WITH XPERT MTB/RIF ULTRA FOR DIAGNOSIS OF INTRATHORACIC PAEDIATRIC TB DIAGNOSIS

- TB-Speed- Stool processing -

[Trial number]

INFORMED CONSENT FORM FOR PARENT(S) OR GUARDIAN(S)

Version 2.0 - 26 June 2019

I, the undersigned, confirm that:

- I have read the information sheet;
- I have had the opportunity to ask questions to the doctor or the study nurse, whose name and signature are shown below, and any question that I have asked have been answered to my satisfaction;
- I understand the benefits and risks of participating in this study.

If Guardian

❑ Furthermore, as the guardian, I certify that I usually assume responsibility for the child's custody, care, and maintenance as

- ❑ a family member (specify: .....)
- ❑ other (specify: .....)
- ❑ the child's caregiver formally mandated by the Director of an institution/orphanage and that the child is not living with his mother and/or father.

I freely agree to take part in this study according to the terms defined in this notice of information
❑ Yes ❑ No

Name of participating child:

_____

Name of Parent(s) or Guardian(s):

_____

Date |__|__| |__|__| |__|__|__|__| Signature of parent(s)/guardian(s):

If Illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Parent(s)/guardian(s) who are illiterate should include their thumbprint as well.

I have witnessed the accurate reading of the consent form to the parent(s)/guardian(s) of the child, and that they have had the opportunity to ask questions. I confirm that parent(s)/guardian(s) have given consent freely.

Name of witness:

_____

Date |__|__| |__|__| |__|__|__|__|

Signature of witness AND thumbprint of parent(s) or guardian(s)

I, study nurse/investigator, hereby certifies having fully explained the relevant details of this study to the parent(s)/guardian(s) named above and believe that they have understood and have knowingly given their consent.
I solemnly promise I will respect all the terms and conditions mentioned in this consent form, keep full confidentiality, and respect the individual's rights and freedom as well as the requirements of the scientific work.

Name of study nurse/investigator taking consent:

_____

Date |__|__| |__|__| |__|__|__|__| Signature: _____

Signed in duplicate (parent(s)/guardian(s) and nurse/investigator). A copy of this Informed Consent Form has been provided to the participant's parent(s) or guardian(s).

19.11. APPENDIX 11: Copy of the insurance policy

19.12. APPENDIX 12: Copy of the Inserm CEEI and WHO ERC approvals

19.13. APPENDIX 13: Copy of the Competent Authority's authorization

19.14. APPENDIX 14: List of required administrative and/or ethical clearance per country

(Subject to further completion)

COUNTRY	Ethical clearance	Administrative research clearance	Institutional Review Board
UGANDA	Uganda National Council of Science and Technology (UNCST) Research Ethics Committee of the Mbarara University of Science and Technology Research Ethics Committee (MUST REC)		
ZAMBIA	National Research Ethics Authority (NREA)		University of Zambia Biomedical Research Ethics Committee