IMPACT OF SYSTEMATIC EARLY TUBERCULOSIS DETECTION USING XPERT MTB/RIF ULTRA IN CHILDREN WITH SEVERE PNEUMONIA IN HIGH TUBERCULOSIS BURDEN COUNTRIES

TB-Speed Pneumonia

International protocol version No 3.2 – 18/12/2020

CONFIDENTIAL

RESEARCH PROTOCOL INVOLVING HUMAN PERSON

INSERM No.	CEEI No.	WHO ERC No.	CNIL	Regulatory Qualification
C18-26	<…> Date: DD/MM/YYYY	<…> Date: DD/MM/YYYY	919186 Date: 06/12/2019	

Corresponding country protocol versions and approvals:

	Côte d'Ivoire	Cameroon	Uganda	Mozambique	Zambia	Cambodia
Country protocol version						
Approved by						
Date						
Approved by						
Date						

Coordinating Investigators		
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	Maryline Bonnet IRD UMI233 / Inserm U1175 Université de Montpellier911 Avenue Agropolis 34394 Montpellier Cedex 05 **Tel**: +33 (0)4 67 41 64 91 **Email**: maryline.bonnet@ird.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**
☐ **French multicentre study**
☐ **European 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 Hamzé, Inserm Project Manager benjamin.hamze@inserm.fr Tel: +33 (0)1 82 53 34 72

This study is funded by Unitaid and 5% Initiative and by the ANRS for the Covid-19 ancillary study.

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:

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:

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	Kampala, Date: Name: Maryline Bonnet 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:

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

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

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 Hamzé
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

Pharmacovigilance:
Noémie MERCIER
Clinical trial safety office
101 rue de Tolbiac
75013 Paris, France
Tel: +33 (0)1 44 23 67 67
E-mail: noemie.mercier@anrs.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 trial manager:* Aurélia VESSIERE E-mail: aurelia.vessiere@u-bordeaux.fr	*Biostatistics:* Hélène FONT E-mail: helene.font@u-bordeaux.fr *Monitoring:* Aurélie BEUSCART E-mail: aurelie.beuscart@ u-bordeaux.fr *System development and data management:* Sophie KARCHER E-mail: sophie.karcher@ u-bordeaux.fr Eric BALESTRE Email: eric.balestre@u-bordeaux.fr

Reference trial microbiologist: **Laboratory coordinator:**

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	Manon LOUNAS 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

Cost-effectiveness specialist:

Eric OUATTARA
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 56 57
Email: eric.ouattara@u-bordeaux.fr

Country principal investigators: (countries appear in alphabetical order)

CAMBODIA Laurence BORAND Epidemiology and Public Health Unit Institut Pasteur du Cambodge No. 5, Monivong Boulevard P.O Box. 983, Phnom Penh Tel: +855 12 333 671 Email: lborand@pasteur-kh.org Mao TAN EANG National Center for Tuberculosis and Leprosy (CENAT/NTP) - Ministry of Health No. 1, Street 278/95, Beung Keng Kang II Chamkar Morn, Phnom Penh Tel: +855 12 916 503 Email: mao@online.com.kh	***CAMEROON*** Jean-Voisin TAGUEBUE Mother and Child Center - Chantal Biya Foundation Rue Henry Dunant P.O. Box 1936, Yaoundé Tel : +237 677 670 788 Email: jvoisintag@yahoo.fr

COTE D'IVOIRE Raoul MOH Programme PAC-CI CHU de Treichville 18 BP 1954, Abidjan Tel: + 225 21 75 59 60 E-mail: raoul.moh@pacci.ci Laurence ADONIS-KOFFY† Service de Pédiatrie CHU de Yopougon 21 BP 632, Abidjan Tel: + 225 23 53 75 50 E-mail: koflaur@hotmail.com ***MOZAMBIQUE*** Celso KHOSA Instituto Nacional de Saúde Av. Eduardo Mondlane, No 1008 Ministério da Saúde PO Box 264, Maputo Tel: +258 21 311 038 E-mail: khosacelso@gmail.com Sandra MAVALE Paediatrics Department Maputo Central Hospital Av. Agostinho Neto, No 1164 Maputo Tel: +258 21 320 449 Email: mavale23@yahoo.co.uk	***UGANDA*** Eric WOBUDEYA MUJHU Care Ltd Upper Mulago Hill Road P.O. Box 23491, Kampala Tel.: +256 712 846 163 Email: ewobudeya@mujhu.org 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 Veronica MULENGA Children's Hospital University Teaching Hospital 1st Nationalist Road Private Bag RW IX, Ridgeway, Lusaka Tel: +260 966 953 792 Email: veromulenga@gmail.com

Associated investigators:

Christophe DELACOURT
Department of Pediatric Pulmonology
Necker University Teaching Hospital
149 rue de Sèvres
75015 Paris, France
Tel: +33 (0)1 44 49 49 55
Email: christophe.delacourt@aphp.fr

Christian LIENHARDT
IRD UMI233 / Inserm U1175
911 Avenue Agropolis, BP 64501
34394 Montpellier Cedex 05, France
Tel: +33 (0)4 67 41 51 41
Email: lienhardtc@who.int

James SEDDON
Department of Medicine
Imperial College
235 Norfolk Place
London, United Kingdom
Tel: +44 (0)20 7594 3179
Email: james.seddon@imperial.ac.uk

Country Clinical Trials Units:

CAMBODIA Institut Pasteur du Cambodge #5 Monivong Boulevard, P.O Box 983, Phnom Penh ***CAMEROON*** IRD Cameroon 782 Rue Joseph Essono Balla BP 1857, Yaoundé ***COTE D'IVOIRE*** Programme PAC-CI CHU de Treichville BP 1954, Abidjan 18	***MOZAMBIQUE*** Instituto Nacional de Saúde Caixa Postal 264 Av. Eduardo Mondlane, No 1008 Maputo ***UGANDA*** MUJHU Care Ltd Upper Mulago Hill Road P.O. Box 23491 Kampala 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

- Olivier MARCY, Coordinating investigator (Inserm U1219, Université de Bordeaux, France)
- Eric WODUBEYA, Coordinating investigator (MUJHU, Kampala, Uganda)
- Maryline BONNET, Coordinating investigator (IRD UMI233 / Inserm U1175 Montpellier, France & Epicentre, Mbarara, Uganda)
- Aurélia VESSIERE, International Trial Manager (Inserm U1219, Université de Bordeaux, France)
- Delphine GABILLARD, Statistician (Inserm U1219, Université de Bordeaux, France)
- Christophe DELACOURT, Pediatric Pulmonologist (Necker University Teaching Hospital, Paris, France)
- James SEDDON, Paediatrician (Imperial College London)
- Christian LIENHARDT, Clinical epidemiologist (IRD UMI233 / Inserm U1175 Montpellier, France)
- Sylvain GODREUIL, Reference trial microbiologist (IRD-Mivegec, Montpellier, France)
- Laurence ADONIS-KOFFY†, co-Principal Investigator (Yopougon University Teaching Hospital, Abidjan, Côte d'Ivoire)
- Laurence BORAND, Principal Investigator (Pasteur Institute in Cambodia)

HISTORY OF PROTOCOL VERSIONS

TB-Speed Pneumonia

Version	Version Date	Amendment Summary
1.0		
2.0	22/11/2018	- Description of study strategies and intervention: clearer distinction between routine care (the WHO Standard of Care) and additional assessments and procedures for research purpose in both arms - Patient schedule and informed consent restructured accordingly - Description of biobank governance and patients' rights
3.1	27/10/2020	- Adaptation of the study design and study schedule to the Covid-19 outbreak - Integration of the TB-Speed Covid sub-study - Cost effectiveness ancillary study: addition of data collection methods - Replacement of a study site in Côte d'Ivoire
3.2	16/12/2020	Core protocol: - Clarifications added to the biobank governance scheme - Addition of Table 6 (Appendix 8) with the list of country Central Laboratories and contractual agreement with CTUs Covid sub-study: - Mention that Cambodia is not participating - Laboratory evaluations: addition of the minimal amount/volume of biological samples in Table 5

LIST OF ABBREVIATIONS

ANRS	French National Agency for Research on HIV/AIDS and Hepatitis
AE	Adverse event
ART	Antiretroviral therapy
Covid-19	Coronavirus disease 2019
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
DBS	Dried blood Spot
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
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
PPE	Personal Protective Equipment
QC	Quality control
RIF	Rifampicin
SAB	Scientific Advisory Board
SAE	Serious Adverse Event
SAM	Severe Acute Malnutrition
SCD	Sickle cell disease
SOC	Standard of Care
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
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

TABLE OF CONTENTS

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 (2). 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].

TB and pneumonia in young children

In high TB burden and resource-limited countries, **children below the age of 5 years are a highly vulnerable group** with an increased risk of developing TB disease following exposure and infection, often through close contact with an infected adult, and a higher case fatality rate compared to older children [6]. In this age group, children with acute severe pneumonia are particularly vulnerable as they have a high risk of mortality and are not systematically investigated for TB.

Pneumonia is the leading cause of death in children under the age of 5 years worldwide [7]. There is growing evidence that, in high TB burden settings, **TB is common in children with pneumonia,** with up to 23% of those admitted to hospital with an initial diagnosis of pneumonia later being diagnosed as TB [8], [9]. This is particularly true in the African and Asian WHO regions, which accounted for 30% and 35% of all paediatric TB cases in 2016 respectively [1]. In these regions, the case fatality rate for childhood pneumonia associated with TB is high, ranging from 4% to 21% [8], with younger age, malnutrition and HIV infection increasing the risk of death [10], [11].

The current standard of care (SOC) for young children with pneumonia considers a diagnosis of TB only if the child has a history of prolonged symptoms or fails to respond to antibiotic treatments [12]. Hence, **TB is often under-diagnosed or diagnosed late in children presenting with pneumonia**. Although TB is a chronic disease in adults, recent data show that the duration of respiratory symptoms before admission can be acute in children with severe pneumonia associated with TB [8]. Hence, Identifying TB cases early and shortening the diagnostic delay to initiate appropriate TB treatment in children with clinical presentation of severe acute pneumonia is likely to reduce mortality.

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 [13]. 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 [14]. 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 [15].

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 [16], [17]. 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 [16]. Although data on the performance of Xpert MTB/RIF in children with pneumonia is limited, in Bangladesh, sensitivity on gastric aspirates or sputum samples compared to culture in this group was equivalent to that reported in other studies [18].

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 [19]. This lower threshold is similar to the detection level of culture and would facilitate the rapid diagnosis of paucibacillary TB disease as seen in children [20], [21]. Retrospective analyses on frozen respiratory samples in children have shown a sensitivity of 71% for Ultra versus 47% for Xpert MTB/RIF [22]. A recently published study reveals, however, a lower specificity of U

ltra in adults, particularly in those with a previous history of TB, potentially resulting in false diagnoses and overtreatment of TB [21]. Though further prospective studies are needed, the risk of false-positive results could be less significant in children as only a small proportion of children have previously had TB [23]. The estimated clinical impact of Ultra is therefore likely to vary depending on the settings, with a recent modelling exercise finding a larger mortality benefit in patient populations with high TB prevalence, high HIV prevalence, and high case fatality ratios for untreated TB [24].

The current WHO recommendations for the use of Xpert MTB/RIF also apply to the use of Ultra as the initial diagnostic test for all adults and children with signs and symptoms of TB. An update of the current guidelines for the use of Ultra is planned for 2018 [25].

Alternative specimen collection methods adapted to children

Young children are frequently unable to expectorate sputum and 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. At the programmatic level, implementation of gastric aspirates and induced sputum can be challenging [26], [27].

Our research group and other groups in Africa and Asia have shown that alternative specimen collection methods such as **nasopharyngeal aspirates (NPA) and stool samples are easier to be implemented in resource-limited settings and are better tolerated in young and sick children** [28]–[33]**.** 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 [34]. In children with presumptive TB, Xpert MTB/RIF has a sensitivity on NPAs close to the one achieved with induced sputum [28], [35]. Recent studies have shown similar sensitivity of Xpert MTB/RIF on the combination of one stool and one NPA as compared to two induced sputum or two gastric aspirates [36], [37]. Stool testing by Xpert MTB/RIF shows results close to respiratory samples in terms of sensitivity but requires a simplified specimen processing methodology for further field use [30], [31], [38]–[40]. The flotation method, based on Sheather's sucrose solution used in the PAANTHER study, showed promising results but relies on centrifugation and other labour-intensive processes [36]. Stool processing will be further optimized in Output 4 of the TB-Speed project to enable implementation at a lower healthcare level.

Most studies on childhood TB diagnostics are early proof-of-concepts or studies that evaluate diagnostic accuracy, collecting and testing multiples samples using existing microbiological tests [35], [41]–[43]. Implementation studies, with patient health outcomes as the primary endpoint of interest, are seldom implemented despite the need for such studies to inform policies [44], [45]. The WHO recommendation to use Xpert MTB/RIF in children was based on diagnostic accuracy, but evidence of its impact on TB outcome has not been evaluated [35], [46], [47]. As in adults, its use in children may have a limited impact on outcome in children with a strong suspicion of TB, due to the common use of empirical treatment in these

populations [48]–[51]. However, this is likely to be different in children with severe pneumonia presenting with acute symptoms, for which TB is usually suspected only after empiric antibiotic treatment has been shown to be ineffective.

The TB-Speed approach to early detection of TB in children with severe pneumonia

In line with the strategies advocated by the National Tuberculosis Programmes (NTPs) in participating countries, the TB-Speed project aims not only to contribute to the reduction of TB-associated mortality, but also to initiate an innovative approach for the early detection and treatment of TB in young children with severe pneumonia to enable access to high-quality healthcare in this highly vulnerable group.

Our hypothesis is that in high TB burden countries, **testing young children with severe pneumonia for TB and starting those who test positive on anti-TB treatment on the day of presentation, could reduce all-cause mortality** through reduction of mortality attributed to TB. In this context, we are proposing a research study to assess the impact on mortality of adding the systematic early detection of TB using Ultra performed on NPAs and stool samples to the WHO SOC for children with severe pneumonia, followed by immediate initiation of anti-TB treatment in children testing positive on any of the samples. If successful, this intervention could be systematically implemented at district hospital level where children with severe pneumonia are referred. Furthermore, we hypothesize that the intervention will raise TB awareness among clinicians and may lead to more empirical TB treatment initiated as compared to the control. This further justifies the stepped-wedge design chosen for this study.

From a health economics perspective, we hypothesize that benefits in terms of survival and Disability Adjusted Life Years (DALYs) will outweigh extra costs incurred by systematic Ultra testing in children with severe pneumonia.

Mortality in children aged 2 to 59 months hospitalized with severe pneumonia and mortality attributable to TB

There were an estimated 120 million pneumonia episodes in children younger than 5 years in 2011, including 14 million severe episodes [52]. 1.3 million pneumonia episodes led to death. In 2014, WHO revised definitions for pneumonia and severe pneumonia, considering ‘chest-indrawing pneumonia’ as non-severe and downgrading ‘pneumonia with severity signs’ from very severe to severe [53][54]. Ambulatory care and oral antibiotics are now recommended for ‘chest-indrawing pneumonia’ and now only severe pneumonia justifies referral from primary health centres to district hospitals for intra-venous (IV) antibiotics and oxygen therapy if needed [53].

There are no global estimates of mortality specifically attributable to severe pneumonia as currently defined by WHO. Overall inpatient mortality was 8.7% in a Kenyan study of 4184 children of median age 8.9 months hospitalized with ‘chest-indrawing pneumonia’ or ‘pneumonia with severity signs’ [55]. In a prospective study of 693 Zambian children below 5 years hospitalized with pneumonia, severity signs were associated with an almost 3 fold increase in the risk of death, and an inpatient case fatality rate of 32% [56]. HIV infection and severe acute malnutrition (SAM), present in 21% and 9% of children, respectively, were associated with a 2 to 3-fold increase in the risk of death. Similarly, in a retrospective study of 113,154 hospitalized Malawian children aged 1 to 59 months, mortality in those with severe pneumonia was 18.0% versus 3.1% in those with pneumonia without severity signs [57].

In children with severe pneumonia, death can also occur after discharge from hospital. Of the 4184 Kenyan children followed in the above study, follow-up was prolonged 1 year after discharge, for a total follow-up time of 2163 child-years. 70 deaths (3.1%) occurred after discharge, representing 34% of overall inpatient and post-discharge mortality in this group. A mid-upper arm circumference (MUAC) below 11.5 cm, which defines SAM, was strongly associated with post-discharge mortality. Half of deaths occurred during the first 3 months after discharge.

Mortality due to TB in undiagnosed and untreated children can be very high. Data from paediatric TB cohorts from the pre-treatment and treatment era showed that the case-fatality ratio in untreated children aged <5 years with TB was 43.6% (95%CI 36.8–50.6), as compared to 1.9% (95%CI 0.5–7.1) in HIV-negative treated children of the same age [58]. This does not take into account the severity of TB, or to individual risk factors such as HIV infection. In HIV-TB co-infected children aged <5 years the case-fatality ratio of untreated children was estimated at 90.0% (95%CI 77.8–96.9) without antiretroviral therapy (ART), and 63.5% (95%CI 30.0–90.1) with ART, in a recent mathematical modelling study [1]. Data from various cohorts of HIV-infected children treated for TB show that mortality remains high despite access to both TB and HIV treatment [59], [60], indicating a need for more systematic and earlier TB detection in this vulnerable group of children. As in adults, bacillary load, as reflected by sputum smear-positivity could be associated to an almost 3-fold increase in the risk of death [61]. Finally, the case fatality rate for childhood pneumonia associated with TB is high, ranging from 4% to 21% [8], with younger age, malnutrition and HIV infection increasing the risk of death [10], [11]. The missed diagnosis of TB or delayed diagnosis in children with TB-associated severe pneumonia is likely to explain this high case fatality rate.

Participating countries and added value of the multi-country aspect

The impact of this innovative approach may vary with TB incidence as well as some geographical and seasonal variability that can affect the prevalence and aetiology of pneumonia in young children. To provide a better basis for the generalizability of results, the project will take place in six countries with different epidemiological and environmental backgrounds, in Sub-Saharan Africa (Cameroon, Cote d'Ivoire, Mozambique, Uganda, and Zambia) and South East Asia (Cambodia) (Table 1). Cambodia, Zambia and Mozambique are among the 30 high TB burden countries according to WHO classification.

Table 1: TB incidence rate in participating countries

Region	Country	TB incidence rate /100,000 population
Western Africa	Cote d'Ivoire	153
Central Africa	Cameroon	203
Eastern Africa	Uganda	201
Southern Africa	Zambia	376
	Mozambique	551
South East Asia	Cambodia	345

Source: WHO Global TB Report, 2017.

National TB Programs (NTPs) from these countries support the TB-Speed project and are members of Country Project Committees (see Chapter 13.3). NTPs will play an instrumental role in the scale up of the TB-Speed strategy, taking the opportunity of the 2020 country dialogue for funding request to the Global Fund. This will be further supported by the WHO-Unitaid TB enabler grant, through which the project will receive support from WHO to ensure country preparedness for accelerated uptake and integration of the TB-Speed approach into national and international guidance for the management of childhood TB.

Due to the Covid-19 pandemic, enrolment of participants in the study was interrupted from April 1st, 2020. The study will be resumed and will take into consideration the possibility of SARS-Cov-2 infection among some of the participants. Therefore, an ancillary study on the prevalence of the SARS-Cov-2 infection was added and is described in Chapter 11.

2. OBJECTIVES

2.1. Primary Objective

To evaluate the impact on all-cause mortality at 12 weeks post inclusion of adding systematic early detection of TB with Ultra, performed on one NPA and one stool sample, to the WHO standard of care (SOC) in young children with severe pneumonia, followed by immediate anti-TB treatment initiation in children with a positive Ultra result, in high TB incidence countries, as compared to the SOC alone.

2.2. Secondary Objectives

- To evaluate the impact of systematic early detection of TB on:
  - TB case detection at 12 weeks
  - TB treatment initiation
  - Time to TB treatment initiation
  - TB treatment exposure during follow-up
  - Inpatient mortality
  - Duration of initial hospitalization
  - Hospital readmission rate
  - Weight gain at 12 weeks

- To compare cost-effectiveness of the two strategies

- Additionally, in the intervention arm:
  - To assess the proportion of microbiologically (Ultra)-confirmed TB and clinically-diagnosed TB
  - To assess the feasibility of NPA and stool samples collection
  - To assess the safety of NPA
  - To assess the acceptability of NPA and stool samples collection in a subset of children
  - To assess the tolerability of NPA in a subset of children

3. STUDY ENDPOINTS

3.1. Primary study endpoint

All-cause mortality 12 weeks after inclusion.

Mortality due to severe pneumonia (related or not to TB) is expected to occur early. A 12 week period is long enough to assess impact on mortality of TB treatment empirically started in children with poor clinical progress during the first weeks of follow-up.

3.2. Secondary study endpoints

- Number of children diagnosed with TB at 12 weeks:
  - based on Ultra results
  - based on the clinician's judgement
- Proportion of children with TB treatment initiated at any time during follow-up
- Time to TB treatment initiation
- Duration of TB treatment at end of trial (week 12 or early termination)
- Number of inpatient deaths
- Duration of initial hospitalization
- Number of readmissions following discharge
- Weight gain at 12 weeks (as compared to body weight at inclusion)
- Incremental cost-effectiveness ratio (ICER)

Additionally, the following endpoints will be assessed **in the intervention group only**:

- Proportion of NPA and stool samples with positive TB detection using Ultra
- Proportion of Ultra-confirmed and clinically-diagnosed TB cases
- Feasibility of NPA and stool samples collection:
  - Proportion of children with samples collected as per protocol
  - Turnaround time between NPA or stool sample collection and result of Ultra
- Safety: adverse events (AEs) collected by study nurses during NPA collection such as vomiting, nose bleeding, low oxygen saturation
- Tolerability of NPA specimen collection procedures: discomfort/pain/distress experienced by the child assessed by the child him/herself (Wong-Baker face scale), by the parents (visual analog scale), by the nurses (FLACC behavioural scale)
- Acceptability of NPA and stool specimen collection procedures by parents and nurses (quantitative and qualitative assessment).

4. STUDY DESIGN

4.1. Study type

TB-Speed Pneumonia is a multicentric, cluster-randomised pragmatic diagnostic trial conducted in six countries with high TB incidence (Cote d'Ivoire, Cameroon, Uganda, Mozambique, Zambia and Cambodia). It aims to assess the impact on mortality of the TB-Speed diagnostic strategy (intervention) added to the SOC compared to the SOC alone (control) in young children newly hospitalised for WHO-defined severe pneumonia. The study only considers community-acquired pneumonia, excluding children already hospitalized who have developed a nosocomial pneumonia.

4.2. Methodology

The TB-Speed Pneumonia study is designed as a stepped wedge trial. It is a type of randomised controlled trial in which clusters successively switch from control to intervention in an order randomly assigned, until all clusters are eventually exposed to the intervention (Figure 1). The stepped wedge cluster-randomised design permits evaluation of the study hypothesis using a pragmatic and operational approach.

A stepped wedge design is justified when the intervention is believed to do more good than harm, where it may be unethical to withhold the intervention from a proportion of patients. Randomisation per hospital rather than per patient is also advantageous due to the difficulty at health facility level to individually randomise children to one of the two strategies, additionally minimising contamination between the two arms. In this study, it is hypothesized that the intervention will raise TB awareness among clinicians and may lead to more empirical TB treatment initiated as compared to the control. The stepped-wedge design is therefore particularly adapted to capture such effects.

The TB-Speed Pneumonia study will be implemented in 15 hospitals. Randomisation will be stratified on the country estimated TB incidence rate (see Table 1): < 300/100,000 patients-years (Cameroon, Cote d'Ivoire and Uganda) vs. ≥ 300/100,000 patients-years (Cambodia, Mozambique and Zambia).

At the start of the study, all hospital will be implementing the WHO SOC for severe pneumonia (control arm). One hospital will then switch to the TB-Speed strategy (intervention arm) at each step, i.e. every 5 weeks. Depending on the time at which they are included into the study, children will receive either - and exclusively - the standard of care, or the TB-Speed intervention.

A total of 3,780 children <5 years old with WHO-defined severe pneumonia will be enrolled over 80 weeks (about 18 months), with a mean of 252 participants per hospital.

Figure 1: Stepped wedge implementation of the intervention in participating hospitals

TB incidence Rate	Hospital number	step (5-week intervals)															
		01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16
Low	01	CT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT
High	**02**	CT	CT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT
Low	03	CT	CT	CT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT
High	**04**	CT	CT	CT	CT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT
Low	05	CT	CT	CT	CT	CT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT
High	**06**	CT	CT	CT	CT	CT	CT	INT	INT	INT	INT	INT	INT	INT	INT	INT	INT
Low	07	CT	CT	CT	CT	CT	CT	CT	INT	INT	INT	INT	INT	INT	INT	INT	INT
High	**08**	CT	CT	CT	CT	CT	CT	CT	CT	INT	INT	INT	INT	INT	INT	INT	INT
Low	09	CT	CT	CT	CT	CT	CT	CT	CT	CT	INT	INT	INT	INT	INT	INT	INT
High	**10**	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	INT	INT	INT	INT	INT	INT
Low	11	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	INT	INT	INT	INT	INT
High	**12**	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	INT	INT	INT	INT
Low	13	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	INT	INT	INT
High	**14**	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	INT	INT
Low	15	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	INT

CT: control (WHO recommended standard of care for children with severe pneumonia); INT: TB-Speed intervention (systematic early detection of tuberculosis in addition to the WHO recommended standard of care for children with severe pneumonia).

- **Study design adaptation to the Covid-19 outbreak**

All enrolments in the TB-Speed Pneumonia study have been stopped as of April 1st, 2020 due to the ongoing Covid-19 outbreak. This was justified by the complete or partial lock down in project countries, the lack of protective equipment to ensure safety of participants and study staff, and the difficulty to conduct proper study monitoring. The decision was supported by the advice of the TB-Speed Pneumonia Independent Data Monitoring Committee (IDMC) and approved by the TB-Speed Scientific Advisory Board. All study sites were instructed: 1) to suspend all enrolments until further notice; 2) to conduct phone-based follow-up when physical visits are not possible, provided the participant's safety is not compromised, based on ad hoc Standard Operating Procedures for remote follow-up; 3) to limit face-to-face contact to that strictly necessary to provide care that cannot be suspended, provided the safety of staff is protected, based on ad hoc Standard Operating Procedures for infection prevention and control during health care, samples collection and samples processing at the laboratory.

As advised by the IDMC, enrolments will resume progressively at sites, on condition that the respective country situations allow for it, in particular the availability of personal protective equipment (PPE), and that a Covid-19 ancillary study is implemented to document SARS-CoV-2 prevalence and its impact on mortality in children, and enable access to specific Covid-19 treatment if needed.

The randomization process will be suspended until all sites have resumed enrolments (i.e. sites will remain in their current phase, control or intervention), date on which the stepped wedge schedule will restart where it left off, unless decided otherwise by the TB-Speed Pneumonia IDMC at its next meeting (expected in October 2020).

4.3. Sample size

Based on evidence from the literature and hypotheses presented in Appendix 5 table A1, we have made the following assumptions on the impact of our intervention, systematic early detection of TB, on mortality in children with severe pneumonia.

In the recent review on TB-related childhood pneumonia in TB endemic areas, 275 of 3644 (7.5%) patients with severe pneumonia had culture-confirmed TB [8]. The proportion of culture positive cases was higher (232/2800, 8% vs 43/844, 5%) in settings with a very high TB incidence (≥300 cases per 100,000 population per year) than in settings with a high incidence (50–299 cases). Assuming that culture-confirmed TB represents roughly 1/3 of all TB cases, the expected proportion of TB (all cases) in children with pneumonia would be 15% and 24%, in high and very high incidence settings, respectively.

Based on results of the first studies evaluating Ultra [21], [23], we hypothesized that the rate of bacteriological confirmation resulting from its use could lead to a 20% increase in the detection yield, as compared to Xpert, resulting from increased sensitivity and potential detection of culture-negative TB cases in children. Assuming that Ultra used on a combination of NPA and stool samples will perform as well as on two gastric aspirates, as earlier shown with Xpert, Ultra testing of NPA and stool samples could detect approximately 90% of culture-confirmed cases [33], [36]. Therefore, our intervention using Ultra on stool and NPA in all children with severe pneumonia on the day of admission could detect the majority of confirmed TB cases, versus a hypothesized TB detection rate of 25% in routine conditions without systematic TB detection. We hypothesized also that the intervention would raise awareness about TB in site clinicians and would lead to an increase in detection rate from 15% to 50% between the control and the intervention arm.

We used estimated mortality rates of 90% for untreated HIV-infected children, 10% in TB-treated HIV-infected children, 43.6% in untreated HIV-negative children, and 1.9% in TB-treated HIV-negative children and we applied a hazard ratio of death of 3.0 associated with culture-confirmation in treated HIV-positive children as well as in all HIV-negative children, and of 1.1 in untreated HIV-infected children (See details in Table A2, Appendix 5).

Based on these assumptions and recent HIV prevalence estimates in paediatric TB cases in participating countries [1], we estimated a proportion of TB deaths in children admitted with severe pneumonia of 8.0% and 3.3% in the control and the intervention arm, respectively, corresponding to an absolute reduction of 4.7% due to the intervention. We estimated that in the control arm the overall mortality would reach 15%, in line with mortality associated to severe pneumonia in previous studies, and that the intervention would therefore lead to a 30% reduction in the overall mortality rate (10.5%) [55]–[57].

TB treatment is highly efficient in young children, including in those with underlying conditions such as HIV infection or malnutrition. Moreover, we expect a low heterogeneity between practices during the study in terms of application of the SOC, management of TB cases and treatment availability. Therefore, in spite of potential differences in the characteristics of study populations between countries (genetic background, HIV and malnutrition prevalence), we may reasonably assess that the intra-cluster correlation coefficient (ICC) will be low.

We have considered ICC values of 0.001, 0.005, and 0.01 as optimistic, median, and pessimistic scenarios, respectively. Using the estimated mortality of 15% in the control arm, an expected reduction in mortality in the experimental arm of 30%, an alpha=0.05, a power of 80%, and 1% of incomplete data, corresponding sample sizes would be: 3 044, 3 780, and 4 254 children, respectively. For financial and logistical reasons, we retained an ICC value of 0.005, corresponding to a design effect of 2.16.

We will therefore randomise 3 780 children across the two strategies, in 15 hospitals over 16 steps, that is a mean of 15.8 children enrolled per hospital per step (or 252 children per hospital for the entire study). The number of participants per stratum will however not be capped.

4.4. Provisional study schedule

- Observational phase: 4th quarter 2018
- First inclusion: March 20, 2019
- Inclusion period: 80 weeks (18 months)
- Duration of follow-up for each participant once enrolled: 12 weeks
- Enrolment stop due to Covid-19 pandemic: April 1st, 2020
- Progressive enrolment restarting: June-August 2020
- Full enrolment restart: October 2020
- Expected last visit of the last participant: 2nd quarter 2021
- Overall duration of the trial (from the first inclusion to the last visit): 116 weeks (27 months)

4.5. Cost-effectiveness ancillary study

A cost-effectiveness study will be performed as part of the TB-Speed Project Output 5 ("Evaluation of cost-effectiveness of the proposed diagnostic approaches"). Cost-effectiveness and budget impact analyses will evaluate the incremental cost-effectiveness ratio (ICER) and the long-term impact of improving TB diagnostics in children with severe pneumonia, guide health authorities' decisions and support the implementation of the TB-Speed approach in resource-limited settings.

A mathematical model will be developed to project health-economic outcomes including TB cases and mortality in children with severe pneumonia. The model will be developed in collaboration with ScHARR of the University of Sheffield (UK) and the CaP-TB project (see Chapter 11.2.3).

The cost-effectiveness analysis will be from the health payer perspective and only direct health care costs will be included. A budget impact analysis will be conducted to evaluate the actual impact of implementing the TB-Speed approach on healthcare budgets at 2- and 5-year horizons in the countries participating in TB-Speed.

A separate analysis plan will be written for the cost-effectiveness analysis. Data collection methods for cost data are outlined in Chapter 10.1.3.

5. STUDY ENROLMENT

5.1. Study population

Inclusion criteria

5.1.1.

- Children aged 2 to 59 months
- Newly hospitalized for severe pneumonia[1] defined using WHO criteria as cough or difficulty in breathing with:
  - Peripheral oxygen saturation < 90% or central cyanosis, or
  - Severe respiratory distress (e.g. grunting, nasal flaring, very severe chest indrawing), or
  - Signs of pneumonia, defined as cough or difficulty in breathing with fast breathing (tachypnea)[2] and/or chest indrawing, with any of the following danger signs:
    - Inability to breastfeed or drink,
    - Persistent vomiting
    - Lethargy or reduced level of consciousness
    - Convulsions,
    - Stridor in calm child
    - Severe malnutrition

5.1.2. ➢ Informed consent signed by parent/guardian

It should be noted that history of TB exposure will be documented but is not part of the inclusion criteria (which are based solely on the WHO definition for severe pneumonia in children).

Non-inclusion criteria

- Ongoing TB treatment or history of intake of anti-TB drugs in the last 6 months

[1] Pneumonia here corresponds to WHO-defined pneumonia with severity criteria requiring referral to district hospital level for O2 therapy and IV antibiotics. Eligibility is not based on confirmed bacterial or viral pneumonia using other criteria and chest radiography.

[2] Children aged 2-12 months: respiratory rate >50/min; >12 months: respiratory rate >40/min

5.2. Recruitment sites

The trial will be implemented in 15 regional or national reference hospitals. All participating hospitals are University Teaching Hospitals (UTH) or equivalent in terms of level of care. Implementing sites were chosen on the basis of previous collaboration on research projects with the relevant country partners, and on a capacity assessment questionnaire including expected numbers of children admitted with severe pneumonia per year.

Table 2 presents indicative recruitment capacities of participating hospitals, based on the number of children <5 years admitted with severe pneumonia in 2017. Based on sample size calculations (see chapter 11.2), we expect a mean of 252 children enrolled per site over 18 months, which is an approximate 202 children per year.

Table 2: Implementing sites and number of children <5 years admitted with severe pneumonia in 2017

Country	Nb sites	Hospitals, City	2017
Côte d'Ivoire	3	Angré UTH, Abidjan	ND
		Treichville UTH, Abidjan	263
		Cocody UTH, Abidjan	293
Cameroon	2	Chantal Biya Foundation, Yaoundé	404
		District Hospital Biyem Assi, Yaoundé	117
Uganda	3	Mulago National Referral Hospital, Kampala	866
		Holy Innocents Childrens' Hospital, Mbarara	238
		Regional Reference Hospital, Jinja	NA[a]
Mozambique	2	Central Hospital, Maputo	659
		Jose Macamo General Hospital, Maputo	490
Zambia	2	UTH, Lusaka	975[b]
		Arthur Davidson Children Hospital, Ndola	522
Cambodia	3	Referral Hospital, Kampong Cham	134
		Referral Hospital, Takeo	54[c]
		National Pediatric Hospital, Phnom Penh	546

[a] NA: disaggregated data by age and severity not available. [b] Not disaggregated by severity. [c] Number corresponds to final pneumonia diagnosis at discharge

6.6.1. STUDY STRATEGIES AND INTERVENTION

6.1. Description of the study strategies and intervention

The WHO standard of care for children with severe pneumonia

All children admitted in the hospital and presenting with WHO-defined severe pneumonia will be immediately managed as part of routine care per the WHO Standard of Care (SOC) for children with severe pneumonia. In order to guarantee that children benefit from the same quality of care across study sites, adherence to the principles and contents of the WHO SOC for severe pneumonia will be reinforced by initial study training and monitored throughout the study implementation. This will therefore benefit to all children hospitalized, beyond research settings.

As recommended in the SOC [12], children should receive:

- broad spectrum intravenous antibiotics

- oxygen therapy, if oxygen saturation <90% or they have signs of hypoxia
- additional supportive care, including airway management, symptomatic fever treatment, bronchodilators or steroids if needed, appropriate maintenance fluids, nutritional support including breastfeeding or nasogastric tube if needed
- specific therapies for comorbidities such as HIV infection or malnutrition
- a chest X-ray if possible.

The child should be checked by a nurse at least every 3 hours and by a doctor at least twice a day, with signs of improvement within 2 days in the absence of complications. A follow-up visit should be arranged 2 weeks after discharge, if possible, to check the child's nutrition.

If the child presents with persistent cough and fever for more than 2 weeks, and signs of pneumonia after adequate antibiotic treatment, he/she should be evaluated for TB using routine procedures. Xpert or Ultra, depending on local availability, could be used in children with clinical suspicion of TB (chronic symptoms, failure to respond to antibiotic treatment or TB exposure) according to the clinician's judgement. This will be done using sample collection methods usually implemented at the inpatient ward.

For research purposes, the child will benefit from HIV testing, malaria testing, and complete blood count (CBC) if not systematically performed as routine care in the country/hospital, as well as from a digitalized chest X-ray. Additionally, samples will be collected for future biomarkers studies in children for whom parent(s)/guardian(s) give their consent for frozen samples (biobank). Data about routine care as well as additional study strategies will be collected.

Follow-up visits will be added at 2 weeks post discharge, if not routinely performed, and 12 weeks post enrolment to collect endpoints.

6.1.2. *The TB-Speed intervention*

The intervention will consist of the WHO standard of care for children with severe pneumonia plus the study intervention consisting in rapid detection of TB on the day of hospital admission using the Ultra assay performed on 1 NPA and 1 stool sample.

The sample flow will be organised in order to reduce time to results to 3 hours. Ultra will be performed at the hospital laboratory to avoid additional delays that may be incurred by transportation to a centralized research laboratory.

If a turnaround time of 3 hours cannot be obtained with the standard GeneXpert device, Ultra testing will be implemented inward using a one-module GeneXpert device (G1 Edge).

Drugs will be available at the inpatient level to enable immediate initiation of TB treatment, as soon as a positive Ultra result is available.

Once implemented, the study intervention will not be discontinued, unless otherwise stipulated by the IDMC (See chapter 14.2). However, the IDMC will not apply stopping rules and interim analyses as per standard clinical trials because the stepped wedge design does not allow for it (see Chapter 11.3.4 for details). To date, no guidance is available on the design of stepped-wedge cluster randomised trials with interim analyses.

For research purposes, the child will also benefit from HIV testing, malaria testing, and complete blood count (CBC) if not systematically performed in routine care in the country/hospital, as well as from a digitalized chest X-ray as done in the control group. Additionally, samples will be collected for future biomarkers studies in children for whom parent(s)/guardian(s) give their consent for frozen samples. Data about routine care as well as additional study strategies and intervention will be collected.

Follow-up visits will be added at 2 weeks post discharge, if not routinely performed, and 12 weeks post-enrolment to collect endpoints.

6.2. Assignment of study strategies and intervention

Randomization

The order in which the strata of units step in to implement the intervention will be determined by computer generated random numbers from a uniform distribution.

Inherent to the stepped wedge design, randomization will be stratified by hospital rather than individuals, i.e. prior to the patient's consent. All hospitals will eventually implement the
6.2.1 intervention during the trial; the random element is the time point at which the switch will occur.

To balance the number of children in the two arms, randomization will be stratified by the country TB incidence rate:

- < 300/100,000 patients-years: Cameroon, Cote d'Ivoire, Uganda
- ≥ 300/100,000 patients-years: Cambodia, Mozambique, Zambia.

Consequently, within each TB incidence rate strata, a hospital will switch to the intervention every 10 weeks. A total of 3,780 children <5 years old with WHO-defined severe pneumonia will be equally distributed across the 2 strategies. This cohort will be enrolled in 80 weeks (i.e., 16 five-week steps), with a mean of 252 participants per hospital. The number of participants per stratum will however not be capped.

Within these strata, a computer-generated random sequence will determine the order in which hospitals move from control to intervention. It is expected that there will be some seasonal variation in the rate of severe pneumonia, hence the decision of having 2 to 3 hospitals per country.

The statistician of the international coordinating Clinical Trials Unit (CTU), based at University of Bordeaux, will prepare the randomization sequence before the start of the trial. The study coordination team will be blinded to the randomization list and informed of the next cluster to switch one month prior to crossover.

6.2.2.

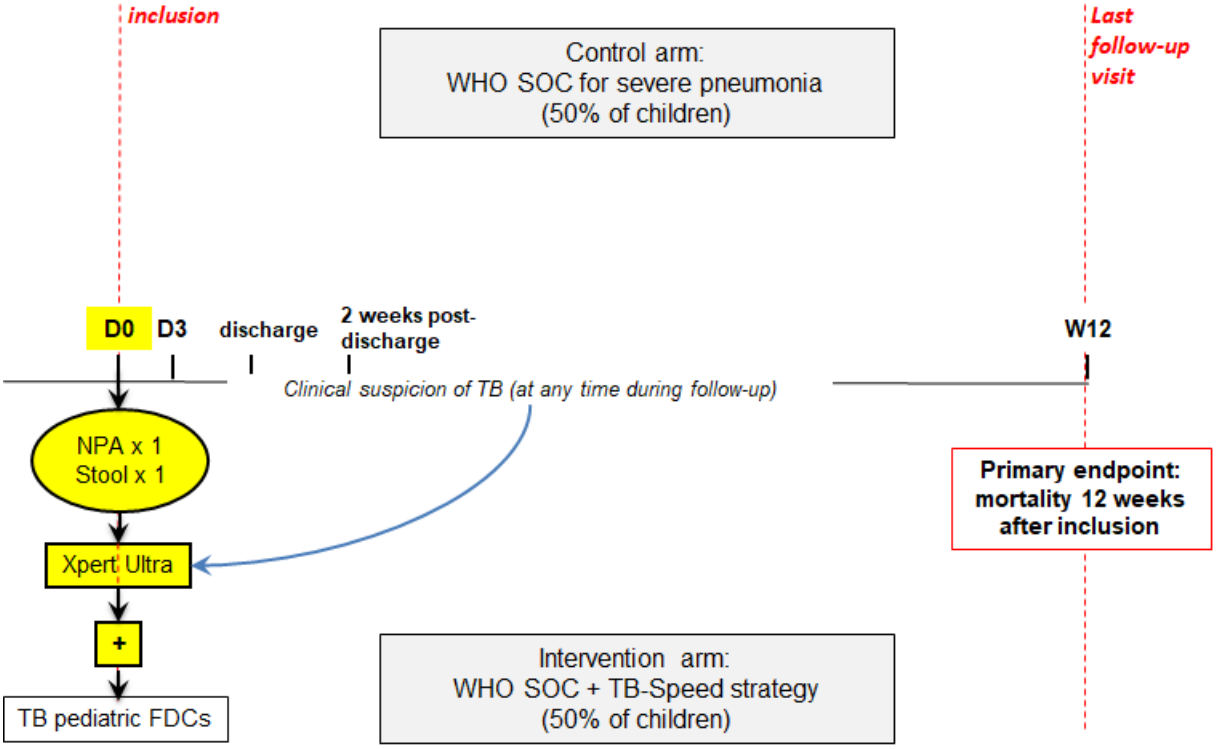
Implementation at site level

Aggregated data on severe pneumonia management will be collected in all hospitals 2 months prior to the initial rollout of the study to document routine practices with the SOC for severe pneumonia in children (number of hospitalisations, inpatient mortality, antibiotics use, access to oxygen therapy and other supportive care).

The country Principal Investigators (PIs) will work with their respective Ministries of Health to ensure that national guidelines are applied in every participating site. It is expected that the provision of equipment for O_2 therapy and training on pneumonia case management in children will reduce the variation in characteristics and practices between the intervention and control group.

Hospitals and country research teams will be notified one month prior to their crossover date to initiate program planning.

6.3. Study flow chart



7. STUDY PROCEDURES

Children will be screened, enrolled and followed up in the paediatric wards of the different hospitals by study nurses and site investigators.

7.1. Selection process

Screening and enrolment will start in all participating hospitals at the same time. Any child younger than 5 years old presenting to outpatient, emergency units, or paediatric departments of the selected hospitals with cough or difficulty in breathing will be managed according to routine procedures based on the WHO SOC, including initiation of IV antibiotics and oxygen therapy if needed. Pulse oximeters will be provided to sites if not available.

Children will be screened for eligibility for the trial as soon as possible by study nurses and site investigators. Assessment for eligibility will be detailed in specific clinical Standard Operating Procedures (SOPs).

7.2. Obtaining informed consent

The purpose, nature of constraints and foreseeable risks and benefits of the trial arm to which the hospital has been randomized at the time of the child's admission, will be 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, without justification, to withdraw from the trial at any moment without consequence on the quality of care and follow-up provided to their child. In addition to oral explanations, a written information sheet in their home language will be systematically provided (see Appendices 6 and 7). Documents with pictures will be available to help the parents to better understand the exams proposed in the study.

Written informed consent will be obtained prior to any trial-specific clinical, biological or radiological exam.

The informed consent process will be implemented by the study nurse or site investigator following specific SOPs. They will ensure that parent(s)/guardian(s) have read and understood the content of the information sheet, and that they have received answers to all their questions before signing the informed consent. If one of the parent(s)/guardian(s) does not agree on the child's participation, the child will not be enrolled. If the participation agreement is given by the parent(s)/guardian(s), the consent form will be completed, signed and dated by the parent(s)/guardian(s) and the study nurses and site investigators. Oral consent in the presence of a witness (not from the medical team) will be acceptable, according to national rules. The consent form will include separate consent for frozen samples storage.

A copy of the signed consent will be given to parent(s)/guardian(s). The original consent 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 will be considered as a guardian if they usually assume responsibility for the child's custody, care, and maintenance even if no court order exists formally appointing that person as the guardian, custodian, or adoptive parent of the child. Should any of the child's parents be alive but not living with the child, the usual caregiver will be considered as a guardian.

7.3. Patient schedule

Table 3: Patient schedule including specimen collection in children

	Protocol visits					
	Inclusion	**D3**	**Discharge**	**2 weeks post-discharge** *(+/- 3 days)*	**(Extra TB visit[1])**	**W12** *(+/- 7 days)*
STANDARD OF CARE (control and intervention arm)						
Treatment of severe pneumonia[2]						
- Antibiotics	X	X	X			
- O_2 saturation[3]	X	X	X			
- O_2 therapy as required	X	X	X			
Treatment and care of co-morbidities if needed[2]:						
- HIV infection	X	X	X	X		X
- Malnutrition	X	X	X	X		X
TB clinical assessment[4]		(X)	(X)	(X)	(X)	
TB treatment if needed[2, 5]		X	X	X	(X)	X
ADDITIONAL STUDY ASSESSMENT AND PROCEDURES (control and intervention arm)						
Eligibility criteria	X					
Informed consent	X					
Clinical evaluation[6]	X	X	X	X	(X)	X
Medical history	X	X	X	X	(X)	X
Digital chest X-Ray	X				(X)	X
TB drug adherence assessment		(X)	(X)	(X)		(X)
TB treatment response				X		X
Lab assessment:						
- HIV test[7]	X					
- Malaria test	X					
- Complete blood count	X					
Biobank (frozen samples):						
- Plasma sample	X					
- Whole blood sample	X					
Maximum number of tubes collected[8]	5					
Maximum volume of blood collected[8,9]	10.5 mL					
TB-SPEED INTERVENTION (intervention arm only)						
Nasopharyngeal aspirate	X					
Stool sample	X					
Xpert MTB/RIF Ultra	X					
Immediate TB treatment initiation if positive Ultra test[2]	X					
Safety/feasibility/tolerability/ acceptability assessment	X					
Biobank: NPA and stool leftovers	X					

(1) An extra TB visit will be performed if the child presents with signs and symptoms in favour of a presumptive TB
(2) According to national treatment guidelines based on WHO recommendations
(3) At inclusion, O_2 saturation will be measured before oxygen therapy.

(4) TB will be evaluated using routine procedures in children with a clinical suspicion of TB (chronic symptoms, failure to respond to antibiotic treatment or TB exposure). Xpert or Ultra, depending on local availability, could be used according to the clinician's judgement, using sample collection methods usually implemented at the ward.
(5) In the intervention arm, TB treatment will be initiated immediately in case of a positive Ultra result. In both arms, TB treatment could be initiated in case of a strong clinical suspicion.
(6) Content of clinical evaluation varies with the visit (detailed in the protocol); includes TB exposure and symptoms assessment at inclusion
(7) Performed if not available in the patient medical chart. In Côte d'Ivoire, should be discriminant for HIV 1 and 2.
*(8) **In children >18 months:** 2 mL of whole blood collected on EDTA or plain tubes for HIV serology; 3 x 2 mL collected on EDTA tubes for malaria test, CBC, and plasma biobank; 2.5 mL collected on Paxgene Blood RNA tubes for whole blood biobank. **In children <18 months**: 500 µL (maximum) dried blood spot or a 2mL EDTA tube for HIV PCR; 3 x 2 mL collected on EDTA tubes for malaria test, CBC, and plasma biobank; 2.5 mL collected on Paxgene Blood RNA tubes for biobank. **In children <18 months weighing <5 kg, or children presenting with signs of severe anaemia (conjunctival or palmar pallor):** plasma sample for biobank will not be collected.*
(9) Volume of blood draw must not exceed 3 ml/kg/visit and 7 ml/kg/6 weeks

7.4. Inclusion visit

The following procedures will be performed during the inclusion visit:

- **Complete clinical evaluation**
  - Demographic information (month and year of birth, sex)
  - Interview of parent/guardian on history of the disease, symptoms, past and current medication, immunization (including BCG, Pneumococcal Conjugate Vaccine, Haemophilus Influenza b, Measles Mumps Rubella)
  - TB assessment: family exposure, fever, cough for more than 2 weeks, weight loss
  - History of chronic diseases (HIV, asthma, diabetes, cardiac disorders, …)
  - Physical examination: central cyanosis, severe respiratory distress, respiratory sounds, adenopathy, hepatomegaly, level of consciousness (Blantyre scale), assessment of nutritional status
  - Vital signs: respiratory rate, heart rate, temperature, weight, height, measurement of oxygen saturation
- **Radiographic exams**
  - Chest X-ray (CXR): standard anteroposterior and lateral view (to be performed within the first 48 hours after inclusion).
- **Blood samples**
  - For HIV testing (in children with unknown HIV status, using national guidelines)
  - For malaria testing (rapid test and/or thick smear)
  - For complete blood count
  - For biobanking (frozen samples)
- **Bacteriological specimen collection (intervention arm)**:

Initial bacteriological specimen collection will be done as soon as possible and within 24 hours of hospital admission, including:

- 1 NPA, collected by the nurse on the day of admission without prior nasal instillation (see Appendix 3); NPA will be done under SpO2 monitoring.
- 1 stool sample, collected as soon as the child is able to produce stool (see Appendix 4).

The tolerability, feasibility, acceptability and AEs linked to bacteriological specimen collection will be assessed by study nurses using qualitative and quantitative tools (Cf Chapter 7.6).

Bacteriological tests performed on collected specimen are detailed in Chapter 8.2.

Extra tests not planned by the protocol will be requested by the clinician in accordance with his/her practice and national recommendations.

- **TB treatment initiation in children with a positive Ultra result**

TB treatment using 2HRZE/4HR[3] with new paediatric formulations according to National Guidelines will be initiated immediately in children with positive Ultra results, without waiting for the next early morning and overnight fasting. To facilitate immediate treatment initiation, the study will provide to implementing sites a drug buffer stock if needed. Children with rifampicin resistance detected by Ultra will have culture and phenotypic DST performed on leftovers from NPA and stool (and additional samples if needed), and will be started on empirical MDR-TB treatment. Arrangements will be made with NTPs for fast-track MDR-TB treatment initiation.

7.5. Follow-up

All children will be followed up for a total duration of 12 weeks, with four systematic trial visits planned at:

- day 3,
- hospital discharge,
- 2 weeks after discharge (as recommended by the WHO SOC),
- 12 weeks after inclusion (Table 3).

Each visit will comprise:

- Clinical evaluation: complete physical examination, vital signs, nutritional status
- Medical history since the last visit: any new clinical and AE with special attention to severe AEs resulting from NPA collection
- Evaluation of adherence to TB treatment, if initiated
- Chest radiography (for W12 visit and extra TB visit if applicable)
- Study drug prescription and dispensation to cover time to the next follow-up visit

At 2 weeks post-discharge, children with HIV or SAM will be assessed for ART and nutritional rehabilitation adherence and co-morbidities. Additionally, treatment response will be evaluated at 2 weeks post-discharge in those initiated on TB treatment. Children deteriorating or showing no improvement on TB treatment will undergo further evaluation by the clinical team.

Parents/guardians will be invited to bring their child back to the hospital in case of new symptoms for an unscheduled visit (see Chapter 7.7)

During the last follow-up visit at 12 weeks, an assessment of TB disease evolution and response to treatment will be made (improvement, treatment failure, death, lost to follow-up) in those diagnosed for TB and initiated on treatment.

The dates of each visit must comply with the provisional patient schedule generated from the date of inclusion of the child. In case of delayed visit at a specific date, dates of subsequent visits must comply with the initial visit schedule. 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 and acceptability assessment of NPA and stool collection procedures

- **Feasibility**

Feasibility will be defined using a series of indicators including the proportion of children with NPA and stool samples performed when the test is expected per study protocol, the proportion of samples collected and tested by Ultra, and the turnaround time.

- **Acceptability and tolerability**

Assessment of the tolerability and acceptability of the TB-Speed strategy at the parent, child, and healthcare level will be assessed using qualitative and quantitative tools. Study nurses will

[3] *Isoniazid + rifampicin + pyrazinamide + ethambutol for 2 months, followed by isoniazid + rifampicin for 4 months*

coordinate and perform data collection on site using pain evaluation tools (Wong-Baker face Scale, Visual Analog Scales, FLACC scales) and auto-questionnaires. A dedicated qualitative junior researcher/interviewer will be specifically hired for semi-structured interviews (SSIs), under the supervision of senior researchers at University of Bordeaux.

- Nurses' acceptability regarding the collection of NPA and stool samples will be evaluated using both quantitative and qualitative methods. Quantitative assessment will be based on a self-reported questionnaire. Qualitative assessment will be done using semi-structured interviews at individual level.
- Children's tolerability will be measured according to the level of discomfort/distress/pain felt assessed by the child, the parents/guardians and the nurses, using a set of validated tools. This evaluation will include: at child level, the Wong-Baker Face scale; at parent(s)/guardian(s) level, the Visual Analog Scale (evaluating child's tolerability); at nurse level, the FLACC (Face Legs Activity Cry Consolability) behavioural scale. These assessments will be conducted in a subset of children in all participating countries.
- Parents' acceptability regarding the whole sampling strategy will also be evaluated using both quantitative (questionnaires) and qualitative methods (semi-structured interviews).

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

Whenever enrolled children become ill, they will have access to medical personnel during the business hours of their respective trial site. If children present with signs and symptoms of presumptive TB, an extra TB visit will be performed. This will include standard bacteriological samples (gastric aspirate or induced sputum according to national guidelines) tested with Ultra and chest X-ray in addition to full clinical assessment. Children will receive care in the form of consultations, inpatient day care and inpatient hospitalizations, dependent on the severity of disease. Care will be provided in accordance with national guidelines and trial specific SOPs. Data will be collected in a similar way to routine protocol visits.

7.8. Management in the case of selected adverse events

7.8.1.

Management in the case of sample collection adverse events

Expected AEs occurring from NPA collection procedure include, by decreasing order of frequency: cough (this induced cough reflex is expected as it is the mechanism by which sample is obtained), nausea, local trauma/nose bleeding, sneezing, vomiting, and in rare cases dyspnea/low O_2 saturation and heart rate deceleration <60/mm [33].

NPA will be performed by study nurses under O_2 saturation monitoring. In case of acute O_2 desaturation or respiratory distress occurring during sample collection, the procedure will be

7.8.2. immediately interrupted and the child started on O_2 therapy. If a sample could not be obtained, a new attempt will be performed as soon as the child's respiratory status allows for it.

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

Management in the case of treatment-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

For each randomized individual, the last visit will occur 12 weeks after inclusion. Once children have completed the 12 weeks follow-up period, they will end their participation in the trial and will benefit from the regular care provided by National Programs.

7.10. End of the research

Definition

Each randomized patient is followed up for 12 weeks. The official end of the trial, except in case of premature termination, is defined by the last visit of the last patient included in the study. Once patients complete 12 weeks of follow-up, they will benefit from the regular care
7.10.1. provided by National Programs of their respective countries.

The sponsor or its representative will notify the end of the trial 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), the IDMC, or the ethical and/or regulatory authorities issuing a decision to discontinue the trial. If the trial 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.

Withdrawal of consent

7.10.2.

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 trial 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 trial.

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 trial 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 analyses.

Withdrawals of consent to participate in the trial must be reported to the country CTU as soon
7.10.3. 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).

Loss to follow-up

When a child for whom parent(s)/guardian(s) have not explicitly withdrawn consent does not show up for routine clinic visits, the trial team must make every effort to contact the parents(s)/guardian(s). With their prior agreement, the trial team 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.

A child who has not withdrawn consent or transferred out, and who does not show up at the W12 visit is considered definitely lost to follow-up in the trial, unless he/she is known to be deceased. The date of lost to follow-up will be the date of his/her last contact with the trial team (either at the hospital, via telephone, or at home).

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.

7.11. Post-trial care conditions

Once they leave the trial, children will continue to receive care and treatment according to the conditions defined by their country authorities. Children with ongoing TB treatment at W12 will be followed-up and treated under the responsibility of the national TB program. Until the ongoing TB episode is cured, the trial investigators, in close collaboration with the TB program, will actively contribute to facilitate their access to the best available TB disease management.

8. LABORATORY AND RADIOLOGIC EVALUATIONS

8.1. Biological specimen collection

NPA, stool and blood samples are collected at inclusion (see Table 4) for laboratory tests performed onsite or at the country reference laboratory, as well as for biobanking (see chapter 8.3).

All specimen collection methods and biological exams procedures will be detailed in a manual of SOPs to be translated into the language used by the staff on site (English, French, Khmer, and Portuguese).

- **Nasopharyngeal aspirates**

The collection of the contents of the oropharynx is done by mechanical suction through a graduated suction tube inserted into the nostril while the child is seated (See appendix 3). The NPA will be performed using a battery-operated device.

- **Stool sample**

Stools cannot be tested with the GeneXpert device without prior processing to avoid indeterminate or falsely negative results due to the presence of PCR inhibitors. In this study, stool processing will be performed using the flotation method based on Sheather's sucrose solution previously used in the PAANTHER 01 study (Appendix 4). Stool processing optimization will be largely developed as a separate work-package of the TB-Speed project (Output 4). It will assess centrifugation-free methods for stool processing hence generating evidence on the diagnostic value of stool as an alternative specimen for TB diagnosis in resource-limited settings.

- **Blood samples**

Table 4: summary of blood samples collected according to age and weight

	Tube	Children <18 months		Children >18 months
		>5 kg	<5 kg or anaemia[a]	
- HIV Serology	Plain/EDTA	NA	NA	2 mL
- HIV PCR	Dried Blood Spot (or EDTA)[b]	0,5 mL (or 2 mL)[b]	0,5 mL (or 2 mL)[b]	NA
Malaria test (thick smear)	EDTA	2 mL	2 mL	2 mL
Complete blood count	EDTA	2 mL	2 mL	2 mL
Biobank:				
- Plasma	EDTA	2 mL	NA	2 mL
- Whole blood	PAXgene	2,5 mL	2,5 mL	2,5 mL
Number of tubes collected		5	4	5
Maximal volume of blood collected		9 mL (or 10.5 mL)	7 mL (or 8.5 mL)	10.5 mL

[a] Plasma sample for biobank will not be collected in children weighing <5 kg or those presenting with signs suggestive of severe anaemia (conjunctival or palmar pallor). Volume of blood draw must not exceed 3 ml/kg/visit and 7ml/kg/6 weeks.

[b] May be performed in Cameroon and Côte d'Ivoire, where Dried Blood Spot (DBS) is not systematically implemented

Blood collection will be performed following specific SOPs guiding nurses on highest priority tests in case sub-optimal volumes of blood are obtained from a child. Tests planned for clinical management of the child will be prioritised over samples for biobank.

8.2. Laboratory assessment

8.2.1.

HIV testing

HIV serology will be performed at inclusion if not available in the patient medical chart, according to national routine practices. In Côte d'Ivoire, the serology test should be discriminant for HIV-1 and 2.

8.2.2. HIV testing in children aged less than 18 months will be based on an HIV RNA or DNA nucleic acid amplification tests (PCR).

TB bacteriological tests

In the intervention arm, the Ultra assay will be performed on the following samples:

- one untreated NPA,
- one stool sample following processing.

Ultra testing will be done at the hospital laboratory on standard G4 platforms for both samples or on the ward using G1 or Omni devices with appropriate infection control procedures.

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 sample, the global result will be given as "MTB detected".

- In case of negativity of Ultra performed on the two samples, 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.

  If volumes are insufficient to repeat testing, no additional sample will be collected unless the child has symptoms or signs suggestive of TB (persistent cough or fever for more than 2 weeks, and failure of antibiotics treatment). The child will be evaluated for TB using routine procedures, which may include sample collection (gastric aspirate or other sample according to country practices) for Xpert testing, as recommended by WHO.
- 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 NPA and stool (with additional samples taken if needed), and will be started on empirical MDR-TB treatment according to national guidelines.

When performed at the hospital laboratory, the result of the Ultra test will be communicated to the study nurse by text message by the laboratory technician and to the treating physician as soon as available. If positive, TB treatment will be initiated immediately.

In case of TB treatment failure or poor treatment response, appropriate samples will be collected and resistance to TB drugs will be assessed using methods available on sites (phenotypic DST or line probe assays).

Routine bacteriological tests will not include smear microscopy or mycobacterial culture. Leftovers of NPA and stool samples will be kept at -80°C for future ancillary/sub-studies. Extra bacteriological tests not planned in the protocol may be requested by the clinician in accordance with his/her practice and national recommendations.

8.2.3. *Laboratory quality control*

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

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.

8.3. Frozen samples and biobank

Leftovers from NPA and stool (collected as part of the intervention), gastric aspirates or sputum (collected as part of routine practice in children with a clinical suspicion on TB), as well as whole blood and plasma samples will be frozen and stored at the Central Laboratory level.

8.3.1. Biobank samples may be used for further retrospective bacteriological analyses, as well as for future immunologic, metabolic, and host genomic studies.

Procedures for preparation of frozen samples and biobank conservation will be detailed in study-specific SOPs.

Justification

Among the WHO-endorsed Priority Target Product profiles (TPP) for TB is a biomarker-based, non-sputum-based rapid test for detecting active TB with the purpose of initiating treatment [62]. Recent efforts in the field of paediatric TB diagnostics have revealed the urgent need for point-of-care diagnostic tools which are more efficient, affordable, and adapted to high-burden settings.

In young children, who presents with a paucibacillary disease and are unable to expectorate sputum, the presence of host markers in accessible non-sputum samples such as peripheral blood would be of great advantage. However, to date few biomarkers have proven to be of value in discriminating childhood TB from other diseases, as well as active TB from latent TB infection. Measurement of immune response molecule concentrations, such as interferon gamma and C-reactive protein, is a complementary strategy to the direct detection of *M. tuberculosis*. However, to this end interferon gamma release assays are not useful as they are unable to discriminate between latent and active TB. Findings from metabolomics studies have provided useful information on the host metabolic response to *M. tuberculosis* infection, but their potential as a TB diagnostic has yet to be confirmed. Emerging research using transcriptional biosignatures in whole blood has been the most promising (accuracy >80%). Studies in cohorts of children from South-Eastern Africa, South America and India have identified mRNA signatures and gene sets capable of distinguishing active disease from latent TB infection [63]–[66], as well as TB from non-TB pneumonia [67]. However, these candidate transcriptomic signatures now require further exploration as well as cross-validation in prospective cohorts of patients from multiple settings and genetic backgrounds [64].

Type of samples and purpose

- **NPA, stool, gastric aspirate, sputum**

8.3.2.

Bacteriological investigations such as culture and mycobacterial antigen assays could be performed retrospectively on NPA, stool, gastric aspirates or sputum leftovers.

- **Whole blood**

2.5 mL of whole blood will be collected on PAXgene® Blood RNA tube.

Whole blood could potentially be used for genomic or transcriptomic analyses to discriminate TB from non-TB pneumonia, including mRNA transcripts and micro RNAs, based on state-of-the-art knowledge subsequent to the study.

- **Plasma**

1 mL of plasma will be aliquoted from a 2 mL ETDA tube collected at inclusion.

The plasma sample for biobank will not be collected in children weighing <5 kg or from those presenting with signs suggestive of severe anaemia (conjunctival or palmar pallor).

8.3.3. Plasma samples could be used to further characterize the proteomic, metabolic and immunologic profile of children presenting with signs of severe pneumonia, with or without TB.

Storage

During the trial, biological samples will be stored at -80°C at the country Central Laboratory (see Appendix 8). Transfer of samples will be done according to the internal procedures of implementing sites, which are checked by the international coordinating CTU before the beginning of the trial. Biobank-related data will be reported in the eCRF, including type of sample, date and volume collected. Each country reference laboratory may maintain his own biobank management system, where study IDs will be the only identifiers.

Subject to approval by relevant Ethics Committees, frozen samples may be sent to external laboratories (inside or outside the country) for additional analyses performed as part of ancillary studies. The transfer of biological materials will be covered by a Material Transfer Agreement submitted to appropriate ethics committees and regulatory authorities of both recipient and supplier countries.

8.4. Radiological assessment

CXR is part of the inclusion and W12 final study visits, as well as the extra-TB visit if applicable (see patient's schedule, chapter7.3).

A 2-view chest radiograph (anteroposterior and lateral) will be performed using standard analogue X-ray machines with digital plates, or digitalized radiography machines where possible.

Digitalized CXRs will be archived on a centralized database accessible through a secured website (the Mereva tool, as described in Chapter 10).

CXRs will be reviewed independently by two readers blinded to clinical and biological data to identify CXR lesions consistent with pneumonia and TB [68]. Discordant opinions will be resolved by a third reader.

9. STUDY VIGILANCE

9.1. Definitions

- **Adverse events**

An 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 trial documentation or the conduct of the trial, or to suspend or interrupt or modify the protocol or similar trials.

Examples: a SAE which could be associated with the trial procedures and which could modify the conduct of the trial, recommendations of the IDMC, if any, where relevant for the safety of subjects.

- **Severity**

The severity of an AE caused by NPA collection 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 trial intervention and any other concomitant intervention/medication.

9.2. Expected adverse events related to the study intervention

Expected AEs occurring from NPA collection procedure include, by decreasing order of frequency: cough (this induced cough reflex is expected as it is the mechanism by which the sample is obtained), nausea, local trauma/nose bleeding, sneezing, vomiting, and in rare cases dyspnea/low O_2 saturation and heart rate deceleration <60/bpm [33].

No AEs are expected from stool sample collection.

Overall, children tolerate anti-TB drugs very well when using currently recommended dosages. SAEs are rare and even mild symptoms such as nausea or vomiting are uncommon. There are occasional case reports of severe hepatotoxicity [70].

Occurrence of AEs will be monitored by study nurses and reported in the Case Report Form (CRF). Management in case of AEs will be detailed in the SOP (see chapter 7.7.1).

9.3. Reporting of adverse events

AEs occurring as a consequence of NPA collection, primary and secondary diagnosis of illness causing initial hospitalisation, death and cause of death, primary and secondary diagnosis of illness causing any hospital readmission will be reported in the CRF.

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

9.4. Notification of serious adverse events

In this diagnostic trial 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, defined as grade 4 clinical AEs, excluding asymptomatic biological AEs of grade 4
- SAEs related to NPA collection.

9.5. Responsibilities of the investigators

The investigators are responsible for:

- Grading the severity of AEs occurring from NPA collection reported by study nurses as severe or potentially life-threatening
- Reporting SAEs, as defined above, to the sponsor and to the appropriate country authorities, according to the procedures described in section 9.4.2.
- Assessing the causality of all SAE in relation to the trial 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 occurs in a participant:

- from the date of signature of the informed consent to the trial;
- during the follow-up of the participant scheduled by the trial;

- until 4 weeks after the end of follow-up when it still could be related to the trial intervention.

9.6. Responsibilities of the sponsor

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 relation to the NPA collection. In the absence of information on causality from the reporting investigator, the
9.6.1. 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.

New fact reporting

9.6.2.

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 trial.

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

10. DATA COLLECTION AND PROCESSING

10.1.1.

10.1. Description of data collected

Aggregated data

In all participating hospitals, baseline information on the standard of care will be collected in a
10.1.2. Microsoft Access database located at country CTU level during a two-month lead-in period prior to the initial rollout of the intervention, including: number of children hospitalized for severe pneumonia, inpatient mortality, antibiotics use, access to oxygen therapy and other supportive care.

Individual patient data

Once enrolled in the study, the following data will be collected for each patient by study nurses, on remote data capture devices:

- Individual identifiers: month and year of birth, sex
- Anthropometric and clinical data: weight, height, vital signs, symptoms, treatments and adherence, AEs

- Data on the tolerability/acceptability of NPA collection (qualitative and quantitative)
- Radiological data: digital images and interpretation
- Laboratory data: HIV testing result, Ultra results
- Samples collected for biobanking
- Pneumonia management: antibiotics, oxygen therapy, other
- TB treatment if initiated
- Comorbidity management: antiretroviral treatment, nutrition therapy, other
- Outcome data: end of trial status or early study termination (death, lost to follow-up, withdrawal)

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.

Cost data

We will collect data to estimate the costs of the systematic TB diagnostic. This will include the costs of collection and testing of samples, and the other direct health costs required to treat patients, including treatment for pneumonia, TB and other comorbidities, use of equipment and staffing costs.

10.1.1

We will measure and record, or where necessary estimate, resource utilization for both systematic TB early detection and for the standard of care in children with severe pneumonia. Unit costs will be collected from relevant sources including clinic and hospital site visits, accounts and invoices from the TB-Speed project and individual facilities, pharmaceutical and medical equipment manufacturers, Ministries of Health and NTPs.

To estimate human labour costs, we will conduct a time and motion study survey to estimate quantities of staff time involved in different health care tasks. To do so, we will ask nurses, doctors and other health workers participating in this study to self-complete timesheets recording the length of time they spend conducting each task.

Costs will be expressed in U.S. dollars, converted using purchasing power parity exchange rates, i.e. market exchange rates adjusted for differences in purchasing power between countries.

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 medical records filled by site physicians:

- Patient’s demographic data (month and year of birth, sex)
- Study name(s) and protocol number(s) in which the patient participates
- Details related to the inclusion criteria
- Date of signing informed consent form
- Dates of follow-up visits
- Medical history and physical examination details
- Laboratory print-outs (HIV testing)
- AEs and concomitant treatments

For the purpose of the trial, specific forms may be developed for source data collection to be inserted into medical records.

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 Output 1.

In the same way, Ultra test result files (.gxx files) will be extracted directly from the GeneXpert software.

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

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 trial SOPs in accordance with good clinical, scientific and data management principles.

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
10.3.1. 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 trial manager in close collaboration with the international data manager and international clinical research assistant.

Data hosting

10.3.2.

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, 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
10.3.3. electronic records and electronic signatures.

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).

10.3.1.

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.

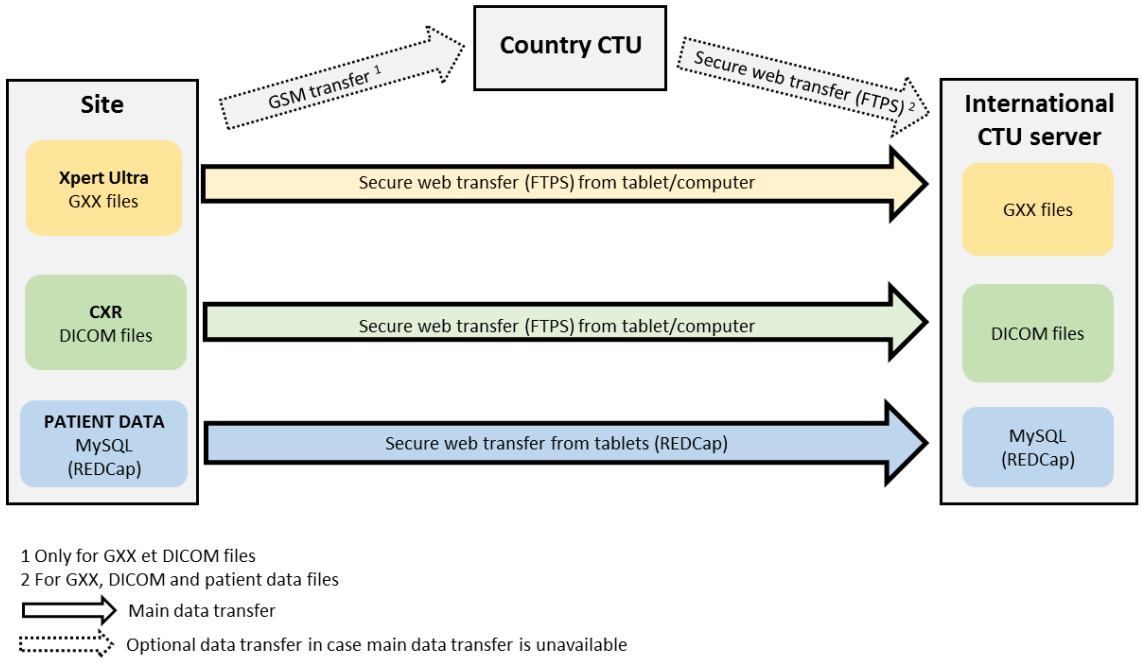
Data entry

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 2: 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.

10.3.2.

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.

10.3.3

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) .

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

A data management plan (DMP) 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 DMP.

A data management system (DMS) will be developed at UBx to enable generation of standardized lists of data management queries at country level. Queries will be programmed by the international data manager for data completeness, integrity and consistency as defined in the DMP. They will be run at the country level on a monthly basis at a minimal. Data management checks will be implemented at the central level on a monthly basis. To further improve quality of the data, centralized correction queries will be sent by the international coordinating CTU to the country CTU, and by the country CTU to trial sites. At country level, queries will be managed by the country data manager or Clinical Research Assistant (CRA). Queries will be solved by data managers along with the country CRA.

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 filling the CRF data, protocol compliance, AEs, 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. Closure of the database will be performed by the international data manager.

10.5. Length of data retention, archiving conditions and management

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 trial documents will be retained at the coordinating centre for 15 years after study completion.

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

10.6. Study documents archiving conditions and management

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

Trial documents constituting the Trial Master File (TMF) 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 trial website. Country CTUs will be responsible for routinely updating national documentation on the trial website.

Investigators will ensure that trial records are not disposed of or removed from the trial 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. TB-SPEED COVID-19 SUB-STUDY

11.1. Background and rationale

Since December 2019, a novel coronavirus emerged in Wuhan, Hubei province, China and has rapidly spread to the rest of the world, becoming a pandemic [71], [72]. The WHO has named it "2019 novel coronavirus" (2019-nCoV or SARS-CoV-2) and has declared Covid-19 a pandemic and public health emergency of international concern. This is the 3rd coronavirus epidemic of large public health threat after the severe acute respiratory syndrome (SARS-CoV-1) and the Middle East respiratory syndrome (MERS-CoV) [73], [74]. Community transmission of SARS-Cov-2 virus has been reported in many African countries, with figures on cases and deaths likely to be underestimated due to the limited access to tests. All countries participating in the TB-Speed Pneumonia study have reported cases. As of June 2nd, 2020, there were 125; 6,397; 2,951; 254; 489 and 1,089 cases reported in Cambodia, Cameroon, Côte d'Ivoire, Mozambique, Uganda, and Zambia, respectively[4] .

Children are largely untouched by Covid-19. Data from China, the United States (US) and Italy showed that only about 2% of all cases were children [75]–[79]. First data from Africa show than only 2.5% of cases were young children below the age of 5 years [79], [80]. No racial predilection has been observed in children, although emerging US data in adults suggest that minority communities may be affected disproportionately [81]. Despite the worldwide spread, the epidemiological, clinical, therapeutic and evolutionary patterns of Covid-19 remain largely unclear among children. In the largest Chinese paediatric case series to date, of 2 143 subjects, children younger than 5 years old represented 40% of all paediatric cases and 60% of the severe cases [82]. In this study, there were more boys (56.6%) than girls. Slight male predominance was also seen in the US data [83]. Few neonate cases have been reported and no cases of intra-uterine transmission was notified [84]. The median incubation period in children is usually 3 ~ 7 days (range of 1-14 days) [85].

Covid-19 has been diagnosed using real-time RT-PCR assays for 2019-nCoV nucleic acids [86], [87] on nasal swabs, sputum, lower respiratory tract secretions, stool, and blood. Few paediatric cases with negative respiratory samples were diagnosed using stool samples, on which RT-PCR is usually less sensitive [88]. Authors from a French study of 99 confirmed cases reported that SARS-CoV-2-positive children exhibited viral loads that do not differ significantly compared to those of adults [89]. In another study from French cluster, the authors have confirmed this similar viral loads in asymptomatic children and have suggested a potential different transmission dynamics in children [90]. Clinical diagnosis has also been proposed using a combination of at least two symptoms, laboratory tests and chest X-ray findings [91], [92]. Chest radiography and computed chest tomography show in the early stage of disease, multiple small plaques and interstitial changes, which are obvious in the lung periphery, that further deteriorate to bilateral multiple ground-glass opacity and/or infiltrating shadows. Lung consolidation may occur in severe cases.

[4] Source: COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University.

Immunological assays for SARS-Cov-2 infection will be useful to assess seroprevalence and seroconversion of infected people. The Institut de Recherche pour le Développement (IRD) has developed a high throughput serological screening assay using the Multiple Analyte Profiling (xMAP) technology (Luminex Corp. Austin, TX, USA). The assay was evaluated on a panel of well documented positive and negative samples (IgG and/or IgM antibodies) obtained from reference laboratories in Europe and collaborators[5]. It is also developed for IgM antibodies to SARS-CoV-2, to allow evaluation of recent infections on plasma, whole blood, DBS, saliva and fecal samples.

There is no data reporting an increase of poor outcome of the Covid-19 infection in people living with HIV when they are well controlled by antiretroviral therapy. Although obesity has been reported as a predictor of poor outcome in adults, there is no data with undernutrition in children [93]. Additionally, children with sickle cell disease who present viral respiratory infection may have an increased risk of bacterial sur-infection and therefore worsening of the clinical presentation as shown in a small study of children with sickle cell disease and H1N1 influenza viral infection [94].

This sub-study will assess the prevalence and impact of the Covid-19 in young children hospitalized with severe pneumonia. The sub-study findings are expected to guide policy makers and clinicians on potential specific screening and management measures for these vulnerable groups of children. They are also key to analysing TB-Speed Pneumonia results on mortality in a context of the Covid-19 outbreak and to take into consideration SARS-CoV-2 infection status in the main study analysis.

11.2. Sub-study objectives

- To assess the prevalence of Covid-19 (confirmed and probable cases) in children below 5 years admitted with WHO-defined severe pneumonia
- To assess the impact of SARS-CoV-2 infection on the clinical outcomes of children with severe pneumonia
- To describe the clinical, laboratory, and radiological characteristics of Covid-19 cases
- To assess the yield of stool as compared to nasal swab for the detection of the SARS-CoV-2 by real time RT-PCR
- To assess the contribution of other viral respiratory infections (at least RSV) on Covid-19 outcome in children with severe pneumonia
- To assess seroprevalence and seroconversion (IgM and IgG to SARS-CoV-2) at Day 0 and Month 3

11.3. Case definition

Confirmed and probable cases are defined according to WHO Covid-19 technical guidance [95].

- **Confirmed case**: A person with laboratory confirmation of Covid-19 infection, irrespective of clinical signs and symptoms (using nucleic acid amplification tests such as RT-PCR).
- **Probable case**:
  - A suspect case for whom testing for the Covid-19 virus is inconclusive. OR
  - A suspect case for whom testing could not be performed for any reason

See detailed definitions of suspect case per country in Appendix 9.

11.4. Sub-study population

- All children enrolled in the TB-Speed Pneumonia study accepting enrolment in the Covid sub-study

[5] Ayouba A, et al. Multiplex detection of IgG antibodies to SARS-CoV2 and the highly pathogenic human Coronaviruses SARS-CoV and MERS-CoV. Clinical Infectious Diseases, 2020. Submitted

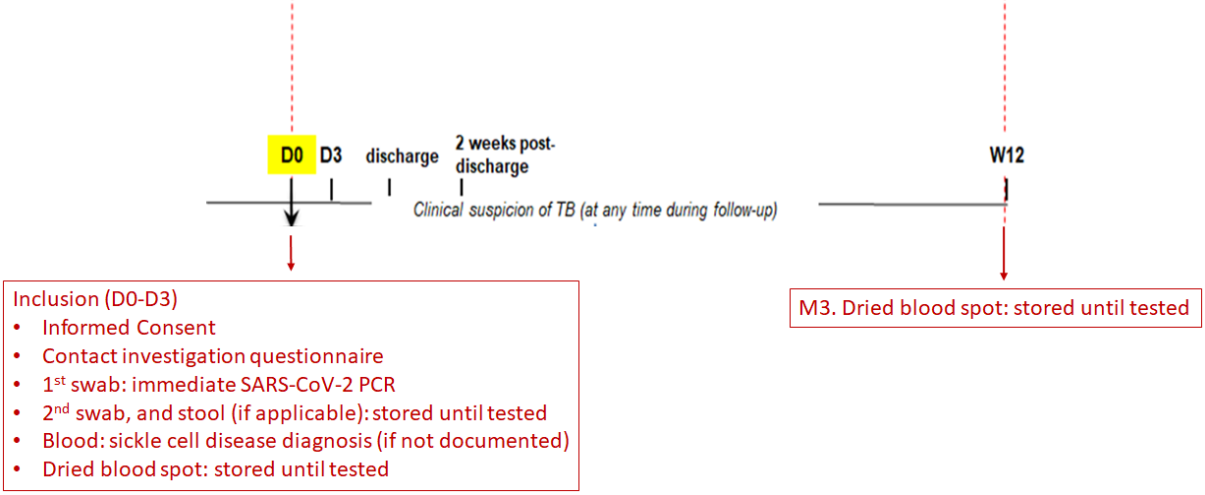
11.5. Sub-Study design

This will be an observational sub-study nested in the TB-Speed Pneumonia trial. The sub-study will be implemented in all participating countries except Cambodia.

At the time of enrolment in the TB-Speed Pneumonia main study, patients will be also offered to participate to the Covid ancillary study. Children will be tested for SARS-Cov-2 on the day of enrolment. The duration of enrolment in the sub-study will be of 6 months in total. Duration of follow-up for a particular participant will be 12 weeks, as in the main Pneumonia study.

The study scheme of the nested Covid-19 study is presented in Figure 3 below.

Figure 3: TB-Speed Covid sub-study flow chart



- **Sample size**

There is no sample size calculated. We propose to enrol all consecutive children included in the TB-Speed Pneumonia study over a 6-months period when enrolment in the study will resume. Based on the average number of inclusions per month between March 2018-March 2020 in the project countries, the expected number of children to be enrolled in the TB-Speed Covid sub-study is 942[VA1][MB2] in 6 months.

- **Statistical analysis**

Prevalence (and 95% CI) of SARS-CoV-2 infection at enrolment will be calculated. Summary statistics of patient characteristics will be described. Differences in patients' characteristics between SARS-Cov-2 infected and non-infected patients will be assessed using Chi-square, Student's t-test or Wilcoxon rank-sum test as appropriate. Bi- and multivariate analysis will be used to identify effect of the Covid-19 disease on the children's outcomes. Factors associated with children's outcomes will be also analysed.

A preliminary analysis of prevalence and outcomes will be performed at 3 months (mid-study) and discuss with the SAB in order to support case detection and management decision.

11.6. Management of Covid-19 cases

At each study site, management of Covid-19 cases will follow national recommendations (see Appendix 9). This will require isolation of cases and potential antiviral treatment in addition to the SOC for severe pneumonia, as well as TB diagnosis on NPA and stool for sites implementing the TB-Speed Pneumonia study intervention. Prescription of specific treatment against Covid-19 will be decided by site clinicians in accordance with country guidelines, subject to modification during the study implementation period.

As soon as a child will be identified a Covid-19 case, the disease control team at site level will be informed in order to ensure that contacts are traced and additional suspect cases tested and isolated according to the national policy.

In the event that a child is transferred to a Covid isolation centre, study staff will ensure compliance and continuity of the main TB-Speed Pneumonia study procedures and follow-up visits, including appropriate TB diagnosis and timely access to TB treatment if needed.

11.7. Sub-study procedures

As part of the overall informed consent process of the TB-Speed Pneumonia study, parent(s)/guardian(s) will be informed about the Covid sub-study (see Appendices 6 and 7). Patient participation in this ancillary study is voluntary; parents will be free to opt out from the Covid sub-study without any consequence on the participation of the child to the main Pneumonia study. Only children whom parents have approved participation to main study will be able to participate in the sub-study.

- **Inclusion visit (D0-D3)**

The following procedures will be performed during the TB-Speed Pneumonia inclusion visit:

- Questionnaire on Covid-19 contact investigation: parent/guardian will be asked questions regarding the child's exposure to the SARS-Cov-2
- Unless national recommendation for SARS-Cov-2 sample collection is different (Appendix 9), one nasopharyngeal (or oropharyngeal) swab will be collected for immediate SARS-Cov-2 RT-PCR
- In children with a positive SARS-Cov-2 RT-PCR only: a second swab will be collected before hospital discharge and stored for further testing of other respiratory viruses (at least RSV) to interpret clinical outcomes (see Table 5)
- A blood sample collected for diagnosis of SCD if not previously documented
- A DBS collected and stored for further serological testing of the SARS-Cov-2 specific antibodies (IgM and IgG)
- A stool sample stored for further SARS-Cov-2 RT-PCR (see Table 5).

- **Month 3 visit**

A second DBS will be collected and stored for further serological testing of SARS-Cov-2.

There will be no additional specific follow-up visits for Covid-19 on top of those planned for children enrolled from the main TB-Speed Pneumonia study (see 7.3. Patient schedule).

11.8. Laboratory evaluations

- **Biological samples collection**

Procedures for sample collection, transportation, and processing at the laboratory will be detailed in specific SOPs.

Table 5: Biological samples collected for the TB-Speed Covid sub-study

Sample type	Minimal amount required	Laboratory test	D0-D3	M3
1st naso/oropharyngeal swab[a]	minor (swabbing of the nasal/pharyngeal mucosa surface)	SARS-CoV-2 RT-PCR	X	
Stool sample (stored)[b]	1 g	SARS-CoV-2 RT-PCR[c]	X	
2nd naso/oropharyngeal swab (stored)[d]	minor (swabbing of the nasal/pharyngeal mucosa surface)	RSV RT-PCR	X	
Blood (EDTA or dry tube)[e]	2 mL[e]	Sickle cell disease	X	
DBS	approximately 50 µL (0.05 mL) collected by finger/heel/toe prick	SARS-CoV-2 serology	X	X

[a] *For young infants (< 6 months) an oropharyngeal swab is preferred due to the small diameter of nasal passage and increased risk of trauma.*
[b] *In the intervention arm, if the volume of the stool sample collected for the TB-Speed Pneumonia study does not allow for 1) Ultra testing, 2) biobanking, and 3) SARS-CoV-2 testing, an additional stool sample will be collected.*
[c] *SARS-CoV-2 PCR on stool will be done only: 1) in children with a positive result on the 1st swab, or 2) in children with a Covid-19 suspicion but a PCR on the 1st swab negative or inconclusive*
[d] *The 2nd swab will be collected only in children with a positive SARS-CoV-2 RT-PCR, before discharge.*
[e] *Possibly performed on the same tube than CBC for the Pneumonia study to avoid additional sampling. Volume of blood draw must not exceed 3 ml/kg/visit and 7 ml/kg/6 weeks.*

The first nasal swab will be referred for immediate testing whenever possible. However, depending on laboratory testing capacities and national policies, it may be possible only for children fitting indication of Covid-19 testing (i.e. meeting suspect case definition, see Appendix 9). If the testing delay is above 72h, the first nasal swab will be stored at -80°C until further testing.

The second nasal swab, the stool sample and the DBS to be tested later on will be stored temporarily at the country Central Laboratory (Appendix 8). There will be no biobanking done in the Covid sub-study. These samples will not be used for other purposes than those mentioned in the protocol, and will be destroyed after testing in accordance with applicable country regulations. At the exception of serodiagnosis on DBS, all laboratory research tests will be done in-country; the recipient laboratory in-country, and in Montpellier, France, will provide a certificate of destruction of samples once the tests have been performed.

- **RT-PCR for SARS-Cov-2**

SARS-CoV-2 virus infection confirmation will be performed by real-time RT-PCR either by in-house or commercial assays at national Covid-19 Reference Centres, namely (subject to modification depending on laboratory capacities and new accreditations at the time of the sub-study implementation): Centre Pasteur in Yaoundé, Cameroun; Pasteur Institute in Abidjan, Côte d'Ivoire; Instituto Nacional de Saude (INS) in Marracuene, Mozambique; 1) Uganda Virus Research Institute/MRC Centre in Entebbe, 2) Makerere Medical and Molecular Laboratories in Kampala, Uganda; and 1) Virology Laboratory, UTH, in Lusaka, 2) TDRC Laboratory in Ndola, Zambia.

- **RT-PCR for RSV and other respiratory viruses**

Detection of the RSV will be performed on the 2nd swab according to usual laboratory practices at site level (only in children with a positive SARS-CoV-2 RT-PCR).

Molecular diagnosis of other respiratory viruses (including influenza, parainfluenza, and adenovirus) could also be performed depending on the capacities of laboratories for multiplex PCR testing.

- **Immunological assays**

DBS collected at Day 0 and Month 3 will be sent to the Virology Laboratory, TransVIHMI Research Unit, IRD (Montpellier, France) for testing with the Multiple Analyte Profiling (xMAP) serological screening assay (Luminex Corp. Austin, TX, USA). Recombinant Nucleocapsid (NC) and Spike (S) proteins from human coronaviruses (SARS-CoV1, SARS-CoV2, MERS-CoV) will be used for a large spectrum screening and to evaluate the immune imprints of past infections with the last two viruses. In addition, synthetic peptides derived from immunodominant regions of the NC, S and membrane proteins (designed by bioinformatics and through literature searches) will be used to discriminate among known coronavirus infections.

- **Diagnosis of sickle cell disease**

Sickle cell disease will be diagnosed by haemoglobin electrophoresis according to usual procedures at site level.

11.9. Safety measures

Risk assessment by study activities and recommended infection prevention and control measures will be detailed in specific SOPs.

Study nurses will be equipped with the appropriate PPE following WHO recommendations [96], including:

- Droplets and contact precautions (clinical examination, blood, stool, and DBS collection): gowns, gloves, googles, surgical masks.
- For aerosol-generating procedures (NPA and GA collection, samples processing at the laboratory): isolation gowns, long gloves, goggles and high filtration masks

Safety boxes will be provided to dispose contaminated sharps and waste appropriately.

Triple packaging boxes will be provided to ensure biosafety during sample transportation.

12. STATISTICAL DATA ANALYSIS

12.1. Statistical analysis manager

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

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

12.2.1.

12.2. Description of the statistical analysis plan

Analysis of the primary endpoint

First, we expect no calendar effect on mortality or on the cluster effect (quantified from the ICC). Second, patient follow-up will be short (12 weeks). Finally, we will adjust on some potential confounding factors in sensitivity analyses. For these reasons, the primary analysis will be carried out with a generalized linear mixed model (GLMM). Using the intention-to-treat principle, we will model individual binary response through a logistic regression with random effect for cluster and fixed effect for step, accounting for TB incidence rate, within-cluster correlation, unequal cluster size and time-trends at the individual level [97]–[99]. We will conclude that the intervention is better than the control if the mortality odds ratio is statistically significant ($p<0.05$).

Data collected during the interruption of randomization due to the Covid-19 pandemic (cf. 4.2. Methodology) will not be considered for the primary analysis, but will be used in a sensitivity analysis as recommended by the IDMC. Furthermore, a specific IDMC meeting is foreseen

after the sites re-opening, in order to decide and validate the best analytical strategy concerning these data.

For sensitivity analyses, we will consider i) adjusting for seasonality (which may increase non-TB pneumonia occurrence and lower the apparent TB incidence), HIV infection, age, and malnutrition; ii) replacing missing outcomes (lost to follow-up and protocol withdrawals) by a failure (death) value.

As exploratory analyses, independent factors associated with death will be identified by similar models.

A detailed statistical analysis plan will be written and validated before half of the clusters have switched to intervention. Analysis will be carried out with version 9.4 or higher of the SAS® software (SAS Institute Inc., Cary, NC, USA). Amendment of the statistical analysis plan will be validated by the IDMC.

Analysis of secondary endpoints

Analysis of secondary endpoints will be detailed in the statistical analysis plan.

12.2.2. For the analysis of qualitative data on the acceptability of the sample collection procedures, SSIs will be transcribed and thematically analysed using codebooks. Quantitative results, including assessment of the child's pain and auto-questionnaires, will be performed using standard statistical tools.

Cost-effectiveness analyses

12.2.3. A mathematical model will be informed by the data collected during the trial and costs from a specific survey to project mortality and costs for the following strategies: 1) the standard of care with current TB diagnostics practice in children with severe pneumonia; 2) the new TB-Speed approach with improved and active TB diagnostics. The projected mortality will be used to estimate Disability Adjusted Life Years saved (DALYs) for each strategy. ICERs will compare the differences in DALYs and costs between the 2 strategies. ICERs will be estimated for each country separately and will be compared to previously published estimated cost-effectiveness thresholds for each country.

The cost-effectiveness analysis will be from the health payer perspective and only direct health care costs will be included. A budget impact analysis will be conducted to evaluate the actual impact of implementing the new TB-Speed approach on health care budgets at 2- and 5-year horizons in the countries participating in the project.

Sensitivity analyses will be conducted to assess uncertainties around the estimates and the robustness of our findings. Variation of parameters such as TB prevalence, TB incidence and costs will help to simulate different scenarios of implementing the TB-Speed approach, adapted to the countries' specific contexts. The latter will be important for the generalization of the results and to inform more general TB guidelines.

12.2.4.

Intermediate analyses

The IDMC will not apply stopping rules and interim analyses as per standard clinical trials because the stepped wedge design does not allow for it, since all clusters will not have received the intervention until the end of the study. As a consequence, interim analyses of stepped wedge trials are less efficient due to the unequal number of measurements in the different study arms. Adjustment of the effect estimates following a group sequential cluster-randomised trial has not be studied [100].

Furthermore, given that the stepped wedge design is most often used when the intervention is thought to do more good than harm, and in implementation research study, stopping early for harmful effects is unlikely.

For the time being, no guidance is available on the design of stepped-wedge cluster randomised trials with interim analyses. However, if the IDMC may decide later to carry out an interim analysis to decide on a potential early termination for futility (i.e., little evidence of a beneficial effect), we will look at Grayling's recent developments to propose interim analyses and stopping rules [101].

13. COMMUNICATION AND PUBLICATION POLICY

13.1. 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. To ensure respect of international standards for authorship, all publications must follow the rules contained in the publication charter defined by the TB-Speed project as part of the project communication plan. 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: "Uniforms Requirements for Manuscripts Submitted to Biomedical Journals" (http: // www.cma.ca/publications/mwc/uniform.htm).

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 and 5% initiative/. All study participants gave their informed written consent to participation, in line with ethical guidelines.*

13.2. Procedure for writing up the final report

The international CTU will establish the final report of the trial as well as summary report within a year after the end date of the trial, 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 trial, the sponsor or its representative will release to the ethical and regulatory authority of each country involved in the trial the final trial report and/or summary including the results of the trial and the scientific publications or communications related to these results.

13.3. Procedure for informing the study participants of the overall research findings

The final trial results will be presented to the investigators and the national authorities of each participating country. A series of documents (written detailed report, and short summary) will be released to help investigators, national authorities and participants to understand the results of the trial. Should the country investigator, national authorities, and patients' representatives consider it desirable, participants may be invited to attend a meeting during which the results will be presented and explained orally.

13.4. 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 site 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. However we will not communicate unsolicited findings which may result from biobank samples.

13.5. 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.

14. STUDY OVERSIGHT

14.1. Output Steering Committee

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

The OSC will consist of the coordinating investigators, country PIs and co-PIs, country project managers, the international trial manager, the laboratory coordinator, the international 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 trial progresses and results (not analysed by trial arm).

14.1. 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;
- The use of data and biological samples, and their utilisation for analyses not listed in the protocol
- 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.

14.2. Independent Data Monitoring Committee

The IDMC is a consultative board for the SAB and the sponsor. It monitors the main safety and efficacy outcome measures and the overall conduct of the trial, with the aim of protecting the safety and the interests of the trial participants. Its members will provide general advice on the progress of the trial, including the rate of inclusions, quality of follow-up, overall rate of AEs, changes in biological markers, overall incidence of primary outcomes, and the number of subjects needed.

It will be responsible for examining data with respect to clinical outcomes and treatment failures and for informing the SAB about any decisions it needs to take to pursue or discontinue the trial, such as:

- Premature discontinuation (because the rate of AEs is high, the trial is no longer feasible, or the available data are sufficient);
- Substantial changes to the protocol that becomes necessary during the inclusion or follow-up phases, or to account for new scientific information.

The IDMC will have access to overall safety and efficacy data, as well as to any information justifying any change affecting the course of the trial. It may request an intermediate statistical analysis.

During the trial, the IDMC may be asked to deliberate on questions relative to the scientific and ethical integrity of the trial, at the request of the SAB, the coordinating investigators, the international coordinating CTU or other participants in the trial. The IDMC will provide a formal written opinion report to the SAB and the sponsor after each IDMC meeting.

IDMC members will be selected in collaboration between the coordinating investigators and the promoter before the beginning of the trial. All IDMC members must be free from any direct involvement with the trial. Any competing interests, both real and potential, must be declared. The list of members will be provided to relevant Ethics Committees when the IDMC is constituted, prior to launching the study.

The IDMC will meet before the beginning of the inclusion phase, and every 12 months until the end of the trial. The sponsor, the SAB or the IDMC may request to increase the frequency of these meetings

14.3. Country Project Committee

At country level, the TB-Speed Country Project Committee (CPC), without any steering role, associates all major TB and child health stakeholders in the country (e.g. implementers, political supports, local NGOs). Under the supervision of the Country PI, the CPC will act as a facilitator for national operations as well as dissemination and communication activities.

14.4. Coordination

The international coordinating CTU, in charge of overall study coordination, data monitoring and management will be the IDLIC/Mereva team at the Inserm U1219 Bordeaux Population Health, located at University of Bordeaux (France). Trial implementation, monitoring and data management activities will be coordinated by an international CRA.

The trial will be conducted and monitored according to a set of Standard Operating Procedures (SOPs). Monitoring will be implemented according to the monitoring plan which is written by the international CTU and validated with Inserm, the trial sponsor. Writing of the SOPs is coordinated by the International CTU.

In each country where the trial will be conducted, the country CTU will be based at the level of the TB-Speed Consortium partner, i.e. PACCI in Côte d'Ivoire, IRD in Cameroon, MU-JHU or Epicentre (Technical Partner) in Uganda, University of Zambia in Zambia, Instituto Nacional de Saúde in Mozambique, and Pasteur Institute in Cambodia. The country CTU will be in charge of trial coordination, monitoring and data management in the country. Trial activities will be coordinated by a country trial manager, who will work in close collaboration with the international trial (output) manager, and monitored by a country CRA who will work in close collaboration with the international CRA.

A consortium agreement, established between UBx and TB-Speed Consortium members, defines task distribution and responsibilities of the different centres during the project.

15. CONFIDENTIALITY

15.1. Procedure for respecting the confidentiality of participants

Each trial subject 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 trial. This will be the only identifier in the electronic trial 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 trial makes having such information absolutely essential, subject to signature of a confidential agreement. This includes routine hospital staff involved in the child clinical management, as well as research study staff when they may be visiting the study site for monitoring, coordination, or event validation purposes. They will not have access to direct personal identifiers outside of the trial site premises.

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

15.2. Procedure for keeping the necessary study data confidential

Individual medical information obtained as result of this trial will be confidential. Trial team members are subject to the obligation of professional secrecy. Individual patient data will be made available upon request to the trial 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 trial 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.

16. PROTECTING RESEARCH PARTICIPANTS

16.1. Ethical justification of the protocol

Risks

Young children with severe pneumonia have a high risk of death, especially if they have comorbidities like HIV infection or malnutrition, which is independent to the study intervention.

16.1.1. Risks due to para-clinical investigations are well known and will be explained to the participants. The potential risks of NPA 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.

- NPA usually causes a reactive cough; epistaxis and discomfort can also occur;
- The NPA does not increase the risk of bronchospasm and should not increase risk of hypoxemia. However sample collection will always be done with access to salbutamol and oxygen. Oxygen is part of the SOC for management of severe pneumonia.
- 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

16.1.2. children identified through the study will be referred to ARV treatment programs.

Benefits

This study is providing the following opportunities for eligible children:

- an improved management and care of severe pneumonia through the WHO SOC
- an improved and early diagnosis of TB, especially by optimizing bacteriological specimen collection and processing for young children;
- an enhanced prognosis thanks to timely TB treatment initiation as recommended by the national health authorities;
- in case of rifampicin resistance detected by Ultra, an access to drug susceptibility testing for *M. tuberculosis* and the opportunity to receive an adapted treatment according to these results, including access to MDR-TB treatment;
- investigation fees per protocol paid by the study (clinical examinations, laboratory, radiology, hospitalization and transportation fees).

Together these factors 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 severe childhood pneumonia in high TB burden countries.

16.2. 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).

16.3. 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, to relevant Institutional Review Boards, 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 PIs will sign the protocol as a commitment to conduct the trial 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.

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

The patient information notice, informed consent form, as well as final protocol version will also be reviewed by local Community Advisory Boards where existing (Cambodia, Cameroon, and Mozambique) in order to ensure greater acceptability at the family and community level.

16.5. Data protection

The data recorded during this trial will be subject to computer processing on behalf of the Sponsor. The protocol has been approved by 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.

16.6. 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, in accordance with the French legal provisions and regulations on research.

The certificate of insurance relating to this Protocol constitutes Appendix 10.

16.7. Participants amenities

Trial investigators will ensure that each subject receives the following benefits throughout the study: reimbursement of transportation fees to the hospital, medical exams, tests, and medications related to the trial.

The amount of reimbursement for transportation fees will be fairly determined at the national level, as either a fixed amount or proportional to the distance between the hospital and the patient's home and will be applied equally to all enrolled patients. In case of withdrawal from the study, any reimbursement due to the participant up to the date of withdrawal will be cleared. In no case will a refund be asked to the participant.

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 trial investigators, within the limits of the available budget.

17. QUALITY ASSURANCE AND MONITORING

17.1. 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 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 subject to source data verification are identified in the monitoring plan. Procedures for monitoring will be detailed in study-specific SOPs developed by the Mereva team at the international CTU.

17.2.1.

17.2. Monitoring (quality control of the study)

General organization

Country CTUs are in charge of the monitoring of 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.

Monitoring by the country CTU

A CRA will regularly visit each implementing site during the all study period, including at set-up, implementation, and at the end of the trial. 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 eligibility criteria, informed consent, patient schedule;

17.2.2.

- 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);
- 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;
- evaluate the progress of patient enrolment;
- check the quality management of samples and biobank
- ensure that quality controls and quality management for laboratory assessments are implemented; monitor CXR interpretation performance
- follow-up with investigator sites centralized correction requests sent by the international coordinating CTU.

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

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

17.2.3. *Monitoring by the international coordinating CTU*

During the trial set-up process, an opening visit will be performed for each site by the international CRA and the project co-investigator on behalf of the promoter. 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 a year. 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.

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

- Informed consent
- Compliance with the trial protocol, SOPs and Good Clinical Practices, including eligibility criteria and reporting of SAEs
- Consistency with the source documents for key data
- Management of samples and biobanking
- Laboratory quality controls

Each visit will be recorded in a written monitoring report, sent to the co-investigators, the clinical and country project managers, the country PIs 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 trial by the international CRA.

Direct access to source data

Participating investigators should agree to allow trial-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.

17.2.4. *17.3. 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.

18. ACCESS TO DATA AND FROZEN SAMPLES

18.1. Data

All data collected in relation to the study will be under the responsibility of the international coordinating CTU.

Data will be utilized according to this protocol. After expertise and opinion by the SAB, any utilization for analyses not listed in the protocol should be approved by the trial coordinating investigators and the sponsor.

Data will be held in a centralized database held at UBx. However, each of the implementing countries will have access to their own data. A Data Sharing Agreement will be signed between UBx and each consortium partner.

The Covid sub-study is subject to the same data governance scheme than the main TB-Speed Pneumonia study.

18.2. Frozen samples

18.2.1.

Biobank governance

The governance structure in place is the same for each country CTU to which the biological material is entrusted.

Samples for which parent(s)/guardian(s) have consented for storage will be under the country CTU's responsibility, during and after the end of the study. The consortium agreement defines the responsibilities of country CTUs, including management of the biobank in their own facilities, or contracting with an external laboratory with adequate biobanking capacity (Appendix 8). Specific SOPs will describe methods and procedures for the collection of biological samples, as well as the Laboratory Quality Assurance system put in place during the TB-Speed Pneumonia study. In addition, each country CTU will be provided with a deep freezer to ensure enough space and good condition for the storage of study samples.

Each Central Laboratory will maintain its own biobank management system. Biobank samples will be identified by a unique, anonymized specimen ID. Data on biobank samples for each patient (including specimen ID, volume, and date of storage/removal) will be recorded in the central study database by the national laboratory coordinator, under the supervision of the international laboratory coordinator. In addition, the management of samples stored for research purposes will be reviewed by the international and country CTUs as part of the global laboratory monitoring process.

Biological samples will be retained for 10 years after study completion, unless objection expressed by parent(s)/guardian(s). Destruction of leftovers or unused samples will be

undertaken by country central laboratories in accordance with local regulations relating to the disposal of biological specimens. In the event of samples shipped to external laboratories, those will be disposed of in accordance with applicable regulations in both recipient and supplier countries. In both cases, the laboratory will provide a certificate of destruction.

Any utilization for tests not listed in the protocol should be approved by the trial co-investigators and the sponsor, after expertise and opinion by the SAB. Each ancillary study will seek ethics approval at national and international level. Subject to approval by relevant Ethics Committees at the national and international level, frozen samples may be sent to external laboratories (inside or outside the country) for additional analyses performed as part of ancillary studies. A Material Transfer Agreement will be submitted to appropriate ethics committees and regulatory authorities of both recipient and supplier countries.

Patients' rights

In line with the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Health-related Research Involving Humans (Guideline 11), authorization from the donor (i.e. parents/guardians of the participating child) for future use of stored biological samples will be sought during the informed consent process. The Information Notice will include: the name and city of the country central laboratory; the purpose of the biobank; the foreseeable use of the samples (extending to a number of yet undefined research studies including genetic analyses); the conditions and duration of storage; the rules of access to the biobank and the protection of data confidentiality. No further consent will be sought from parents/guardians in case of post-trial studies.

18.2.2

The donor can retract his authorisation for sample storage at any time. In such case, biological material will be destroyed.

Biobank samples will be collected from children benefitting from a 3-month cohort follow-up. Any undiagnosed TB at baseline will most likely be detected by the end of follow-up. We therefore do not expect unsolicited findings, and will not report any results from biobank samples to the patient.

However, as mentioned in the Information Notice, knowledge generated by the research will be shared with participants if they are willing to. Site investigators will be responsible for informing study participants by using the most appropriate mean that research results are available and can be communicated to them if they wish so.

19. 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 PIs 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 promoter 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.

20. BIBLIOGRAPHY/REFERENCES

[1] P. J. Dodd, C. M. Yuen, C. Sismanidis, J. A. Seddon, and H. E. Jenkins, “The global burden of tuberculosis mortality in children: a mathematical modelling study,” *Lancet Glob. Health*, vol. 5, no. 9, pp. e898–e906, 2017.

[2] World Health Organization, “Global Tuberculosis Report 2017,” Geneva, 2017.

[3] B. J. Marais, R. P. Gie, H. S. Schaaf, N. Beyers, P. R. Donald, and J. R. Starke, “Childhood Pulmonary Tuberculosis: Old Wisdom and New Challenges,” *Am. J. Respir. Crit. Care Med.*, vol. 173, no. 10, pp. 1078–1090, May 2006, doi: 10.1164/rccm.200511-1809SO.

[4] C. M. Perez-Velez and B. J. Marais, “Tuberculosis in Children,” *N. Engl. J. Med.*, vol. 367, no. 4, pp. 348–361, Jul. 2012, doi: 10.1056/NEJMra1008049.

[5] World Health Organization, UNICEF, and C. for D. Control, “Roadmap for childhood tuberculosis: towards zero deaths,” 2013.

[6] World Health Organization, “Guidance for national tuberculosis programmes on the management of tuberculosis in children - 2nd ed.,” Geneva, 2014.

[7] L. Liu *et al.*, “Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals,” *The Lancet*, vol. 388, no. 10063, pp. 3027–3035, 2016.

[8] J. N. Oliwa, J. M. Karumbi, B. J. Marais, S. A. Madhi, and S. M. Graham, “Tuberculosis as a cause or comorbidity of childhood pneumonia in tuberculosis-endemic areas: a systematic review,” *Lancet Respir. Med.*, vol. 3, no. 3, pp. 235–243, Mar. 2015, doi: 10.1016/S2213-2600(15)00028-4.

[9] J. M. Nantongo *et al.*, “High incidence of pulmonary tuberculosis in children admitted with severe pneumonia in Uganda,” *BMC Pediatr.*, vol. 13, no. 1, p. 16, 2013.

[10] M. J. Chisti *et al.*, “Post-Discharge Mortality in Children with Severe Malnutrition and Pneumonia in Bangladesh,” *PLoS ONE*, vol. 9, no. 9, p. e107663, Sep. 2014, doi: 10.1371/journal.pone.0107663.

[11] P. M. Enarson, R. P. Gie, D. A. Enarson, C. Mwansambo, and S. M. Graham, “Impact of HIV on standard case management for severe pneumonia in children,” *Expert Rev. Respir. Med.*, vol. 4, no. 2, pp. 211–220, Apr. 2010, doi: 10.1586/ers.10.14.

[12] World Health Organization, Ed., *Pocket book of hospital care for children: guidelines for the management of common childhood illnesses*, Second edition, 2013 edition. Geneva, Switzerland: World Health Organization, 2013.

[13] C. C. Boehme *et al.*, “Rapid molecular detection of tuberculosis and rifampin resistance,” *N. Engl. J. Med.*, vol. 363, no. 11, pp. 1005–1015, 2010.

[14] C. C. Boehme *et al.*, “Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study,” *Lancet Lond. Engl.*, vol. 377, no. 9776, pp. 1495–1505, Apr. 2011, doi: 10.1016/S0140-6736(11)60438-8.

[15] World Health Organization, “Policy statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system.,” Geneva, 2011.

[16] World Health Organization, “Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update,” Geneva, 2014.

[17] A. K. Detjen *et al.*, “Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis,” *Lancet Respir. Med.*, vol. 3, no. 6, pp. 451–461, Jun. 2015, doi: 10.1016/S2213-2600(15)00095-8.

[18] M. J. Chisti *et al.*, “A Prospective Study of the Prevalence of Tuberculosis and Bacteraemia in Bangladeshi Children with Severe Malnutrition and Pneumonia Including an Evaluation of Xpert MTB/RIF Assay,” *PLoS ONE*, vol. 9, no. 4, p. e93776, Apr. 2014, doi: 10.1371/journal.pone.0093776.

[19] S. Chakravorty *et al.*, “The new Xpert MTB/RIF Ultra: improving detection of Mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing,” *MBio*, vol. 8, no. 4, pp. e00812–17, 2017.

[20] Zar HJ, Workman L, and Nicol MP, “Diagnosis of pulmonary tuberculosis in HIV-infected and uninfected children using Xpert MTB/RIF Ultra. Abstr A7610,” presented at the International Conference of the American Thoracic Society, Washington, DC, USA, May 2017.

[21] S. E. Dorman *et al.*, “Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study,” *Lancet Infect. Dis.*, vol. 18, no. 1, pp. 76–84, Jan. 2018, doi: 10.1016/S1473-3099(17)30691-6.
[22] FIND, “Report for WHO. A multicentre non-inferiority diagnostic accuracy study of the Ultra assay compared to the Xpert MTB/RIF assay,” Geneva, 2017.
[23] M. P. Nicol *et al.*, “Accuracy of Xpert MTB/RIF Ultra for the Diagnosis of Pulmonary Tuberculosis in Children,” *Pediatr. Infect. Dis. J.*, p. 1, Feb. 2018, doi: 10.1097/INF.0000000000001960.
[24] E. A. Kendall, S. G. Schumacher, C. M. Denkinger, and D. W. Dowdy, “Estimated clinical impact of the Xpert MTB/RIF Ultra cartridge for diagnosis of pulmonary tuberculosis: A modeling study,” *PLOS Med.*, vol. 14, no. 12, p. e1002472, Dec. 2017, doi: 10.1371/journal.pmed.1002472.
[25] World Health Organization, “WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF,” Geneva, 2017.
[26] M. J. A. Reid, S. Saito, R. Fayorsey, R. J. Carter, and E. J. Abrams, “Assessing capacity for diagnosing tuberculosis in children in sub-Saharan African HIV care settings [Short communication],” *Int. J. Tuberc. Lung Dis.*, vol. 16, no. 7, pp. 924–927, Jul. 2012, doi: 10.5588/ijtld.11.0816.
[27] H. A. Moore, P. Apolles, P. J. T. de Villiers, and H. J. Zar, “Sputum induction for microbiological diagnosis of childhood pulmonary tuberculosis in a community setting,” *Int. J. Tuberc. Lung Dis. Off. J. Int. Union Tuberc. Lung Dis.*, vol. 15, no. 9, pp. 1185–1190, i, Sep. 2011, doi: 10.5588/ijtld.10.0681.
[28] H. J. Zar *et al.*, “Rapid Molecular Diagnosis of Pulmonary Tuberculosis in Children Using Nasopharyngeal Specimens,” *Clin. Infect. Dis.*, vol. 55, no. 8, pp. 1088–1095, Oct. 2012, doi: 10.1093/cid/cis598.
[29] S. Owens *et al.*, “Nasopharyngeal aspiration for diagnosis of pulmonary tuberculosis,” *Arch. Dis. Child.*, vol. 92, no. 8, pp. 693–696, Apr. 2007, doi: 10.1136/adc.2006.108308.
[30] S. I. B. Cadmus *et al.*, “Mycobacterium tuberculosis and Mycobacterium africanum in stools from children attending an immunization clinic in Ibadan, Nigeria,” *Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis.*, vol. 13, no. 6, pp. 740–744, Nov. 2009, doi: 10.1016/j.ijid.2008.11.016.
[31] M. P. Nicol *et al.*, “Xpert MTB/RIF testing of stool samples for the diagnosis of pulmonary tuberculosis in children,” *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.*, vol. 57, no. 3, pp. e18-21, Aug. 2013, doi: 10.1093/cid/cit230.
[32] E. Walters, R. P. Gie, A. C. Hesseling, S. O. Friedrich, A. H. Diacon, and R. P. Gie, “Rapid Diagnosis of Pediatric Intrathoracic Tuberculosis From Stool Samples Using the Xpert MTB/RIF Assay: A Pilot Study,” *Pediatr. Infect. Dis. J.*, vol. 31, no. 12, p. 1316, Dec. 2012, doi: 10.1097/INF.0b013e318266c21c.
[33] B. Dim, T. Tran, and L. Borand, “Feasibility, safety and tolerability of nasopharyngeal aspirates and string tests for diagnosis of TB with Xpert® MTB/RIF in HIV-infected children: ANRS 12229 PAANTHER 01 study,” presented at the 48th Union World Conference on Lung Health, Guadalajara, Mexico, Oct. 2017.
[34] N. S. Planting, G. L. Visser, M. P. Nicol, L. Workman, W. Isaacs, and H. J. Zar, “Safety and efficacy of induced sputum in young children hospitalised with suspected pulmonary tuberculosis,” *Int. J. Tuberc. Lung Dis. Off. J. Int. Union Tuberc. Lung Dis.*, vol. 18, no. 1, pp. 8–12, Jan. 2014, doi: 10.5588/ijtld.13.0132.
[35] H. J. Zar, L. Workman, W. Isaacs, K. Dheda, W. Zemanay, and M. P. Nicol, “Rapid diagnosis of pulmonary tuberculosis in African children in a primary care setting by use of Xpert MTB/RIF on respiratory specimens: a prospective study,” *Lancet Glob. Health*, vol. 1, no. 2, pp. e97–e104, Aug. 2013, doi: 10.1016/S2214-109X(13)70036-6.
[36] O. Marcy *et al.*, “Performance of Xpert MTB/RIF and Alternative Specimen Collection Methods for the Diagnosis of Tuberculosis in HIV-Infected Children,” *Clin. Infect. Dis.*, vol. 62, no. 9, pp. 1161–1168, May 2016, doi: 10.1093/cid/ciw036.
[37] Song et al., “Diagnostic yield of Xpert® MTB/RIF assay and Mycobacterium tuberculosis culture on respiratory and non-respiratory specimens among Kenyan children.,” presented at the 46th Union Conference on World Health, Cape Town, South Africa, Dec. 2015.
[38] S. M. LaCourse *et al.*, “Stool Xpert MTB/RIF and urine lipoarabinomannan for the diagnosis of tuberculosis in hospitalized HIV-infected children.,” *AIDS*, vol. 32, no. 1, pp. 69–78, Jan. 2018, doi: 10.1097/QAD.0000000000001662.
[39] M. Chipinduro, K. Mateveke, B. Makamure, R. A. Ferrand, and E. Gomo, “Stool Xpert ® MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis at primary clinics in Zimbabwe,” *Int. J. Tuberc. Lung Dis.*, vol. 21, no. 2, pp. 161–166, Feb. 2017, doi: 10.5588/ijtld.16.0357.

[40] E. Walters *et al.*, “Xpert MTB/RIF on Stool Is Useful for the Rapid Diagnosis of Tuberculosis in Young Children With Severe Pulmonary Disease:,” *Pediatr. Infect. Dis. J.*, vol. 36, no. 9, pp. 837–843, Sep. 2017, doi: 10.1097/INF.0000000000001563.
[41] D. Portevin *et al.*, “Assessment of the novel T-cell activation marker–tuberculosis assay for diagnosis of active tuberculosis in children: a prospective proof-of-concept study,” *Lancet Infect. Dis.*, vol. 14, no. 10, pp. 931–938, Oct. 2014, doi: 10.1016/S1473-3099(14)70884-9.
[42] M. P. Nicol *et al.*, “Urine lipoarabinomannan testing for diagnosis of pulmonary tuberculosis in children: a prospective study,” *Lancet Glob. Health*, vol. 2, no. 5, pp. e278–e284, May 2014, doi: 10.1016/S2214-109X(14)70195-0.
[43] S. T. Anderson *et al.*, “Diagnosis of Childhood Tuberculosis and Host RNA Expression in Africa,” *N. Engl. J. Med.*, vol. 370, no. 18, pp. 1712–1723, May 2014, doi: 10.1056/NEJMoa1303657.
[44] P. M. M. Bossuyt, J. B. Reitsma, K. Linnet, and K. G. M. Moons, “Beyond Diagnostic Accuracy: The Clinical Utility of Diagnostic Tests,” *Clin. Chem.*, vol. 58, no. 12, pp. 1636–1643, Dec. 2012, doi: 10.1373/clinchem.2012.182576.
[45] J. G. Lijmer, M. Leeflang, and P. M. M. Bossuyt, “Proposals for a Phased Evaluation of Medical Tests,” *Med. Decis. Making*, vol. 29, no. 5, pp. E13–E21, Sep. 2009, doi: 10.1177/0272989X09336144.
[46] M. P. Nicol *et al.*, “Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study,” *Lancet Infect. Dis.*, vol. 11, no. 11, pp. 819–824, Nov. 2011, doi: 10.1016/S1473-3099(11)70167-0.
[47] M. Bates *et al.*, “Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirates in children in sub-Saharan Africa: a prospective descriptive study,” *Lancet Infect. Dis.*, vol. 13, no. 1, pp. 36–42, Jan. 2013, doi: 10.1016/S1473-3099(12)70245-1.
[48] G. Theron *et al.*, “Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial,” *Lancet Lond. Engl.*, vol. 383, no. 9915, pp. 424–435, Feb. 2014, doi: 10.1016/S0140-6736(13)62073-5.
[49] A. Trajman *et al.*, “Impact on Patients’ Treatment Outcomes of XpertMTB/RIF Implementation for the Diagnosis of Tuberculosis: Follow-Up of a Stepped-Wedge Randomized Clinical Trial,” *PLOS ONE*, vol. 10, no. 4, p. e0123252, Apr. 2015, doi: 10.1371/journal.pone.0123252.
[50] C. Yoon *et al.*, “Impact of Xpert MTB/RIF Testing on Tuberculosis Management and Outcomes in Hospitalized Patients in Uganda,” *PLoS ONE*, vol. 7, no. 11, p. e48599, Nov. 2012, doi: 10.1371/journal.pone.0048599.
[51] M. O. Akanbi *et al.*, “Evaluation of gene xpert for routine diagnosis of HIV-associated tuberculosis in Nigeria: A prospective cohort study,” *BMC Pulm. Med.*, vol. 17, no. 1, Dec. 2017, doi: 10.1186/s12890-017-0430-6.
[52] C. L. F. Walker *et al.*, “Global burden of childhood pneumonia and diarrhoea,” *Lancet*, vol. 381, no. 9875, pp. 1405–1416, Apr. 2013, doi: 10.1016/S0140-6736(13)60222-6.
[53] *Revised WHO Classification and Treatment of Pneumonia in Children at Health Facilities: Evidence Summaries*. Geneva: World Health Organization, 2014.
[54] World Health Organization, *Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources*, 2nd edition. Geneva, 2005.
[55] M. M. Ngari *et al.*, “Mortality after Inpatient Treatment for Severe Pneumonia in Children: a Cohort Study,” *Paediatr. Perinat. Epidemiol.*, vol. 31, no. 3, pp. 233–242, May 2017, doi: 10.1111/ppe.12348.
[56] C. G. Sutcliffe *et al.*, “A clinical guidance tool to improve the care of children hospitalized with severe pneumonia in Lusaka, Zambia,” *BMC Pediatr.*, vol. 16, p. 136, 20 2016, doi: 10.1186/s12887-016-0665-z.
[57] M. Lazzerini *et al.*, “Mortality and its risk factors in Malawian children admitted to hospital with clinical pneumonia, 2001–12: a retrospective observational study,” *Lancet Glob. Health*, vol. 4, no. 1, pp. e57–e68, Jan. 2016, doi: 10.1016/S2214-109X(15)00215-6.
[58] H. E. Jenkins *et al.*, “Mortality in children diagnosed with tuberculosis: a systematic review and meta-analysis,” *Lancet Infect. Dis.*, Dec. 2016, doi: 10.1016/S1473-3099(16)30474-1.
[59] O. Marcy *et al.*, “Mortality and its determinants in antiretroviral treatment-naive HIV-infected children with suspected tuberculosis: an observational cohort study,” p. 9, 2017.
[60] W. C. Buck *et al.*, “Risk factors for mortality in Malawian children with human immunodeficiency virus and tuberculosis co-infection,” *Int. J. Tuberc. Lung Dis. Off. J. Int. Union Tuberc. Lung Dis.*, vol. 17, no. 11, pp. 1389–1395, Nov. 2013, doi: 10.5588/ijtld.13.0030.

[61] E. W. Tiemersma, M. J. van der Werf, M. W. Borgdorff, B. G. Williams, and N. J. D. Nagelkerke, “Natural history of tuberculosis: duration and fatality of untreated pulmonary tuberculosis in HIV negative patients: a systematic review,” *PloS One*, vol. 6, no. 4, p. e17601, 2011, doi: 10.1371/journal.pone.0017601.
[62] World Health Organization, “High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Available at: http://apps.who.int/iris/bitstream/10665/135617/1/WHO_HTM_T- B_2014.18_eng.pdf,” Geneva, 2014.
[63] S. T. Anderson *et al.*, “Diagnosis of Childhood Tuberculosis and Host RNA Expression in Africa,” *N. Engl. J. Med.*, vol. 370, no. 18, pp. 1712–1723, May 2014, doi: 10.1056/NEJMoa1303657.
[64] G. Walzl *et al.*, “Tuberculosis: advances and challenges in development of new diagnostics and biomarkers,” *Lancet Infect. Dis.*, Mar. 2018, doi: 10.1016/S1473-3099(18)30111-7.
[65] M. Zhou, G. Yu, X. Yang, C. Zhu, Z. Zhang, and X. Zhan, “Circulating microRNAs as biomarkers for the early diagnosis of childhood tuberculosis infection,” *Mol. Med. Rep.*, vol. 13, no. 6, pp. 4620–4626, Jun. 2016, doi: 10.3892/mmr.2016.5097.
[66] J. E. Gjøen *et al.*, “Novel transcriptional signatures for sputum-independent diagnostics of tuberculosis in children,” *Sci. Rep.*, vol. 7, no. 1, Dec. 2017, doi: 10.1038/s41598-017-05057-x.
[67] L. M. Verhagen *et al.*, “A predictive signature gene set for discriminating active from latent tuberculosis in Warao Amerindian children,” *BMC Genomics*, vol. 14, no. 1, p. 74, 2013, doi: 10.1186/1471-2164-14-74.
[68] S. M. Graham *et al.*, “Evaluation of Tuberculosis Diagnostics in Children: 1. Proposed Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease. Consensus From an Expert Panel,” *J. Infect. Dis.*, vol. 205, no. suppl_2, pp. S199–S208, May 2012, doi: 10.1093/infdis/jis008.
[69] U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS., “Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1.,” 2017.
[70] A. R. Frydenberg and S. M. Graham, “Toxicity of first-line drugs for treatment of tuberculosis in children: review,” *Trop. Med. Int. Health TM IH*, vol. 14, no. 11, pp. 1329–1337, Nov. 2009, doi: 10.1111/j.1365-3156.2009.02375.x.
[71] Q. Li *et al.*, “Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia,” *N. Engl. J. Med.*, Jan. 2020, doi: 10.1056/nejmoa2001316.
[72] C. Huang *et al.*, “Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China,” *The Lancet*, vol. 395, no. 10223, pp. 497–506, Feb. 2020, doi: 10.1016/S0140-6736(20)30183-5.
[73] N. S. Zhong *et al.*, “Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People’s Republic of China, in February, 2003.,” *Lancet Lond. Engl.*, vol. 362, no. 9393, pp. 1353–8, Oct. 2003, doi: 10.1016/s0140-6736(03)14630-2.
[74] A. M. Zaki, S. Van Boheemen, T. M. Bestebroer, A. D. M. E. Osterhaus, and R. A. M. Fouchier, “Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia,” *N. Engl. J. Med.*, vol. 367, no. 19, pp. 1814–1820, Nov. 2012, doi: 10.1056/NEJMoa1211721.
[75] Z. Wu and J. M. McGoogan, “Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention,” *JAMA*, Feb. 2020, doi: 10.1001/jama.2020.2648.
[76] J. F.-W. Chan *et al.*, “A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster,” *Lancet Lond. Engl.*, vol. 395, no. 10223, pp. 514–523, 15 2020, doi: 10.1016/S0140-6736(20)30154-9.
[77] X. Lu *et al.*, “SARS-CoV-2 Infection in Children,” *N. Engl. J. Med.*, Mar. 2020, doi: 10.1056/NEJMc2005073.
[78] CDC MMWR, “Coronavirus Disease 2019 in Children — United States, February 12–April 2, 2020,” *MMWR Morb. Mortal. Wkly. Rep.*, vol. 69, 2020, doi: 10.15585/mmwr.mm6914e4.
[79] E. Livingston and K. Bucher, “Coronavirus Disease 2019 (COVID-19) in Italy,” *JAMA*, Mar. 2020, doi: 10.1001/jama.2020.4344.
[80] WHO Africa, “Situation reports on COVID-19 outbreak, WHO AFRO region,” *WHO | Regional Office for Africa*. https://www.afro.who.int/health-topics/coronavirus-covid-19 (accessed May 17, 2020).
[81] S. Garg, “Hospitalization Rates and Characteristics of Patients Hospitalized with Laboratory-Confirmed Coronavirus Disease 2019 — COVID-NET, 14 States, March 1–30, 2020,” *MMWR Morb. Mortal. Wkly. Rep.*, vol. 69, 2020, doi: 10.15585/mmwr.mm6915e3.

[82] Y. Dong *et al.*, “Epidemiological Characteristics of 2143 Pediatric Patients With 2019 Coronavirus Disease in China,” *Pediatrics*, Mar. 2020, doi: 10.1542/peds.2020-0702.
[83] FDA, “Coronavirus (COVID-19) Update: FDA Issues Emergency Use Authorization for Potential COVID-19 Treatment,” *FDA*, Apr. 05, 2020. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment (accessed May 17, 2020).
[84] H. Chen *et al.*, “Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records,” *Lancet Lond. Engl.*, vol. 395, no. 10226, pp. 809–815, 07 2020, doi: 10.1016/S0140-6736(20)30360-3.
[85] J. Cai *et al.*, “A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features,” *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.*, Feb. 2020, doi: 10.1093/cid/ciaa198.
[86] W. Wang *et al.*, “Detection of SARS-CoV-2 in Different Types of Clinical Specimens,” *JAMA - Journal of the American Medical Association*. American Medical Association, 2020, doi: 10.1001/jama.2020.3786.
[87] Z. M. Chen *et al.*, “Diagnosis and treatment recommendations for pediatric respiratory infection caused by the 2019 novel coronavirus,” *World Journal of Pediatrics*. Institute of Pediatrics of Zhejiang University, 2020, doi: 10.1007/s12519-020-00345-5.
[88] A. Tang *et al.*, “Detection of Novel Coronavirus by RT-PCR in Stool Specimen from Asymptomatic Child, China,” *Emerg. Infect. Dis.*, vol. 26, no. 6, Jun. 2020, doi: 10.3201/eid2606.200301.
[89] COLSON Philippe *et al.*, “Children account for a small proportion of diagnoses of SARS-CoV-2 infection and do not exhibit greater viral loads than adults.,” *2020*. https://www.mediterranee-infection.com/wp-content/uploads/2020/03/Ms_Raoult_SARS-CoV-2_Age_Mar2020_vL.pdf (accessed Apr. 09, 2020).
[90] K. Danis *et al.*, “Cluster of coronavirus disease 2019 (Covid-19) in the French Alps, 2020,” *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.*, Apr. 2020, doi: 10.1093/cid/ciaa424.
[91] Y. Dong *et al.*, “Epidemiological Characteristics of 2143 Pediatric Patients With 2019 Coronavirus Disease in China.,” *Pediatrics*, Mar. 2020, doi: 10.1542/peds.2020-0702.
[92] E. Livingston and K. Bucher, “Coronavirus Disease 2019 (COVID-19) in Italy.,” *JAMA*, Mar. 2020, doi: 10.1001/jama.2020.4344.
[93] CDC COVID-19 Response Team, “Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) - United States, February 12-March 16, 2020,” *MMWR Morb. Mortal. Wkly. Rep.*, vol. 69, no. 12, pp. 343–346, Mar. 2020, doi: 10.15585/mmwr.mm6912e2.
[94] F. B. Piel, M. H. Steinberg, and D. C. Rees, “Sickle cell disease,” *New England Journal of Medicine*, vol. 376, no. 16. Massachussetts Medical Society, pp. 1561–1573, Apr. 20, 2017, doi: 10.1056/NEJMra1510865.
[95] WHO, “Unity Studies: Early Investigation Protocols,” *2020*. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/early-investigations (accessed Apr. 09, 2020).
[96] World Health Organization, “Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected. Interim guidance.,” Geneva, Mar. 2020.
[97] K. Hemming, T. P. Haines, P. J. Chilton, A. J. Girling, and R. J. Lilford, “The stepped wedge cluster randomised trial: rationale, design, analysis, and reporting,” *Bmj*, vol. 350, p. h391, 2015.
[98] K. Hemming and M. Taljaard, “Sample size calculations for stepped wedge and cluster randomised trials: a unified approach,” *J. Clin. Epidemiol.*, vol. 69, pp. 137–146, Jan. 2016, doi: 10.1016/j.jclinepi.2015.08.015.
[99] M. A. Hussey and J. P. Hughes, “Design and analysis of stepped wedge cluster randomized trials,” *Contemp. Clin. Trials*, vol. 28, no. 2, pp. 182–191, Feb. 2007, doi: 10.1016/j.cct.2006.05.007.
[100] E. de Hoop *et al.*, “The need to balance merits and limitations from different disciplines when considering the stepped wedge cluster randomized trial design,” *BMC Med. Res. Methodol.*, vol. 15, no. 1, Dec. 2015, doi: 10.1186/s12874-015-0090-2.
[101] M. J. Grayling, J. M. Wason, and A. P. Mander, “Group sequential designs for stepped-wedge cluster randomised trials,” *Clin. Trials J. Soc. Clin. Trials*, vol. 14, no. 5, pp. 507–517, Oct. 2017, doi: 10.1177/1740774517716937.
[102] M. Yotebieng *et al.*, “Effect on mortality and virological response of delaying antiretroviral therapy initiation in children receiving tuberculosis treatment,” *AIDS Lond. Engl.*, vol. 24, no. 9, pp. 1341–1349, Jun. 2010, doi: 10.1097/QAD.0b013e328339e576.

21. APPENDICES

21.1. APPENDIX 1: Protocol summary

Clinical trial ID number:

Title: Impact of systematic early tuberculosis (TB) detection using Xpert MTB/RIF Ultra in children with severe pneumonia in high tuberculosis burden countries

Short title: TB-Speed Pneumonia

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

Participating countries: Cambodia, Cameroon, Côte d'Ivoire, Mozambique, Uganda, Zambia

Primary Objective

To evaluate the impact on all-cause mortality at 12 weeks of adding systematic early detection of TB with Xpert MTB/RIF Ultra performed on one NPA and one stool sample to the WHO standard of care (SOC) in young children with severe pneumonia, followed by immediate anti-TB treatment initiation in children with a positive Ultra result, in high TB incidence countries, as compared to the SOC alone.

Secondary Objectives:

- To evaluate the impact of systematic early detection of TB on:
  - TB case detection at 12 weeks
  - TB treatment initiation
  - Time to TB treatment initiation
  - TB treatment exposure during follow-up
  - inpatient mortality
  - duration of initial hospitalization
  - hospital readmission rate
  - weight gain at 12 weeks
- To compare cost-effectiveness of the two strategies
- Additionally, **in the intervention arm**:
  - To assess the proportion of microbiologically (Ultra)-confirmed TB and clinically-diagnosed TB
  - To assess the feasibility of NPA and stool samples collection
  - To assess the safety of NPA
  - To assess the acceptability of NPA and stool samples collection in a subset of children
  - To assess the tolerability of NPA collection in a subset of children

METHODS

- **Study design**: multicentric, stepped wedge cluster-randomised diagnostic trial
- **Implementing sites**: 15 hospitals from six countries with high TB incidence rate: Côte d'Ivoire, Cameroon, Uganda, Mozambique, Zambia and Cambodia
- All hospitals will start managing children using the WHO SOC for severe pneumonia [12]; one hospital will switch to the TB-Speed intervention every 5 weeks following the randomization list.
- **Randomisation**: 3,780 children will be equally distributed across the two strategies using a computer generated random sequence. Randomisation will be stratified on the country estimated TB incidence rate (cut-off value of 300 cases/100 000 person-years)
- **Follow-up**: children will be followed for 12 weeks after enrolment, with systematic trial visits at day 3, discharge, 2 weeks post-discharge, and week 12. An extra TB visit will be performed if children present with signs and symptoms of presumptive TB.

Sample size: 3,780 children <5 years old with WHO-defined severe pneumonia.

Primary endpoint: all-cause mortality 12 weeks after inclusion.

Secondary endpoints:

- Number of children diagnosed with TB at 12 weeks:
  - based on Ultra results
  - based on the clinician's judgement
- Proportion of children with TB treatment initiated at any time during follow-up
- Time to TB treatment initiation
- Duration of TB treatment at end of trial (week 12 or early termination)
- Number of inpatient deaths
- Duration of initial hospitalization
- Number of readmissions following discharge

- Weight gain at 12 weeks (as compared to body weight at inclusion)
- Incremental cost-effectiveness ratio (ICER)

Additionally, the following endpoints will be assessed **in the intervention group only**:

- Proportion of NPA and stool samples with positive TB detection using Ultra
- Proportion of Ultra-confirmed and clinically-diagnosed TB cases
- Feasibility of NPA and stool samples collection:
  - Proportion of children with samples collected as per protocol
  - Turnaround time between NPA or stool sample collection and result of Ultra
- Safety: adverse events collected by study nurses during NPA collection such as vomiting, nose bleeding, low oxygen saturation
- Tolerability of NPA specimen collection procedures: discomfort/pain/distress experienced by the child assessed by the child him/herself (Wong-Baker face scale), by the parents (visual analog scale), by the nurses (FLACC behavioural scale)
- Acceptability of NPA and stool specimen collection procedures by parents and nurses

Inclusion criteria:

- Children aged 2 to 59 months
- Severe pneumonia defined using WHO criteria as cough or difficulty in breathing with:
  - Peripheral oxygen saturation < 90% or central cyanosis, or
  - Severe respiratory distress, or
  - Signs of pneumonia with any of the following danger signs:
    - Inability to breastfeed or drink,
    - Persistent vomiting
    - Lethargy or reduced level of consciousness
    - Convulsions,
    - Stridor in calm child
    - Severe malnutrition
- Informed consent signed by parent/guardian

Non-inclusion criteria: ongoing TB treatment or history of intake of anti-TB drugs in the last 6 months.

Trial strategies and intervention:

- **Control arm**: All children admitted in the hospital and presenting with WHO-defined severe pneumonia will be immediately managed as part of routine care per the WHO SOC, including broad spectrum antibiotics, oxygen therapy if required, additional supportive care and specific therapies for comorbidities such as HIV infection.
- **Intervention arm**: the TB-Speed strategy consists on the WHO SOC plus the study intervention consisting in rapid TB detection on the day of hospital admission using the Ultra assay performed on 1 NPA and 1 stool sample. The sample flow will be organised in order to reduce time to results to 3 hours. Drugs will be available at the inpatient level to enable immediate initiation of TB treatment, as soon as a positive Ultra result is available.
- **For research purposes**, children will benefit from HIV testing, malaria testing, and complete blood count (CBC) if not systematically performed as routine care in the country/hospital, as well as from a digitalized chest X-ray. Additionally, samples will be collected for future biomarkers studies.
- Xpert testing could be done in children with clinical suspicion of TB according to the clinician's judgement, using sample collection methods usually implemented at the inpatient ward.

Trial agenda:

- Observational phase: 4th quarter 2018
- First inclusion: 1st quarter 2019
- Inclusion period: 80 weeks (18 months)
- Duration of follow-up for each participant once enrolled: 12 weeks
- Enrolment stop due to Covid-19 pandemic: April 1st, 2020
- Progressive enrolment restarting: June-August 2020
- Full enrolment restart: October 2020
- Last visit of the last patient: 2nd quarter 2021
- Overall duration of the trial (from the first inclusion to the last visit): 116 weeks (27 months)

Statistical analysis:

The primary analysis will be carried out with a generalized linear mixed model (GLMM). Using the intention-to-treat principle, we will model individual binary response through a logistic regression with random effect for cluster and fixed effect for step, accounting for TB incidence rate, within-cluster correlation, unequal cluster size and time-trends at the individual level. We will conclude that the

intervention is better than the control if the mortality odds ratio is statistically significant ($p<0.05$). For sensitivity analyses, we will consider i) adjusting for seasonality, HIV infection, age, and malnutrition; ii) replacing missing outcomes (lost to follow-up and protocol withdrawals) by a failure (death) value. As exploratory analyses, independent factors associated with death will be identified by similar models.
Ancillary study: cost-effectiveness study performed as part of the TB-Speed project Output 5 ("Evaluation of cost-effectiveness of the proposed diagnostic approaches").
Expected results: reduction of mortality through systematic early detection of TB in young children presenting with severe pneumonia; modification of national recommendations for the diagnosis of paediatric TB.

21.2. APPENDIX 2: Description of study sites

COUNTRY	**CAMBODIA**		
SITE	Takeo Provincial Hospital	Kampong Cham Provincial Hospital	National Paediatric Hospital
CITY	Takeo, Takeo Province	Kampong Cham, Kampong Cham Province	Phnom Penh - capital city
LEVEL	Provincial Referral Hospital	Provincial Referral Hospital	District general hospital National referral centre for paediatrics
CATCHMENT AREA (population)	984 567	1 071 187	1 573 544 (and whole country)
NB PAEDIATRIC BEDS	32	25	150
NB CHILDREN ADMITTED	1 200 /month	3 492 in 2017	19 223 in 2017
SPECIALIZED WARDS		TB ward	Pulmonology ward HIV & TB ward Nutrition ward
CLINICAL RESEARCH EXPERIENCE	GCP training: part of the staff	Yes GCP training: part of the staff	Yes (HIV, rotaviruses, malnutrition, asthma, surgery) GCP training: part of the staff
HUMAN RESOURCES	Paediatric ward: 11 nurses, 3 doctors	Total hospital staff: 323	Total hospital staff: 340
TECHNICAL RESOURCES	CXR. Laboratory: HIV serology, mycobacteriology, Xpert TB test.		

COUNTRY	**CAMEROUN**	
SITE	Mother and Child Center of the Chantal Biya Foundation	Biyem Assi District Hospital
CITY	Yaoundé - capital city	Yaoundé - capital city
LEVEL		Provincial Referral Hospital
CATCHMENT AREA (population)	3 500 000 (Yaoundé)	300 000 (Health District)
NB PAEDIATRIC BEDS	205	42
NB CHILDREN ADMITTED	5 000 /year	3 500 /year
SPECIALIZED WARDS	HIV	HIV Treatment Center Basic TB Management Unit
CLINICAL RESEARCH EXPERIENCE	Yes (Mother & Child HIV)	
HUMAN RESOURCES	Total hospital staff: 282	Paediatric ward: 2 Pediatricians, 5 General Practitioners, 9 Nurses and 14 Nurse-assistants
TECHNICAL RESOURCES		1 oxygen extractor, 2 oxygen tanks and 1 nasopharyngeal aspirator. Laboratory Unit.

COUNTRY	**COTE D'IVOIRE**		
SITE	Treichville	Yopougon	Cocody
CITY	Abidjan – capital city	Abidjan – capital city	Abidjan – capital city
LEVEL	Teaching Hospital	Teaching Hospital	Teaching Hospital
CATCHMENT AREA (population)	6 000 000 (Abidjan) 137 000 (Treichville area)	6 000 000 (Abidjan) 1 375 000 (Yopougon)	6 000 000 (Abidjan) 447 055 (Cocody area)
NB PAEDIATRIC BEDS	86	41	47

NB CHILDREN ADMITTED	4 520 in 2017	1 839 in 2017	1 394 in 2017
SPECIALIZED WARDS	HIV treatment centre Nutritional Rehabilitation Unit	HIV treatment centre Nutritional Rehabilitation Unit	HIV treatment centre Nutritional Rehabilitation Unit
CLINICAL RESEARCH EXPERIENCE	No	Yes (HIV, viral hepatitis, TB)	Yes (HIV, viral hepatitis)
HUMAN RESOURCES	Paediatric ward: 1 professor, 10 assistant professors, 11 doctors, 16 nurses	Paediatric ward: 1 professor, 11 assistant professors, 23 doctors, 15 nurses	Paediatric ward: 1 professor, 14 assistant professors, 10 doctors, 14 nurses
TECHNICAL RESOURCES	39 oxygen wall outlets, 19 oxygen bubblers, 09 nasopharyngeal aspirators. CXR. Laboratory: routine blood tests, mycobacteriology, Xpert TB test, PCR.	25 oxygen wall outlets, 06 oxygen bubblers, 04 aspirators. CXR. Laboratory: routine blood tests, mycobacteriology.	37 oxygen wall outlets, 20 oxygen bubblers, 06 aspirators. CXR. Laboratory: routine blood tests, mycobacteriology.

COUNTRY	**MOZAMBIQUE**	
SITE	José Macamo General Hospital	Maputo Central Hospital
CITY	Maputo - capital city	Maputo - capital city
LEVEL	Secondary level hospital Teaching Hospital	National referral hospital Teaching Hospital
CATCHMENT AREA (population)	451 888	
NB PAEDIATRIC BEDS	65 including 11 beds for lung diseases	335 including 36 beds for lung diseases
NB CHILDREN ADMITTED	2 400 /year	8 000 /year
SPECIALIZED WARDS	TB ward Nutrition ward	InfectiousDdiseases ward (including TB and HIV) Pulmonology ward Nutrition ward
CLINICAL RESEARCH EXPERIENCE	Yes (HIV)	Yes
HUMAN RESOURCES	Paediatric ward: 6 paediatricians, 4 GPs, 18 nurses	Paediatric ward: 26 paediatricians, 14 GPs, 26 resident physicians, 121 nurses
TECHNICAL RESOURCES	Emergency and wards equipped with oxygen wall outlets. CXR. Laboratory: HIV and routine blood tests, mycobacteriology, Xpert TB test.	Emergency and wards equipped with oxygen wall outlets. CXR. Laboratory: HIV and routine blood tests, mycobacteriology, Xpert TB test.

COUNTRY	**UGANDA**		
SITE	Mulago National Regional Hospital	Holy Innocents Childrens' Hospital	Jinja Regional Referral Hospital
CITY	Kampala - capital city	Mbarara, South Western Ankole Region	Jinja
LEVEL	National Referral Hospital Teaching Hospital	General Children's hospital	Regional Referral Hospital
CATCHMENT AREA (population)	1 507 000 (Kampala)	100 000	486 256 (Jinja District)
NB PAEDIATRIC BEDS	250 including 40 beds for lung diseases	60	
NB CHILDREN ADMITTED	150-200 children with severe pneumonia /month	238 children <5 years with severe pneumonia in 2017	10 /day
SPECIALIZED WARDS	HIV (Baylor College Centre of Excellence) Pneumonia Ward Nutrition Ward		HIV Treatment Center Emergency room for children with severe pneumonia

CLINICAL RESEARCH EXPERIENCE		Yes	
HUMAN RESOURCES		2 permanent paediatricians	2 resident paediatricians
TECHNICAL RESOURCES		Digital CXR. Oxygen generator, C-PAP. Laboratory: routine blood tests, mycobacteriology.	Laboratory: HIV and routine blood tests

COUNTRY	**ZAMBIA**	
SITE	University Teaching Hospital	Arthur Davison Children's Hospital
CITY	Lusaka - capital city	Ndola, Copperbelt Province
LEVEL	national referral hospital	Provincial referral hospital Only standalone paediatric 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 Research Clinic	
CLINICAL RESEARCH EXPERIENCE	Yes (HIV) GCP training: all staff from the Research Clinic	
HUMAN RESOURCES	Research Clinic: PI, co-PI, and 15 staff (doctors, research nurses, data managers).	
TECHNICAL RESOURCES		

21.3. APPENDIX 3: Summary procedure for nasopharyngeal aspirates collection

Nasopharyngeal aspirates consist in the collection of 2-5 ml of throat contents through a catheter tube connected to a mucus aspirator. The procedure is performed in a child in supine position on his/her back or side, or sitting on his family member/guardian's lap. In order to avoid child injury due to movement, young children are wrapped in a piece of cloth, and the child's head id hold throughout procedure.

After connecting a mucus extractor to the suction pump and catheter, the suction pressure is adjusted. Recommended pressure and catheter size are based on the child's age as follows: in children aged < 1 year, 8 CH catheter with 80-100 mmHg (0.10 bar) suction pressure; in children aged 1 to 10 years, 8 CH catheter with 100-120 mmHg (0.15 bar) suction pressure. After measuring the length of tube necessary to reach the posterior pharynx, equal to the distance between the tip of the nose and the external opening of the ear, the catheter is inserted into the child's nose, without applying suction, along the nostril floor to the posterior pharyngeal wall.

This usually induces cough and sputum expectoration that can be aspirated by applying suction and slowly withdrawing catheter, using a rotating movement, without pushing the catheter forward while aspirating to reduce the risk of local trauma. The catheter should remain in nasopharynx for a minimal period of time, not to exceed 10 seconds.

This procedure should aspirate 2 to 5 ml of secretions. If the volume is not reached by the first aspiration, the procedure is repeated in the other nostril. This procedure is not repeated more than twice. After recapping and cleaning of the specimen container with alcohol/chlorhexidine

to prevent cross-infection, and appropriate labelling, the specimens should be transported to the laboratory within 4 hours.

The entire procedure is performed under peripheral oxygen saturation monitoring with an oximeter.

21.4. APPENDIX 4: Summary procedure for the preparation of stool samples for Xpert MTB/RIF Ultra

(Subject to further modifications)

Stool samples are prepared for Ultra testing by emulsification of 0.5 g of material in Sheather's solution, filtering through funnel gauze and centrifugation.

Sheather's solution is prepared by dissolving 454 g of sucrose in 355 mL of distilled water over low-heat on a stove. After autoclaving for 15 min at 110°C, 10 mL aliquots of this solution are prepared in sterile 15 ml Falcon tube and kept at 4° C to prevent mold contamination.

Stool samples are processed by adding 10 mL of the 50% Sheather's solution to 0.5 g of fresh stool specimen or frozen stool specimen thawed at room temperature into a 15 ml Falcon tube, emulsifying stool manually with two wooden sticks, and vortexing for 30 seconds. The emulsion obtained is poured through funnel-gauze into a new 15 mL Falcon tube, and centrifuged at 100g for 1 minute (no brake). After careful removal of the tube from the centrifuge to avoid disturbing the suspension, 0.5 mL of suspension is retrieved from the top of the specimen and added to 1.8 mL of Xpert MTB/RIF Ultra Sample Reagent, shaken vigorously 10 - 20 times, and incubated for 15 minutes at room temperature. After 5 to 10 minutes of incubation, the specimen is shaken again vigorously 10 to 20 times. The specimen obtained is then directly tested with Ultra.

21.5. APPENDIX 5: Supporting data for sample size calculation

Table A1. Data and hypotheses for the calculation of estimated mortalities in the control and intervention arm

	Very high TB incidence settings[1]	**High TB incidence settings[2]**	**References/hypotheses**
TB prevalence	**24%**	**15%**	
Culture confirmed TB	8%	5%	Oliwa, 2015 [8]
Unconfirmed TB	16%	10%	33% culture-confirmed TB
HIV prevalence			Dodd, 2017 [1]
Cambodia	2,6%		3 hospitals (756 children)
Cameroon		21,1%	2 hospitals (504 children)
Côte d'Ivoire		16,2%	3 hospitals (756 children)
Mozambique	22,6%		2 hospitals (504 children)
Uganda		17,1%	3 hospitals (756 children)
Zambia	32,6%		2 hospitals (504 children)
Mean HIV prevalence	16,9%	**17.8%**	Weighted mean
Mortality rate when treated			
HIV-infected overall	**10%**		Marcy, 2018 [59]; Yotebieng [102]
HR of death in Xpert+ versus Xpert negative	*3.0*		Tiemersma, 2011 [61]
HIV-infected Xpert+	18%		Calculated
HIV-infected Xpert negative	6%		Calculated
HIV-negative overall	**2%**		Jenkins, 2017 [58]
HR of death in Xpert+ versus Xpert negative	*3.0*		Tiemersma, 2011 [61]
HIV-negative Xpert+	3%		Calculated
HIV-negative Xpert negative	1%		Calculated
Mortality rate when not treated			
HIV-infected overall	90%		Dodd, 2017 [1]
HR of death in Xpert+ versus Xpert negative	1,1		Tiemersma, 2011 [61]
HIV-infected Xpert+	96%		Calculated
HIV-infected Xpert negative	87%		Calculated
HIV-negative overall	44%		Jenkins, 2017
HR of death in Xpert+ versus Xpert negative	3		Tiemersma, 2011
HIV-negative Xpert+	79%		
HIV-negative Xpert negative	26%		
Detection rate			
Control			
Culture confirmed TB	25%		Hypothesis
Unconfirmed TB	15%		Hypothesis
Intervention			
Culture confirmed TB	90%		Dorman, 2017[21]; Nicol, 2018 [23]
Unconfirmed TB	50%		Hypothesis

[1]≥300 cases/100,000 population per year; [2]50 - 299 cases/100,000 population per year

Table A2. Estimated mortalities in the intervention and control arm and difference between both arms

	Very high TB incidence settings[1]			**High TB incidence settings[2]**	
	Intervention	Control		Intervention	Control
Probabilities					
HIV-infected	16,9%	16,9%		17,8%	17,8%
Culture-confirmed TB	8,0%	8,0%		5,0%	5,0%
TB detected	90,0%	25,0%		90,0%	25,0%
Mortality rate in treated	18,0%	18,0%		18,0%	18,0%
TB not detected	10,0%	75,0%		10,0%	75,0%
Mortality rate not treated	96,1%	96,1%		96,1%	96,1%
Unconfirmed TB	16,0%	16,0%		10,0%	10,0%
TB detected	50,0%	15,0%		50,0%	15,0%
Mortality rate in treated	6,0%	6,0%		6,0%	6,0%
TB not detected	50,0%	85,0%		50,0%	85,0%
Mortality rate not treated	87,1%	87,1%		87,1%	87,1%
Expected percentage of HIV+ TB death	1,6%	3,1%		1,1%	2,0%
HIV-negative	83,1%	83,1%		82,2%	82,2%
Culture-confirmed TB	8,0%	8,0%		5,0%	5,0%
TB detected	90,0%	25,0%		90,0%	25,0%
Mortality rate in treated	3,4%	3,4%		3,4%	3,4%
TB not detected	10,0%	75,0%		10,0%	75,0%
Mortality rate not treated	78,6%	78,6%		78,6%	78,6%
Unconfirmed TB	16,0%	16,0%		10,0%	10,0%
TB detected	50,0%	15,0%		50,0%	15,0%
Mortality rate in treated	1,1%	1,1%		1,1%	1,1%
TB not detected	50,0%	85,0%		50,0%	85,0%
Mortality rate not treated	26,2%	26,2%		26,2%	26,2%
Expected percentage of HIV-negative TB death	2,5%	7,0%		1,6%	4,3%
Overall expected TB deaths	4,2%	10,0%		2,6%	6,3%

Overall expected probability of death due to TB all settings	**3,3%**	**8,0%**
Differential mortality	**-4,7%**	

[1]≥300 cases/100,000 population per year; [2]50 - 299 cases/100,000 population per year

21.6. APPENDIX 6: Information sheet and informed consent form: control arm

21.7. APPENDIX 7: Information sheet and informed consent form: intervention arm

21.8. APPENDIX 8: List of country Central Laboratories

Table 6: National Central Laboratories and contractual agreement with the CTU

COUNTRY	Central Laboratory	Agreement with country CTU
CAMBODIA	Pasteur Institute in Cambodia, Phnom Penh	Own CTU facility
CAMEROON	Centre Pasteur du Cameroun, Yaoundé	Research agreement with IRD CTU
CÔTE D'IVOIRE	Centre de Diagnostic et de Recherche sur le Sida et les autres maladies infectieuses (CeDReS), Abidjan	General MoU with PAC-CI CTU
MOZAMBIQUE	Instituto Nacional de Saúde (INS), Maputo	Own CTU facility
UGANDA	Medical and Molecular Laboratory Repository, Makerere College of Health Sciences Medical School, Kampala	Agreement for sample storage with MUJHU CTU (Kampala and Jinja sites)
	Epicentre Mbarara Research Center, Mbarara	Own CTU facility (Mbarara site)
ZAMBIA	National TB Laboratory, University Teaching Hospital, Lusaka	Own CTU facility

21.9. APPENDIX 9: Covid-19 suspect case definition, testing indications and management of Covid-19 in children per country

Country	Covid-19 suspect case definition	Testing indication for children with severe pneumonia	National recommendations for sample collection	Treatment guidelines	Management/ isolation of paediatric cases
Cameroon	WHO case definition*	Yes (community transmission in study sites area)	One nasal swab	Hospitalized cases: respiratory distress management, fluid management, prevention of complications, ATB. Antiviral treatment: HCQ + azm	Centralised. ORCA Covid Centre, Yaoundé (not study sites)
Côte d'Ivoire	WHO case definition*	Yes (community transmission in study sites area)	One nasal swab	Hospitalized cases: Respiratory distress management, fluid management, ATB, antiviral treatment (LPV/r), and telmisartan	Centralised. Tropical Infectious Diseases Unit at Treichville UTH.
Mozambique	WHO case definition*	Yes (community transmission in study sites area)	One nasal swab	Hospitalized cases: Respiratory distress management, fluid management, prevention of complications, HCQ + azm	Centralised. Polana Canica Hospital, Maputo (not study site)
Uganda	WHO case definition*	Yes (community transmission in study sites area)	One nasal swab	Respiratory distress management, fluid management, prevention of complications, ATB (azm or amx or ceftriaxone for severe cases)	Centralised. Covid units at Mulago Hospital, Kampala, and Mbarara Regional Hospital
Zambia	WHO case definition*	Yes (community transmission in study sites area)	One nasal swab	Respiratory distress management, fluid management, prevention of complications, ATB (azm) + corticoids, and anticoagulant for severe cases	Centralised. Lusaka UTH is a transfer centre only.

ATB: antibiotics; amx: amoxicillin; azm: azithromycin; HCQ: Hydroxychloroquine; LPV/r: lopinavir/ritonavir

*** WHO COVID-19 CASE DEFINITION (published 7 August 2020)**

1. SUSPECTED CASE

A) A person who meets the clinical AND epidemiological criteria:

Clinical criteria:

- Acute onset of fever AND cough;
OR
*- Acute onset of **ANY THREE OR MORE** of the following signs or symptoms: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnoea, anorexia/nausea/vomiting, diarrhoea, altered mental status.*

AND

Epidemiological criteria:

- Residing or working in an area with high risk of transmission of virus: closed residential settings, humanitarian settings such as camp and camp-like settings for displaced persons; anytime within the 14 days prior to symptom onset;
OR
*- Residing or travel to an **area with community transmission** anytime within the 14 days prior to symptom onset;*
OR
- Working in any health care setting, including within health facilities or within the community; any time within the 14 days prior of symptom onset.

B) A patient with severe acute respiratory illness (acute respiratory infection with history of fever or measured fever of ≥ 38 C°; and cough; with onset within the last 10 days; and requires hospitalization).

2. PROBABLE CASE

*A) A patient who meets **clinical criteria above** AND is a **contact of a probable or confirmed case**, or epidemiologically linked to a cluster with at least one confirmed case;*
*B) **A suspect case with chest imaging** showing findings suggestive of Covid-19 disease;*
C) A person with recent onset of anosmia (loss of smell) or ageusia (loss of taste) in the absence of any other identified cause.
*D) **Death**, not otherwise explained, in an adult with **respiratory distress** preceding death AND **was a contact of a probable or confirmed case** or epidemiologically linked to a cluster with at least one confirmed case.*

3. CONFIRMED CASE

A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms.

21.10. APPENDIX 10: Copy of the insurance policy

21.11. APPENDIX 11: Copy of the Inserm CEEI and WHO ERC approvals

21.12. APPENDIX 12: Copy of the Competent Authority's authorization

21.13. APPENDIX 13: List of required administrative and/or ethical clearance per country

(Subject to further completion)

COUNTRY	Ethical clearance	Administrative research clearance	Institutional Review Board
CAMBODIA	National Ethics Committee for Health Research (NECHR)		Clinical Research Committee (CoRC) of the Pasteur Institute
CAMEROON	National Ethics Committee for Research in Human Health (CNERSH)	Administrative Authorization for Research (AAR) (Division of Operational Health Research (DROS) of the Ministry of Public Health	
CÔTE D'IVOIRE	National Ethics Committee (CNER)		
MOZAMBIQUE	Comité Nacional de Bioética para Saúde de Moçambique (CNBS)		Comité Institucional de Bioética para Saúde-INS (CIBS-INS)
UGANDA	- Uganda National Council of Science and Technology (UNCST) - Joint Clinical Research Centre Research & Ethics Committee (JCRC REC/IRB)		
ZAMBIA	National Research Ethics Authority (NREA)		University of Zambia Biomedical Research Ethics Committee