TB-SPEED (STRENGTHENING PEDIATRIC TB SERVICES FOR ENHANCED EARLY DETECTION

IMPACT OF AN INNOVATIVE CHILDHOOD TB DIAGNOSTIC APPROACH DECENTRALIZED TO DISTRICT HOSPITAL AND PRIMARY HEALTH CARE LEVELS ON CHILDHOOD TUBERCULOSIS CASE DETECTION AND MANAGEMENT IN HIGH TUBERCULOSIS INCIDENCE COUNTRIES

TB-Speed Decentralisation

International protocol version No -6.1 – 25/06/2021

CONFIDENTIAL

RESEARCH PROTOCOL INVOLVING HUMAN PERSON

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☐ **Monocentric study**
☐ **French multicentre study**
☐ **European multicentre study**
☒ **International multicentre study**

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HISTORY OF PROTOCOL VERSION

TB-SPEED (STRENGTHENING PEDIATRIC TB SERVICES FOR ENHANCED EARLY DETECTION)

IMPACT OF AN INNOVATIVE CHILDHOOD TB DIAGNOSTIC APPROACH DECENTRALIZED AT DISTRICT HOSPITAL AND PRIMARY HEALTH CARE LEVEL ON CHILDHOOD TUBERCULOSIS CASE DETECTION AND MANAGEMENT IN HIGH TUBERCULOSIS INCIDENCE COUNTRIES

TB-Speed Decentralisation

Version NO.	Version Date	Amendment Summary
Protocol v2.0	21/5/2019	Patient level diagnostic approach refined to differentiate between standard of care and research. Parental consent will only be sought for NPA and stool collection, access to individual medical records, use of individual data for study purposes , storage of data and biological samples
Protocol v3.0	25/6/2019	Community engagement approach clarified Consenting guidance for children reaching 18 years during the sample storage refined
Protocol v4.0	21/10/2019	Exclusion of all biobanking in Sierra Leone and blood biobanking in Cameroon Placement of oxygen concentrator in all the PHC implementing PHC-focused strategy in Cameroon in compliance with local IRB recommendation and pulse oximeters in all DH all countries Change in PHC facilities in Ivory Coast and Mozambique
Protocol v5.0	13/01/2020	TB diagnosis will be standardized using the TB-Speed Diagnostic algorithms Included collection of plasma in M2 for the cohort in the patient schedule Included that children with presumptive TB diagnosed with TB will be asked to consent to join the study as part of the nested prospective cohort Clarified that facilities would ensure access to oxygen and salbutamol within routine care for children who may require it following NPA Revised patient flow charts
Protocol v6.0	10/05/2021	Section 4.3 Revised study period from sept 2021 to Sept 2022 Section7.2.4: Biobanking left over samples for stool and NPA will be restricted to the participants in the cohort study Section 8.2.3 Include CXR re-reading by an external team as part of the CXR EQA process. Section 7.5: Revision of acceptability, feasibility and fidelity data collection timeline and method (interview data can be collected on the phone if face-to-face interview not feasible) Section 16.2.3: Added virtual International monitoring visit Section 20.4: Changed of one of the PHC in Sierra Leone from Jembe PHC to New police barracks

Protocol v6.1	28/05/2021	Section 7.2.3: Stool processing to also be done in PHCs that have the capacity to process samples onsite Section 7.3.2 Presumptive cases can also be identified from IPD Section 7.4.2: Cohort follow ups to be done at the PHCs in the DH focused strategy, Cohort follow up visits can also be done via the phone. Section 8.1.0: collection of blood at M2 to also be done at the PHCs in the PHC focused strategy Section 20.5: Changed from systematically referring all children not diagnosed with TB from the routine to only children with persistent symptoms after 7 days of antibiotic treatment

LIST OF ABBREVIATIONS

ANRS	French National Agency for Research on HIV/AIDS and Hepatitis
CAB	Community Advisory Board
CME	Continuing Medical Education
CNIL	Commission Nationale de l'Informatique et des Libertés
CPC	Country Project Committees
CPM	Country Project Manager
CRA	Clinical Research Associates
CREDIM	Centre de Recherche et Développement en Informatique Médicale
CRF	Case Report Form
CTU	Clinical Trials Unit
CXR	Chest radiography or chest X-ray
DH	District Hospital
DMP	Data Management Plan
DR	Digital Radiography
eCRF	Electronic Case Report Form
EMB	Ethambutol
ERC	Ethical Review Committee
eTMF	electronic Trial Master File
FDC	Fixed-dose combinations
FRA	Field Research Assistant
GCP	Good Clinical Practices
GPM	Global Project manager
HC	Health Centre
HIV	Human Immunodeficiency Virus
HW	Health Workers
ICER	Incremental Cost Effectiveness Ratio
IDLIC	Infection Diseases in Low Income Countries
IMCI	Integrated Management of Childhood Illnesses
INS	Instituto Nacional de Saude (National Institute of Health Mozambique)

IPC	Institut Pasteur in Cambodia
IRB	Institutional Review Board
IRD	Institut de Recherche pour le Développement (Research Institute for Development)
IT	Information technology
KAP	Knowledge Attitude and Practices
LYS	Life Years Saved
MOH	Ministry of Health
MSF	Médecins Sans Frontières (Doctors without Borders)
MTB	Mycobacterium tuberculosis
NPA	Nasopharyngeal aspirate
NTP	National Tuberculosis Program
OPD	Outpatient Department
OSC	Output Steering Committee
PCC	Project Coordination Committee
PHC	Primary Health Care
PI	Principal Investigator
POC	Point-of-care
QA	Quality assurance
QC	Quality control
RIF	Rifampicin
SAB	Scientific Advisory Board
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
SSRA	Social Science Research Assistant
TB	Tuberculosis
TeAM/SPI	Technical Assistance for Management/Soutien Pneumologie International
UBx	University of Bordeaux
WG	Working Group
WHO	World Health Organization

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1. INTRODUCTION

Childhood tuberculosis burden

The burden of childhood tuberculosis is still high, at approximately one million new cases and 253,000 deaths in 2016 (1, 2). Of the 253,000 estimated TB deaths, the vast majority were the result of lack of access to treatment mainly due to under-diagnosis, especially among young children (1). Recent mathematical modelling estimated that 96% of childhood TB deaths occur in children not receiving TB treatment (3). Most of these deaths may be preventable with access to treatment since childhood TB is a highly curable disease. Indeed, data from large TB paediatric cohorts show that, with access to treatment, the pooled case fatality ratio can be as low as 0.9% (95% CI 0.5–1.6) compared to 21.9% (95% CI 18.1–26.4) in the pre-treatment era (4). Treatment of childhood TB will improve with access to child-friendly fixed-dose combinations (FDC) developed through the Global Alliance/Unitaid Step-TB project.

The WHO End TB Strategy aims to reduce overall TB-related mortality by 75% and TB incidence by 50% by 2025(5). Pillar 1 of this strategy includes early TB diagnosis, treatment of all diagnosed cases, and screening of high-risk and vulnerable groups. However, in 2016, only 42% (about 438,000) of the estimated childhood TB cases were notified to the WHO, largely due to the low detection rates (1).

Challenges and lack of decentralisation of childhood TB diagnosis

Several factors can explain the low detection of childhood TB. One of these factors is the poor performance of current diagnostic tools in children. Although, as in adults, pulmonary TB is the most common form of childhood TB, children usually present paucibacillary disease, which significantly contributes to the low microbiological detection yield from respiratory samples (6). On top of this low detection yield, current assays such as Xpert MTB/RIF are underutilized due to the difficulty of obtaining sputum and other respiratory samples in children (7). Children are frequently unable to expectorate sputum. WHO recommends using other sputum collection methods as induced sputum and gastric aspiration, which are operationally challenging for most low resource settings and are poorly accepted by parents and health care workers (8, 9). These methods often require overnight admission, trained staff, along with robust and affordable equipment and material, the absence of which leads to poor implementation (10). Besides the paucibacillary nature of the disease leads to low diagnostic yield of sputum on existing tests including smear microscopy and culture. Simpler sputum and non-sputum collection methods are needed to maximize bacteriological confirmation of TB.

Another challenge for the bacteriological confirmation of TB in children is the low roll-out of GeneXpert machines in many high TB incidence settings, particularly at secondary and primary health care facilities (11) . Current GeneXpert machines need electricity and computers, which limits their deployment at primary health care level (11) . As a consequence, microbiological tests are not available at the level where children will access care leading to need for referral of children or their samples to a higher level, therefore increasing the risk of drop-outs during the diagnostic pathway and of delayed treatment initiation (11) .

In the absence of bacteriological confirmation, treatment decisions are based on a combination of a history of TB contact, suggestive clinical and radiological features, and the presence of comorbidities such as HIV-infection. There are no pathognomonic clinical signs of TB since other infections can mimic its clinical presentation (12) . The large proportion of children receiving empiric TB treatment without bacteriological confirmation can pose serious problems in regions with increasing incidence of drug resistance.

Capacity for proper diagnosis and treatment decision is often lacking as health care providers are not always trained on the clinical diagnosis of childhood TB and mentoring on TB care is often lacking mostly especially at primary health care levels. Access to good quality chest X-rays (CXR) is another major limitation in many low resource settings. There is both limited access to CXR services for TB diagnosis and inequalities in the distribution of such services (13) . Radiography is usually positioned at secondary health care level, therefore requiring referral of the patient, and the cost of CXRs is usually not covered by the National TB Programs (NTPs), resulting in potentially

substantial financial costs for the families and so low uptake (10) . When available, the quality of CXRs is poor due to lack of reagents, old equipment, and poor training of staff (14) . CXR interpretation skills are limited among most health workers due inadequate training and lack of mentoring (15-18) . Besides, the non-specific CXR lesions in children, especially in the context of HIV infection, and the presence of CXR features such as lymph nodes lead to significant disagreement between X-ray readers (14, 19).

Other challenges contributing to under-diagnosis of TB in children are structural and arise from the way health care services are organised. In most resource-limited countries, childhood TB services continue to be centralised at secondary and tertiary care levels, limiting the access to care for children at peripheral level. Primary Health Centres (PHC) are not routinely involved in TB care in many countries and are supposed to refer cases with clinical suspicion of TB. However, in many PHCs staff are not trained to identify potential TB cases and referral is poor.

WHO guidance on the diagnosis of TB is stepwise, with screening as the first step to identify patients with presumptive TB who will require further investigation. However, the screening step is not well characterised and there is often confusion between screening and clinical evaluation which is part of the diagnostic package for presumptive TB cases (20) . In most documents, TB screening includes a clinical evaluation. There is no clear recommendation on how, where, and by whom TB screening should be done. Therefore, there is usually no systematic TB screening of children attending health care facilities.

Despite recent policy changes in high and very high TB incidence countries and implementation of childhood TB diagnosis at PHC level in some countries, childhood TB stills remains underdiagnosed under treated and under reported as compared to adult TB.

Opportunities to improve childhood TB diagnosis

Despite the lack of tools, several actions could be implemented to improve the diagnosis of childhood TB. Regarding specimen collection, several studies have shown that nasopharyngeal aspirate (NPA) is easier to perform and better tolerated than induced sputum and can achieve a detection yield close to that of gastric aspirate or induced sputum (21, 22) . There is also evidence that stool samples could be an alternative to gastric aspirate to detect TB bacteria swallowed by the child. Studies report sensitivity of between 50 and 80% of Xpert used on stool samples as compared to standard respiratory specimens (23-27).

Recent work by our group and others has shown than the use of Xpert on the combination of one NPA and one stool could achieve the same detection yield as 2 gastric aspirates or 2 induced sputum samples (22) . These methods do not require overnight fasting and are easier to implement in children with severe respiratory distress than induced sputum (28) . The newly developed Xpert cartridge, the Xpert MTB/RIF Ultra (Ultra) has an increased sensitivity compared with Xpert (29, 30).

Furthermore, Cepheid is developing a new battery-operated machine with results available from a smartphone, the GeneXpert Omni, that has the potential to be used at PHC level and that may be available at the end of 2019. Meanwhile the one-module GeneXpert platform (G1 Edge) is already available and has the potential to be introduced in peripheral settings. The combination of the Ultra cartridge and GeneXpert Omni/G1 (Edge) could facilitate the near point-of-care deployment of sensitive molecular diagnostics in peripheral settings. Furthermore there is evidence that the Xpert machines can be successfully operated by a nurse in the peripheral centres in resource limited settings (28, 31) .

There is limited literature on the feasibility of decentralisation of TB services in children(32). However, some studies reporting decentralising adult TB services have shown improved patient access and quality of care. Where decentralisation of TB services was implemented, the case notification rates increased along with the improvement of treatment outcomes (32-34) . Training tools for decentralization have been developed to improve clinical diagnosis and radiological interpretation of CXR in children and guidance has been incorporated into WHO guidelines (35, 36). Experiences from pilot projects have shown that mentoring on top of training is important to strengthen and maintain technical skills (32, 37).

Simple symptom-based TB screening at the children entry point to a health facility could identify children requiring further TB investigations. The definition of 'TB screening' however is not standardised in most settings and symptom screening consistent with the WHO guidelines often leads to confusion (20) . Systematic symptom-based screening using lay providers at health care entry points could identify large numbers of children with presumptive TB. The impact of this screening on TB case detection and its feasibility still needs to be demonstrated in high burden countries.

Lastly, digital radiography could solve the problem of poor quality of X-rays due to poor quality of reagents, could facilitate the interpretation of X-rays and the transfer of images for double reading and quality control for research purposes (38) .

The TB-Speed approach to decentralization of childhood TB diagnosis

The TB-Speed Decentralisation study aims to increase childhood TB case detection at district hospital (DH) and PHC levels using adapted and child-friendly specimen collection methods, i.e. NPA and stool samples, sensitive microbiological detection tests (Ultra) close to the point-of-care (Omni/G1(Edge)), reinforced training on clinical diagnosis, and standardized CXR quality and interpretation using digital radiography. Optimised CXR reading is a structured deliberate process to minimise the disadvantages of the existing poor quality film-based X-rays and reading skills amongst the clinicians. Digital X-ray could facilitate the interpretation of X-rays and the transfer of a sample of images for quality control at national level for in-country monitoring and quality improvement and re-reading of all CXR at international level for validation of study hypothesis.

Unlike the management of childhood pneumonia, which is well defined at each district health system level (39), evidence is lacking on the best decentralization model for diagnosis of childhood TB. The TB-Speed Decentralisation study will evaluate the impact of an innovative patient care level diagnostic approach deployed at DH and PHC levels, namely the DH focused and the PHC focused decentralization strategies. This is aimed at, improving case detection in 6 high TB incidence in low/moderate resource countries: Cambodia, Cameroon, Côte d'Ivoire, Mozambique, Sierra Leone, and Uganda, and compare effectiveness and cost-effectiveness of the two different decentralization approaches.

Our hypothesis is that, in countries with high and very high TB incidence (100–299 and ≥300 cases/100,000 population/year, respectively), a systematic approach to the screening for and diagnosis of TB in sick children presenting to the health system will increase childhood TB case detection, especially pulmonary TB, which represents the majority of the disease burden (>75% of case)(40). We also hypothesize that sputum collection using battery-operated suction machines and microbiological TB diagnosis using Omni/G1 (Edge) can be decentralized to PHC level, thus enabling TB diagnosis and treatment in children at PHC level.

2. OBJECTIVES

2.1. PRIMARY OBJECTIVE

To assess the impact of an innovative childhood TB diagnostic approach decentralized at district hospital and PHC levels on childhood TB case detection compared to the pre-intervention status.

2.2. SECONDARY OBJECTIVES

1. To compare the DH-focused and the PHC-focused decentralization strategies of an innovative childhood TB diagnostic approach in terms of:
    a. TB case detection
    b. TB screening in outpatient children
    c. Feasibility of implementing the different diagnostic approach components
    d. TB treatment uptake and time to TB treatment initiation

e. Cost-effectiveness from the health services perspective

f. Acceptability by health care providers, NTPs and health authorities, and beneficiaries

g. Fidelity of the implementation of the diagnostic approach as compared to the protocol and study procedures

2. To determine, within a nested cohort of children with presumptive and diagnosed TB, and compare between the decentralization strategies:

   a. Performance of the diagnostic approach at patient level

   b. TB treatment outcome

3. To assess the CXR component of the intervention in terms of:

   a. Diagnostic performance of CXR reading by clinicians at DH and PHC levels

   b. Added value of CXR in the diagnosis of TB in children as compared to microbiology and clinical evaluation only

   c. Uptake of the quality control of the CXR reading

3. STUDY ENDPOINTS

3.1. PRIMARY STUDY ENDPOINT

Proportion of TB cases detected among sick children routinely attending outpatient services before and after the intervention.

3.2. SECONDARY STUDY ENDPOINTS

1. Decentralisation strategy specific secondary endpoints

   a. Proportion of TB cases (confirmed and unconfirmed) detected among children identified as presumptive TB

   b. i) Proportion of children screened for TB among sick children attending outpatient services

      ii) Proportion of children identified with presumptive TB among children screened

   c. i) Proportion of children with presumptive TB enrolled in the study receiving the different components of the innovative childhood TB diagnostic approach (NPA and stool or sputum sampling attempt and success, sample testing with Ultra and results, clinical evaluation, CXR and interpretation, full diagnostic package)

      ii) Time to sample test and results delivery to clinician

      iii) Number of visits to the health facility until final diagnosis

   d. i) Proportion of children initiating TB treatment among those diagnosed as TB

      ii) Time from positive TB screening to TB treatment initiation

   e. Incremental-Cost Effectiveness Ratio (ICER) of the diagnostic approach

   f. Acceptability endpoints:

      i) Perceptions and experience of the intervention by healthcare workers (HCWs), the NTP and health authorities, and the beneficiaries (parents/guardians)

   g. Fidelity endpoints:

      i) Changes in the intervention implementation as compared to 1) study standard implementation procedures and 2) country implementation procedures

These changes could be related to NTP guidelines dispositions, adaptation to local context and constraints not initially planned per standard and country implementation procedures.

ii) Proportion of clinical mentoring visits performed per study procedures; proportion of health facilities implementing NPA and stool sample collection and performing sample processing and Ultra testing per study procedures

2. Nested-cohort specific secondary endpoints
    a. Sensitivity and specificity of the diagnostic approach as compared to the reference diagnosis based on the Case Definitions for Classification of Intrathoracic Tuberculosis in Children for clinical research (see section 3.3)
    b. TB treatment outcome as defined by WHO (41)
3. CXR secondary endpoints
    a. Sensitivity and specificity of CXR reading by clinicians at DH and PHC to detect lesions suggestive of TB as compared to the reference reading (independent reading by external radiologist experts)
    b. Proportion of children diagnosed with TB based on CXR and incremental yield of TB detection with CXR results as compared to microbiological (Ultra on NPA and stool or sputum) and clinical evaluation, respectively
    c. Proportion of CXR selected for quality review assessed by the reference reviewer and time to results of the quality control to the clinic

3.3. REFERENCE DIAGNOSIS FOR THE COHORT STUDY AND VALIDATION BY THE EXPERT COMMITTEE

All children participating in the prospective cohort study will be classified in 3 categories at the end of the follow up, according to the revised Classification of Intrathoracic Tuberculosis Case Definitions for Diagnostic Evaluation Studies in Children (30), detailed in Table 1 or any subsequent published update at the time of final case review if feasible and approved by the Scientific Advisory Board (SAB).

Table 1: Updated Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children (adapted from Graham et al, 2015 [55]***)***

Case definition	Refined criteria
Confirmed tuberculosis	Bacteriological confirmation obtained (*Mycobacterium tuberculosis* confirmed by culture or Xpert MTB/RIF assay from at least 1 respiratory specimen[1])
Unconfirmed tuberculosis	Bacteriological confirmation NOT obtained AND at least 2 of the following: • Symptoms/signs suggestive of tuberculosis[2] • CXR consistent with tuberculosis[3] • Close tuberculosis exposure or immunologic evidence of *MTB* infection[4] • Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment—no time duration specified) AND no spontaneous improvement of symptoms in the absence of antituberculosis treatment

Unlikely tuberculosis	Bacteriological confirmation NOT obtained AND criteria for "unconfirmed tuberculosis" NOT met (including spontaneous improvement of symptoms in the absence of antituberculosis treatment)

1 Stool is an alternative to gastric aspirate to collect swallowed respiratory samples and is therefore considered a respiratory sample in this project.
2 Clinical signs/symptoms suggestive of tuberculosis include: (a) Persistent cough: persistent (>2 wk), unremitting cough. (b) Weight loss/failure to thrive: (b1) Unexplained weight loss: >5% reduction in weight compared with the highest weight recorded in last 3 months OR (b2) Failure to thrive defined as (i) Clear deviation from a previous growth trajectory, and/or (ii) Documented crossing of percentile lines in the preceding 3 months, and/or (iii) Weight-for-age z score of ≤−2 in the absence of information on previous/recent growth trajectory, and/or (iv) Weight-for-height z score of ≤−2 in the absence of information on previous/recent growth trajectory AND (b3) Not responding to nutritional rehabilitation (or antiretroviral therapy if HIV infected). (c) Persistent unexplained fever: Persistent (>1 wk) and unexplained fever (>38°C) reported by a guardian or objectively recorded at least once. (d) Persistent, unexplained lethargy or reduced playfulness: persistent, unexplained lethargy or decrease in playfulness/activity reported by the parent/caregiver. (e) Infants 0–60 d (or neonate): additional signs and symptoms suggestive of tuberculosis include: (e1) neonatal pneumonia or (e2) unexplained hepatosplenomegaly or (e3) sepsis-like illness.
3 CXR will be considered consistent with tuberculosis if reviewers agree on the presence and location (right/left) of ≥ 1 lesion among the following: alveolar opacity, bronchial compression, excavation, Ghon focus, gibbus, miliary, nodular infiltrates, paratracheal nodes, peri-hilar nodes, pleural effusion, tracheal compression (as suggested by Graham et al. 2012 [45]).
4 TST and IGRA: the possibility of having documented immunologic evidence of M. tuberculosis infection will not be possible as neither TST nor IGRA will be performed in the study.

CXRs will be graded by two independent readers experienced in reviewing CXRs in children. The readers will be blinded to the clinical information, site and to each other's interpretation. In case of disagreement, a third reader will break the tie and the majority opinion will be taken as the consensus.

An Expert Committee will be set up at national level for each country for the purpose of case review and validation of TB diagnosis for the prospective cohort study. The Expert Committee will not review all cases systematically. We will use an algorithm to select children who will be reviewed. The Committee will not review bacteriologically confirmed cases, or true negative TB cases (untreated children with Ultra-negative results who are alive at 6 months with normal M6 follow-up CXR). The Committee will review and validate the TB diagnosis for cases basing on clinical data (both initial clinical and follow up data), microbiological data and radiological features.

The Committee will classify children using the Updated Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children (30) (Table 1), using standard operating procedures (SOPs) developed for the study. An International Expert Committee will review a subset of cases reviewed at country level to ensure homogeneity of classification across participating countries.

4. STUDY DESIGN

4.1. STUDY TYPE

This will be an operational research study using:

- a before and after cross-sectional design to assess the impact of decentralizing an innovative childhood TB diagnostic approach
- a cross-sectional and nested cohort design to compare two different decentralization strategies at DH and PHC levels
- quantitative and qualitative methods

The intervention will be at two levels: at patient care level where an innovative childhood TB diagnostic approach will be implemented, and at health system level where two distinct decentralization strategies will be implemented. The patient care level TB diagnostic approach consists of systematic TB screening, clinical evaluation, NPA and stool or sputum testing using Xpert Ultra, and optimised CXR reading. The two decentralization strategies are the DH-focused and the PHC-focused implementation of the innovative childhood TB diagnostic approach. Two districts per participating countries will be randomly assigned to implement the DH or PHC-focused strategies (See Figure 1).

The study will also include a nested cohort at both the DH and PHC during the intervention phase for a selected sub-set of children with presumptive TB and all children with a diagnosis of TB that consent to participate. This prospective cohort will enable to further document study endpoints related to follow up (TB treatment outcome) and to document TB diagnosis by assessing spontaneous resolution or resolution under TB treatment.

4.2. METHODOLOGY

The study will comprise an observation phase, during which there will be no interference with the routine TB childhood diagnosis processes, followed by an intervention phase in participating districts. During the last month of the observation phase, each district will be randomly assigned to implement either DH or PHC focused decentralisation. There will be no individual patient level randomisation.

4.2.1. OBSERVATION PHASE

During this 3-month observation phase, in each of the study health facilities, we will i) describe the childhood TB diagnosis data and practices; ii) describe the referral processes and outcomes of referrals for TB diagnosis and treatment where feasible and iii) assess existing challenges in childhood TB diagnosis and treatment, as well as readiness (including potential challenges) for the study intervention implementation.

Mixed-methods (quantitative and qualitative) will be used including the collection of retrospective and prospective aggregated data by study nurses (staff) from facility registers, observations, HCW self-administered questionnaire and individual interviews.

4.2.2. INTERVENTION PHASE

At the beginning of the intervention phase, a preparation period will set up decentralization by providing equipment, materials, and reagents, training health workers in childhood TB care, in NPA and stool collection and testing on Ultra, setting up G1(Edge) or Omni at PHC level and G4 at DH if not already available, and digital CXR and CXR quality control. This preparation period will be of 3 months' duration with each facility starting implementation of the patient care level TB diagnostic approach as soon as they have been appropriately trained and have equipment in place.

Following the preparation phase, the study will initiate the innovative childhood TB diagnostic approach at the selected DH and PHC as soon as sites are equipped and HCWs are trained in childhood TB care and NPA and stool collection, and will implement continued capacity building at sites, regular clinical mentoring visits with NTP or their representative, and continued CXR quality control. The prospective 6-month follow-up cohort study will be initiated immediately and will consecutively enrol every tenth child with presumptive TB and all children diagnosed with TB.

Individual data collection in the study will be initiated as soon as the innovative childhood TB diagnostic approach is implemented in the site and will be conducted throughout the intervention phase to document secondary endpoints. Aggregated data will be collected throughout the study.

Feasibility, acceptability, and fidelity of the intervention will be assessed by mixed methods including observations, and self-administered questionnaires of HCW, interviews with HCWs, health authorities, and beneficiaries.

4.3. PROVISIONAL STUDY SCHEDULE

The project is planned to be implemented over a 31 months period, including 3 months of observation phase, 22 months of intervention phase (including a 3-month preparation phase), and 6 month follow-up for children enrolled in the prospective cohort. These timelines take into account extension from initial plan to cover the delayed start and the study suspension due to Covid-19 restrictions in the countries.

- Observation phase: Q3 2019

- Intervention phase: Q4 2019 to Q3 2021 (including preparation phase Q4 2019)
- Last visit of the last patient in cohort follow-up: Q1 2022

5. STUDY ENROLLMENT

5.1. STUDY POPULATIONS

The study populations will be in 4 categories for the 4 different components of the study:

5.1.1. FOR THE PRIMARY ENDPOINT AND SYSTEMATIC TB SCREENING

This study population will consist of all sick children below 15 years entering the Outpatient Department (OPD) of the selected health facilities (DH and PHC).

- Inclusion criteria
  - Sick children seeking care at OPD of DH or PHC
  - Age <15 years
- Non-inclusion criteria for this group
  - None

Generally, no informed consent will be sought because only aggregated data will be collected from routine reports and registers. However, for those children whose consultation will be observed during observation phase, parent(s)/guardian(s) informed consent will be sought.

5.1.2. FOR THE SECONDARY ENDPOINTS COMPARING THE DH AND PHC-FOCUSED STRATEGIES

This study population will include all children routinely attending services below 15 years meeting criteria for presumptive TB after systematic TB screening and/or routine clinical assessment during the intervention phase.

- Inclusion criteria
  - Age <15 years
  - Presumptive TB defined as children presenting ≥1 systematic screening criteria among the following:
    - Cough with a duration of >2 weeks
    - Fever with a duration of >2 weeks
    - Documented weight loss
    - History of TB contact with any duration of cough
  - Presumptive TB identified by the site clinician irrespective of the above criteria, especially presumed extra-pulmonary TB cases
  - Informed consent signed by parent/guardian
  - Child's assent obtained in those aged >7 years
- Non-inclusion criteria
  - Children who have received TB treatment in the past 6 months

5.1.3. FOR THE SECONDARY ENDPOINTS FROM THE NESTED COHORT

This study population will be a subset of the patient care level TB diagnostic approach population enrolled during the intervention phase.

- Inclusion criteria

- Presumptive TB as defined above and identified as 1/10 by selection process

OR

- Diagnosed TB

AND

- Informed consent signed by parent/guardian to participate to the prospective cohort follow-up
- Child's assent to participate to the prospective cohort follow-up obtained in those aged >7 years

- Non-inclusion criteria
  - Children who have received TB treatment in the past 6 months

5.1.4. FOR THE SECONDARY ENDPOINTS TO ASSESS FEASIBILITY, ACCEPTABILITY AND FIDELITY ON THE DIAGNOSTIC APPROACHES

These non-patient study population groups invited to participate will be as follows:

- Parents/guardians of children with presumptive TB
- Health care providers delivering the innovative childhood TB diagnostic approach
- Health care managers such as National TB program and health authorities

All non-patient participants will provide consent to participate in the study.

5.2. STUDY SETTINGS

The study will be conducted in six high (>100 cases/100,000 pop) or very high (>300 cases/100,000 pop) TB incidence countries (Table 2): Cambodia, Cameroon, Cote d'Ivoire, Mozambique, Sierra Leone, and Uganda.

Table 2: TB incidence rate in participating countries

Region	Country	TB incidence rate /100,000 population
Western Africa	Cote d'Ivoire	153
	Sierra Leone	307
Central Africa	Cameroon	203
Eastern Africa	Uganda	201
Southern Africa	Mozambique	551
South East Asia	Cambodia	345

Source: WHO Global TB Report, 2017.

All NTPs of these countries are willing to participate in the TB-Speed Decentralisation study and all countries have existing links with TB-Speed research partners. NTPs are members of the Country Project Committees (CPC) and will play an instrumental role in the scale up of the TB-Speed Decentralisation study strategy, if it is shown to be successful, prior to integration of the recommendations in WHO guidance.

In each country, the study will be implemented in two rural or semi-rural health districts. The selection of the two districts was based on results of a baseline assessment for diagnostic capacities in five

to ten districts per country, using a standardized site assessment tool that was conducted during the 1st semester of 2018. The selection was undertaken jointly by the NTP, the TB-Speed Coordinating Investigators and the country principal investigators (PIs) based on the information from the baseline assessment. The selected districts had to be relatively similar with the following minimum requirements or characteristics: 1) one DH and 4 PHC facilities could participate in the study; 2) CXR services or opportunities to have CXR services were available at DH; 3) minimum human capacity were available at all facilities; and 4) support from NGOs or other institutions, if existing, should be limited to national recommendations as per NTP guidelines. Furthermore, TB case notification and population size of districts were considered in the selection of the districts (see Table 3).

In countries where multiple levels of health centers are found below the DH, the PHC level was identified as the lowest level of care corresponding to the protocol definition of a PHC (see 6.1.2). In several countries, the PHC may not be the lowest level of care where smaller health posts, not to mention Community Health Workers (CHW) exist.

The full list of participating DH and PHC and selected characteristics is presented in Appendix 8.

Table 3. Estimated annual paediatric presumptive TB and diagnosed TB cases by country by selected Districts based on actual paediatric Outpatient (OPD) numbers of 2017

Country	Districts	Annual Paediatric OPD attendance in selected facilities	Estimated presumptive TB cases (10% of OPD cases)	Estimated TB Cases (10% to 20% of presumptive TB)
Uganda	Kanungu	26,676	2,668	267 to 534
	Rakai	25,664	2,566	257 to 513
Sierra Leone	Port Loko	20,503	2050	205 to 410
	Bo	28,033	2,803	208 to 561
Ivory Coast	Danane	26,930	2,693	269 to 539
	Sassandra	18,178	1,817	182 to 364
Mozambique	Manjacaze	29, 050	2,905	291 to 581
	Chokwe	35, 096	3,510	351 to 702
Cameroon	Obala	2,155	215	22 to 43
	Bafia	5,100	510	51 to 102
Cambodia	Batheay	23,189	2,318	232 to 464
	Angroka	17,339	1,734	173 to 347

6. STUDY INTERVENTION

6.1. DESCRIPTION OF THE STUDY INTERVENTION

6.1.1. PATIENT CARE LEVEL: INNOVATIVE CHILDHOOD TB DIAGNOSTIC APPROACH

The intervention will consist of the following 4 components:

- Systematic TB screening: refers to asking simple specific questions at health care OPD entry point to identify children with symptoms suggestive of TB.
- Clinical evaluation: refers to detailed history taking, relevant physical examination, severity of illness. Clinical evaluation will be for all children with presumptive TB.
- Xpert Ultra testing of NPA and stool (or expectorated sputum) for microbiological diagnosis (genotypic detection of *M.tb* and rifampicin resistance).
- Optimised CXR reading: using digital radiography, improvement of reading skills, simplified reading tool, and quality control of CXR reading.

These components will provide guidance to the clinician for the decision whether or not to treat for TB, and when to start treatment. Treatment decision will be either based on individual clinical judgement of urgency and certainty, or on the TB-Speed TB Diagnostic algorithms adapted from the algorithms proposed in the Union desk-guide and that have been adapted for use in many countries (see Figure 1 & 2)(42).

All components of this innovative childhood TB diagnostic package are recommended by the WHO and national guidelines for diagnosis of childhood TB, at the exception of NPA and stool sample collections. The intervention will therefore replace/reinforce standard of care TB diagnostic procedures in implementing health facilities, at the exception of NPA and stool, that will be tested in the context of the research. All children, regardless of whether or not they are enrolled in the study, will therefore be given the best diagnostic approach for TB offered in the context of the project as “standard of care”, i.e. systematic screening, Ultra testing on any sample collected, chest X-ray, and reinforced clinical diagnosis. The parent(s)/guardian(s) will therefore provide consent for NPA collection and stool collection for TB testing as well as access to personal clinical records if they exist and use of the routine clinical data collected for study purposes. Children whose parent(s)/guardian(s) do not consent for enrolment to the study, will have access to the above mentioned improved diagnostic approach – with exceptions of NPA and stool sampling – and to the same standards of care, but their individual data will not be included in data collection or analysis as per specific study protocol.

Figure 1: TB-Speed TB Diagnosis algorithm - HIV Negative children

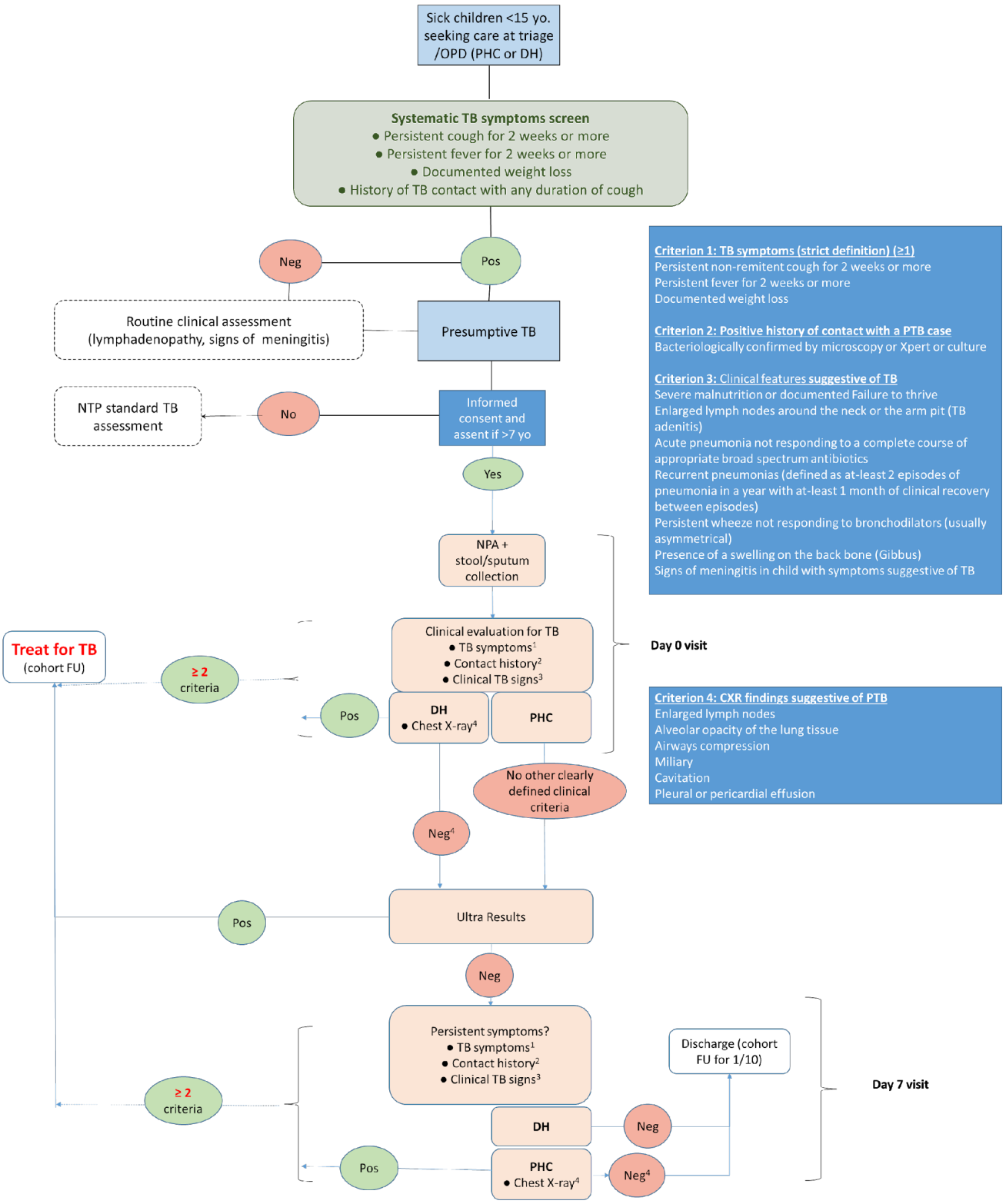
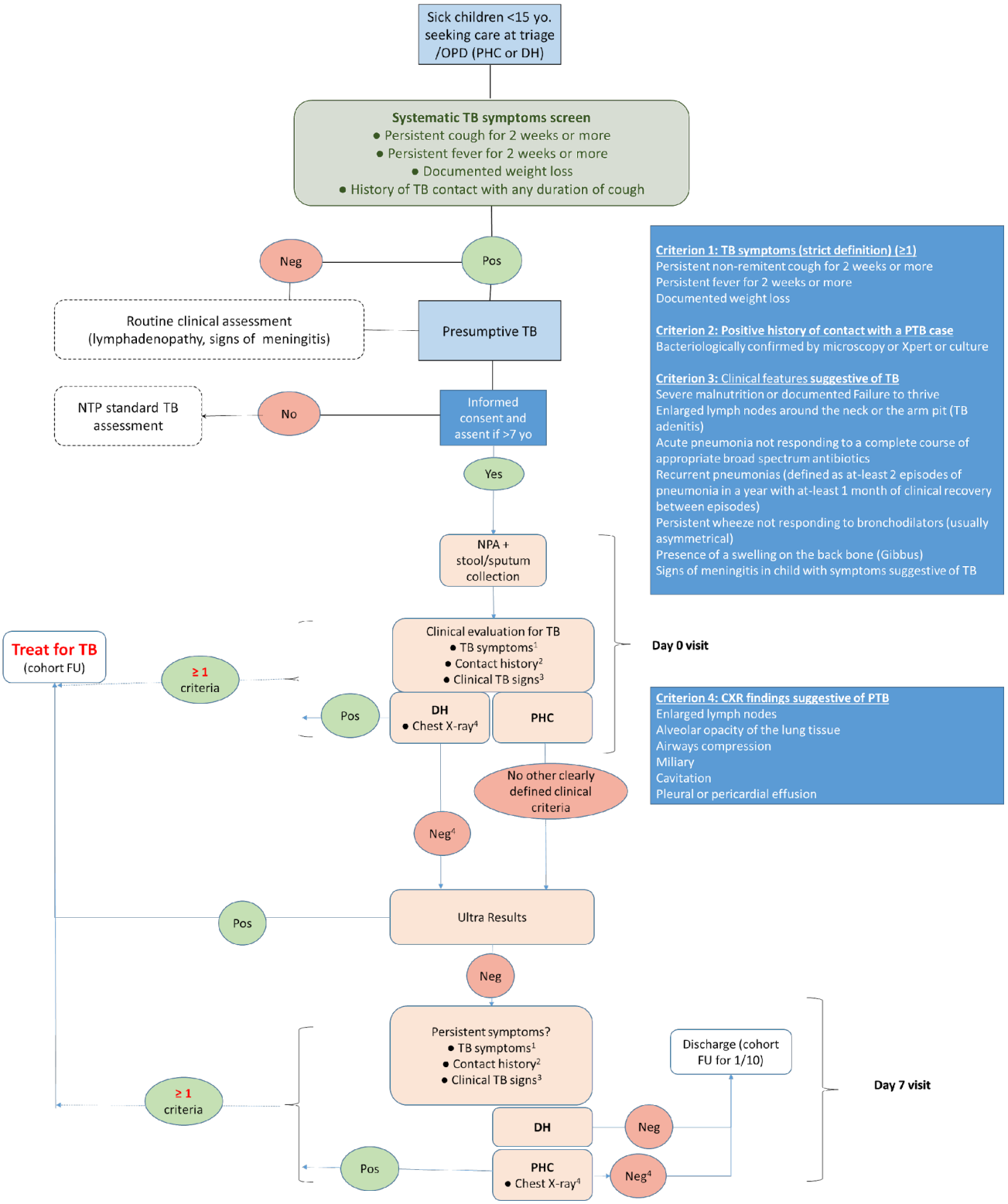

Sick children <15 yo. seeking care at triage /OPD (PHC or DH)
Systematic TB symptoms screen
• Persistent cough for 2 weeks or more
• Persistent fever for 2 weeks or more
• Documented weight loss
• History of TB contact with any duration of cough
Neg
Pos
Routine clinical assessment (lymphadenopathy, signs of meningitis)
Presumptive TB
NTP standard TB assessment
No
Informed consent and assent if >7 yo
Yes
NPA + stool/sputum collection
Clinical evaluation for TB
• TB symptoms[1]
• Contact history[2]
• Clinical TB signs[3]
Treat for TB (cohort FU)
≥ 2 criteria
Pos
DH
• Chest X-ray[4]
PHC
Day 0 visit
No other clearly defined clinical criteria
Neg[4]
Pos
Ultra Results
Neg
Persistent symptoms?
• TB symptoms[1]
• Contact history[2]
• Clinical TB signs[3]
Discharge (cohort FU for 1/10)
≥ 2 criteria
DH
Neg
Pos
PHC
• Chest X-ray[4]
Neg[4]
Day 7 visit
Criterion 1: TB symptoms (strict definition) (≥1)
Persistent non-remitent cough for 2 weeks or more
Persistent fever for 2 weeks or more
Documented weight loss
Criterion 2: Positive history of contact with a PTB case
Bacteriologically confirmed by microscopy or Xpert or culture
Criterion 3: Clinical features suggestive of TB
Severe malnutrition or documented Failure to thrive
Enlarged lymph nodes around the neck or the arm pit (TB adenitis)
Acute pneumonia not responding to a complete course of appropriate broad spectrum antibiotics
Recurrent pneumonias (defined as at-least 2 episodes of pneumonia in a year with at-least 1 month of clinical recovery between episodes)
Persistent wheeze not responding to bronchodilators (usually asymmetrical)
Presence of a swelling on the back bone (Gibbus)
Signs of meningitis in child with symptoms suggestive of TB
Criterion 4: CXR findings suggestive of PTB
Enlarged lymph nodes
Alveolar opacity of the lung tissue
Airways compression
Miliary
Cavitation
Pleural or pericardial effusion

Figure 2: TB-Speed TB Diagnosis algorithm- HIV Infected children



6.1.2. HEALTH SYSTEMS LEVEL – DECENTRALIZATION STRATEGIES

- ***Definitions***

  - **District hospital (DH)** refers to an inpatient health care facility with a doctor, a nurse, laboratory services with TB laboratory capacity (smear microscopy or Xpert MTB/RIF), CXR services or capacity for CXR services, and childhood TB diagnosis and treatment capacity.
  - **Primary Health care (PHC)** refers to an outpatient health facility with a minimum of a clinician (doctor, clinical officer, medical technician, assistant nurse or nurse), and offers OPD services, closer to the community.

- ***DH-focused decentralisation strategy***

In this strategy, the patient care level innovative childhood TB diagnostic approach will be implemented at the DH level. PHCs in this district, will only conduct systematic TB screening (Figure 3). In PHCs **not already** implementing childhood TB diagnosis and treatment at the study start, all children will be screened and those identified as presumptive TB cases will be referred to the DH for clinical evaluation, Ultra testing on NPA and stool or expectorated sputum, and CXR if indicated (see section 7.3.4).

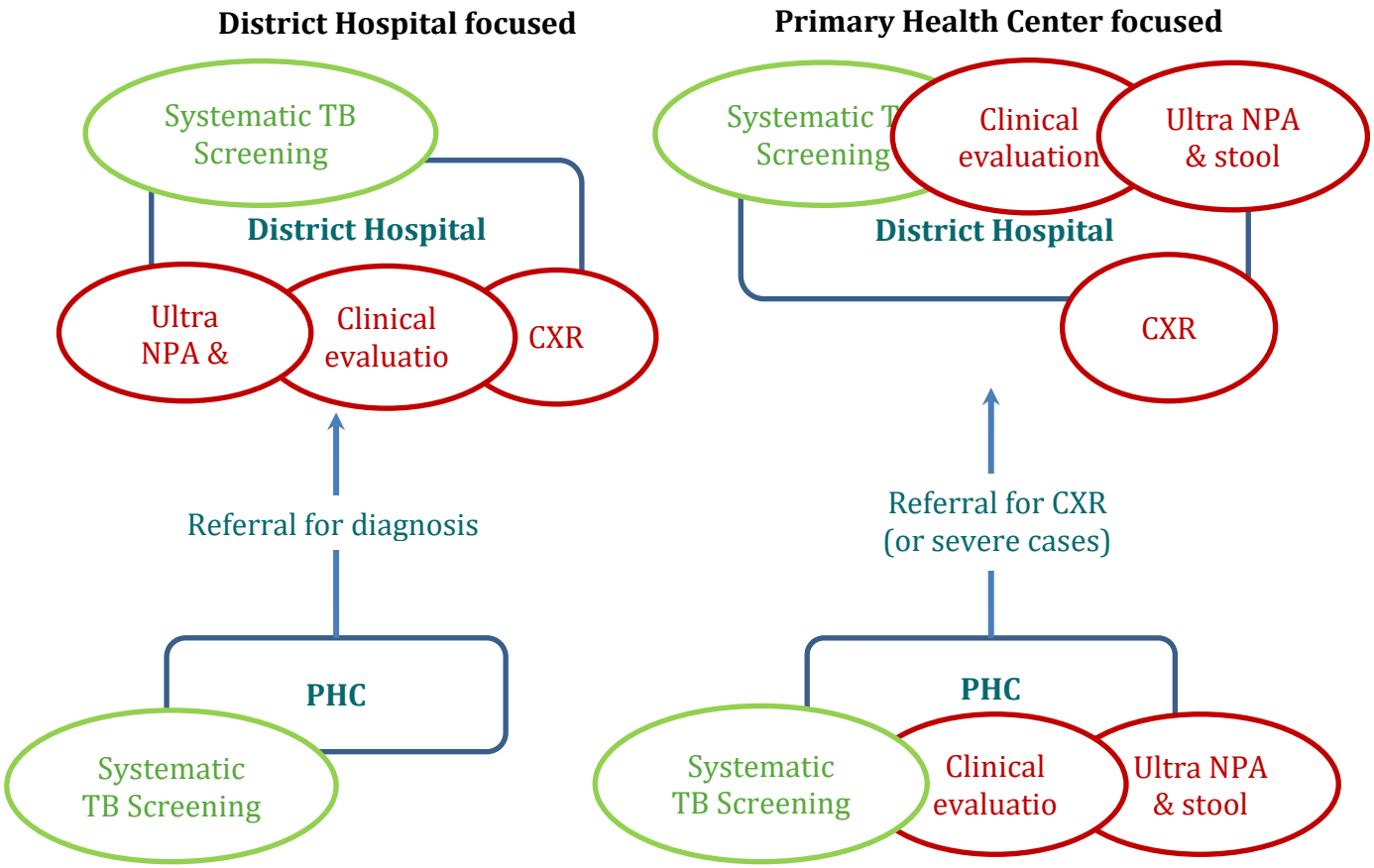
In participating countries that have already decentralized TB diagnosis and treatment initiation in children at PHC level using clinical evaluation and sputum collection (for children able to expectorate), these PHCs will continue to provide these services. Only children in whom clinicians decide NOT to treat for TB according to national guidelines shall be referred to the DH for further investigations and for those cases the decision to initiate treatment will be taken at the DH. All the referrals will be documented and tracked. Children that are diagnosed with TB at PHC level will be offered to participate in the nested-cohort. Children whose symptoms have resolved at day 7 will not be referred to the DH , only children with persistent symptoms at day seven will be referred to the DH for further assessment and enrolment into the study.

- ***PHC-focused decentralization strategy***

In this strategy, the patient care level innovative childhood TB diagnostic approach will be done at the PHC. This will include systematic TB screening, clinical evaluation, and testing of NPA and stool or expectorated sputum with Ultra. NPA or sputum samples collected at the PHC will be tested using G1 (Edge) or Omni or G4 where possible. The stool samples will be referred to the DH for testing until a simplified stool testing kit becomes available for use at the PHC level except for the PHCs that have capacity to process stool onsite. Children will be referred to the DH for CXR only, when indicated (see section 7.3.4).

The DH in the districts implementing the PHC decentralization strategy will also implement the patient care level diagnostic approach for children screened with presumptive TB at DH level and will be supported with digital radiography plates and G1 (Edge) or Omni or G4 where necessary as well as training in child TB diagnosis and treatment.

Figure 3. Diagrammatic representation of the two decentralization strategies (DH focused and PHC focused)



- ***Random allocation of decentralisation strategy to districts***

The decentralization strategy will be randomly allocated between the 2 districts at country level. The random allocation will determine which district will implement the DH or PHC decentralization strategy. The random allocation will be done by the central research unit at University of Bordeaux. The districts and country CTU and principal investigators will only know which strategy to implement in the last month of the observational phase.

6.2. IMPLEMENTATION OF THE STUDY AT SITE LEVEL (DH OR PHC)

6.2.1. OBSERVATION PHASE

During this observation phase, in DHs and PHCs, childhood TB diagnosis data, referral data and the outcomes of all routine referrals for TB care where possible will be collected and analysed. Diagnosis and referral practices, challenges and readiness to the project interventions will also be assessed and documented. This phase will inform the number of children and reasons for screening for TB at a particular PHC facility but been diagnosed for TB at another facility.

We will prospectively, over 3 months, collect aggregated quantitative data from routine facility registers. This includes number of sick children attending OPD, number screened for TB, number with presumptive TB, number tested for TB, number bacteriologically confirmed for TB, number diagnosed with TB, number initiated on TB treatment. In order to determine representativeness of the registered children in routine registers compared to the actual number of sick children entering the health facility general OPD, the study will conduct register exhaustiveness checks. If the quality and exhaustiveness of the registers allow, we will also collect aggregated retrospective data for a period of 9 months (before the 3 months observational period), which will provide a full 12-month dataset before the start of the intervention period.

In order to describe diagnosis and referral practices and processes, we will implement a mixed methods survey in all or selected PHCs and DHs including:

- Knowledge-Attitudes-Practices (38) self-administered questionnaire, to be used during the observation phase and repeated at the end of the study
- Observation of childhood TB consultations while clinicians conduct childhood TB assessment
- Individual interviews among HCWs and key informants

6.2.2. INTERVENTION PHASE:

The intervention phase includes a 3-month preparation period followed by a 19-month implementation period. During the implementation period, the study will implement the patient care level innovative childhood TB diagnostic approach in the selected districts.

In order to decentralize the diagnostic approach, the preparation period of the intervention phase will include the following main activities:

- Capacity building - Equipment, material and reagents: implementing health facilities will be equipped to implement the microbiological (DH and PHC) and radiological (DH only) component of the diagnostic approach
- Capacity building – Training on the four components of the diagnostic approach including reinforcement of the CXR interpretation skills, and training on TB treatment management according to NTP guidelines jointly conducted by the NTP and the study staff for PHC that will newly initiate TB drug management and treatment within the study
- Establishment of a CXR quality control

After the 3-month preparation period, and as soon as site are equipped and trained for NPA collection, all sick children seeking care at the participating facilities will be screened and diagnosed for TB following the innovative childhood TB diagnostic approach, according to the decentralization strategy to which the district facility was allocated. Data will be collected to document the implementation and outcomes of the interventions. The children enrolled in the nested cohort will each have a follow-up duration of 6 months. Monthly clinical mentoring visits will be performed by experienced clinicians from country coordination team, NTP, and referral facilities, during the first quarter, and quarterly thereafter.

6.3. STUDY IMPLEMENTATION FIGURE AND PATIENT FLOW CHART

Figure 4. Study implementation

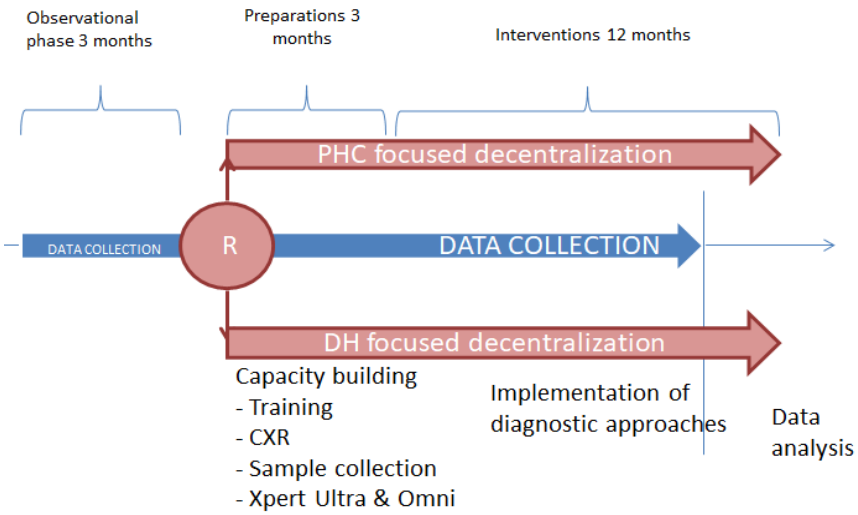
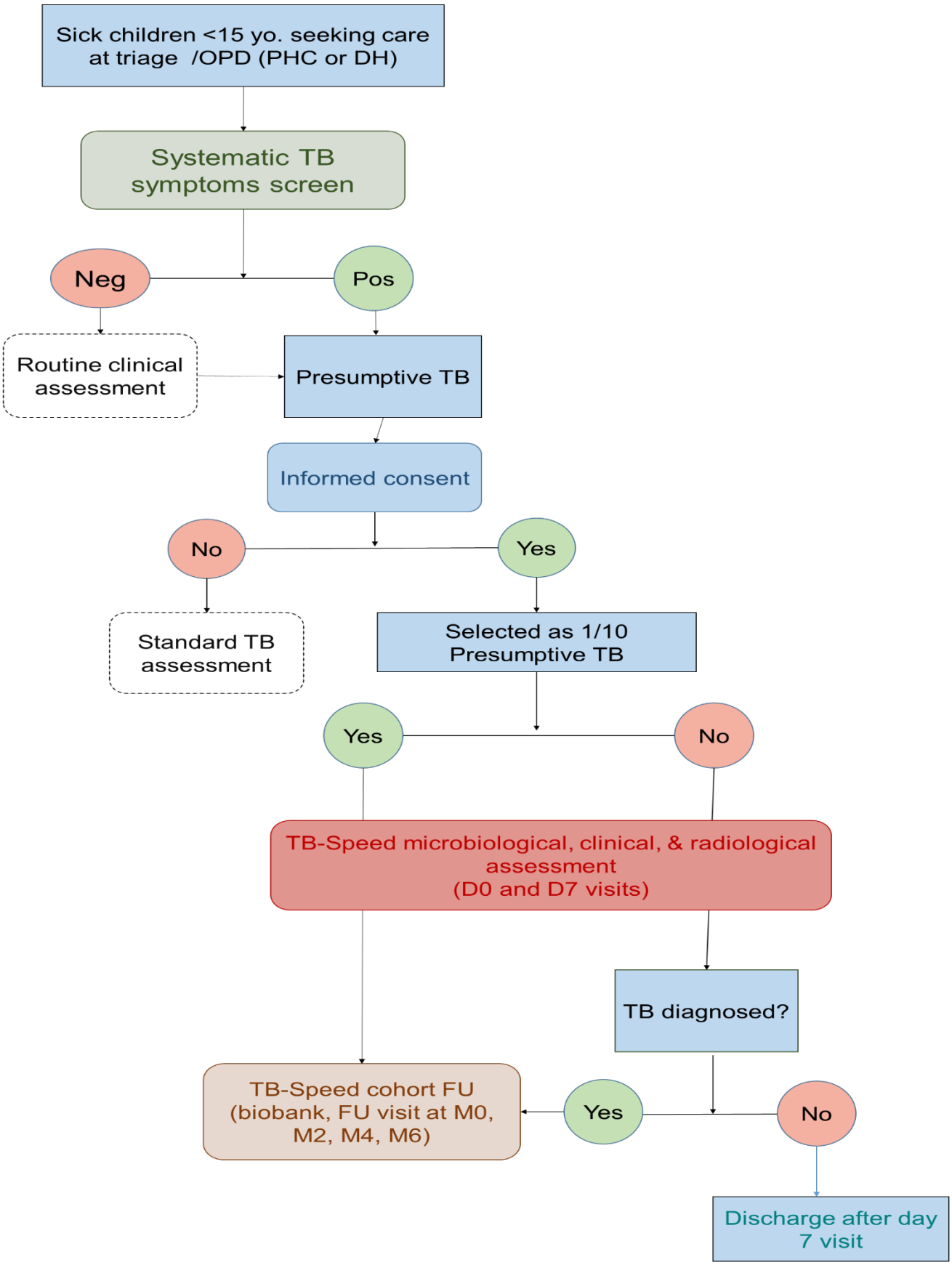


Figure 5. Overall Study flow chart at PHC or DH



7. STUDY PROCEDURES

7.1. OBSERVATION PHASE

7.1.1. ASSESSMENT OF THE CHILDHOOD TB DIAGNOSIS AND REFERRAL DATA AND PRACTICES

Aggregated quantitative data will be collected by external study staff (study nurses, field workers, or clinical research assistants (CRAs)) from routine outpatient registers, screening registers if they exist, TB facility registers, laboratory registers as well as other existing data collection tools, in order to describe and analyse outpatient attendance, diagnosed TB cases, type of TB, TB treatment initiation, and child referral. If the quality of the registers allow, we will also collect aggregated retrospective data for a period of 9 months (before the 3 months observational period), which will provide a full 12-month dataset of process data documented before the start of the intervention period. Permission will be sought from local health authorities for the collection of these routine data.

A mixed methods survey will be implemented by the country study coordination team, with the support of associated investigators in the field of social sciences. It will be conducted in a minimum of 2 PHCs and 1 DH per district (if feasible, we aim for exhaustiveness of study sites):

- All HCWs involved in childhood TB management in each of the study sites will be invited and consented to respond to a short self-administered questionnaire that will capture their knowledge, attitudes and practices (KAP). This KAP questionnaire will be developed based on a literature review and assessment of existing and validated KAP questionnaires; it will be repeated at the end of the study.
- A random sample of child outpatient and childhood TB consultations (an average of 25 per district, n=10 maximum per health facility included in the study) will be observed. Observations will be conducted at different time-points (e.g. during the first month and then the last month of the observation phase, and during random days of the week), after consent by both the HCWs and parent(s)/guardian(s) of the children. These will be guided by a checklist on the expected standard of care for TB in children as defined in the NTP guidelines and will include observation of routine practices on the following activities: history taking including TB screening questions, clinical evaluation including signs and symptoms assessment, TB diagnosis and treatment decision, treatment education provided to the caregiver, communication and interpersonal skills, bacteriological sample collection, microbiological testing, and patient referral for further investigation.
- A purposive sub-sample of HCWs (n=6-10 per district, including TB focal points), in addition to selected key informants (such as the district TB supervisor, n=3 on average per country), will be invited and consented for an individual interview to describe current challenges (potentially discuss results from case studies review) and readiness to the project interventions.

7.1.2. EXHAUSTIVENESS CHECKS

Exhaustiveness checks will be performed on the registers used at the OPD entry points in health care facilities. This is done to determine representativeness of the registered children in routine registers compared to the actual number of sick children entering the health facility. During these checks, the study staff will count and record numbers of sick children entering the health facility for a pre-defined period of time and figures will be compared with the number of children recorded in the facility register at the entry point during the same period.

7.2. INTERVENTION PHASE – DECENTRALIZATION PROCEDURES

Implementation of the study in the different countries will be guided by standard study SOPs and country specific implementation plans secondarily developed by each country research unit (CTU) and validated centrally by the Coordinating Investigators and the international study coordination team.

7.2.1. EQUIPMENT OF STUDY SITES AND PROCUREMENT OF MATERIAL AND REAGENTS:

During the preparation phase and implementation phase, health facilities will be equipped according to the level in the health care pyramid, and according to their needs as assessed during the baseline assessment and observation phase, as follows.

- ***Equipment for PHCs applying the PHC-focused decentralisation strategy***

The following equipment will be provided only if the facility is not already equipped:

- Battery operated Aspiration/suction machines for NPA
- GeneXpert Omni (once available) or GeneXpert Edge
- Tablets (2 to 3 per site) to access and enter patient data
- Transportation cool boxes
- Equipment for basic vital signs measurement
  - Thermometers
  - Weighing scales for children and infants
  - MUAC bracelets
  - Measuring board

Oxygen concentrators will be provided in PHCs implementing the PHC-focused strategy in Cameroon only.

The following consumables/reagents will be provided regularly throughout the duration of the project by the country coordination team:

- Mucus extractors for NPA,
- Sputum and stool sample collection containers
- Ultra-cartridges
- Consumables needed for laboratory processing of stool and NPA samples where needed (Falcon tubes 15/50 mL, transfer pipets, surgical or N95 masks as relevant, sterile gauzes, waste disposal, disposable gloves, lab coat)

- ***Equipment for PHCs applying the DH-focused decentralisation strategy***

The following equipment will be provided only if the facility is not already equipped:

- Equipment for basic vital signs measurement
  - Thermometers
  - Weighing scales for children and infants
  - MUAC bracelets
  - Measuring board

- ***At DH level for both strategies***

The following equipment will be provided only if the facility is not already equipped:

- Battery operated aspiration/suction machines for NPA
- GeneXpert G4
- Digital radiography (DR) plates and mobile X-ray machines
- Tablets (2 to 3 per site) to access and enter patient data

- Oxygen concentrators
- Pulse oximeters
- Equipment needed to perform stool sample processing using the sucrose flotation method (refrigerator, vortex agitator, centrifuge)
- Refrigerator with freezer for samples and plasma storage before transfer to the central laboratory biobank
- Transportation cool boxes

The following consumables/reagents will be provided regularly throughout the duration of the project by the country coordination team (CTU):

- Mucus extractors for NPA,
- Sputum and stool sample collection containers
- Ultra-cartridges
- Sheather's solution (prepared centrally by the country reference laboratory)
- Other material/consumables needed for laboratory processing of stool and NPA samples where needed (Falcon tubes 15/50 mL, transfer pipets, surgical or N95 masks as relevant, sterile gauzes, waste disposal, disposable gloves, lab coat)

7.2.2. TRAINING

The initial training implemented during the preparation period is intended to build/strengthen capacity for childhood TB diagnosis at lowest level of care and will include the following components:

- Nurses or other HCWs working at triage/OPD from DHs and PHCs, and community healthcare workers (CHW) or lay persons when relevant according to local context, will be trained on the standardised TB screening tool.
- Nurses will be trained on NPA and stool sample collection and monitoring adverse events of NPA.
- Nurses and or laboratory technicians will be trained to process samples and test them with Ultra using Gx Edge or standard GX machines.
- Clinicians (nurses, clinical assistants, medical doctors, or any other HCW when relevant according to local context) will be trained on clinical evaluation and global childhood TB diagnosis and treatment skills using:
  - Country childhood TB training curricula adapted OR
  - Study-specific training tool based on the Union Desk-guide and current WHO Childhood TB Treatment guidelines taking into account the country specific NTP guidelines.

  A specific training working group involving members of the WHO - GTB paediatric team, in the framework of the Unitaid funded WHO TB enabler grant will validate the content of the training modules and specific add-ons to national curricula for the study.
- HCWs from PHCs that are involved in the PHC-focused decentralization strategy and that were previously not initiating TB treatment for children and adults, will be trained on anti-TB drug management, TB treatment initiation and follow-up procedures, registration and reporting.
- Clinicians at the DH and PHC levels, and radiographers at DH level will be trained also on CXR interpretation for diagnosis of childhood TB using a study specific training developed by the TB-Speed CXR working group based on previous training

curricula developed by TeAM-SPI and other CXR WG (Working Group) members. A simplified CXR reading tool designed by the CXR WG will be used.

The training will be conducted by the study coordination team staff together with NTP or their collaborators. The CXR training will be conducted by TeAM-SPI.

7.2.3. INTEGRATION OF THE CHILDHOOD TB DIAGNOSTIC APPROACH INTO ROUTINE CARE

The study intervention will be implemented within the existing health care system and involve existing HCWs, and will be adopted as routine TB diagnostic procedures in implementing health facilities, according to the decentralization strategy, at the exception of NPA and stool sample collection which will be subject to parent(s)/guardian(s)' consent.

The TB-Speed decentralization study will train all health workers in systematic TB screening and paediatric TB diagnosis, and treatment. Training for study specific components will focus on the designated staff along the care pathway. Training of all staff will limit interruption of provision of routine childhood TB care services at the implementing facilities.

HCWs working at lower level of care, i.e. community health posts, or CHWs working directly in the community will be informed about the project and sensitized about the need to refer to PHC level children with symptoms evocative of TB.

The study will work within the framework of the NTP and district health system in engaging the communities. The community leaders and the CAB, where they exist, will be introduced to the study so that they can link and also support the study at community level. The CPC at national level will be regularly given updates and shall also link the study to the communities.

- ***Staff support and supervision***

For the purpose of the study, District, DH, and PHC TB focal persons will be trained on the TB-Speed Decentralisation study by the country project coordination teams. These teams will work closely with the TB focal person from the district health office with at least monthly meetings to review progresses and challenges.

At the specific implementing facility, the health facility manager or delegated staff, and the TB focal persons will be involved in planning for study set up, selection of health workers to be trained, identifying space for the study requirements, handling of logistics and other supplies for the study, day to day supervision and monitoring to ensure that the regular health services are preserved even as the study is going on.

- ***Role of different staffs***

Different staff from selected health facilities will be involved in the implementation of the intervention, the study monitoring, or data collection.

The health facility TB focal person will work closely with the country project manager and field workers to oversee the implementation at facility level and ensure that all the required documentation is done and reports submitted. The TB focal person will also receive reports from the CXR and Lab Quality Assurance teams and will be involved in clinical mentoring process.

CHWs working at health facility level may be involved in systematic screening and informed consent taking, especially in PHCs participating in the DH-focused decentralisation, where they can record participant consent to data collection and referral at DH level after TB screening.

Nurses will be in charge of sample collection and Ultra testing for NPA at PHC, if there are no laboratory technicians available at this level of care. Depending on the health facility, they will also be involved in triage, vital signs check, clinical assessment, supervision and delivery of TB treatment. They may be involved in inviting parents and recording their consent for individual interviews, as part of the acceptability and feasibility assessment.

Laboratory technicians will be in charge of Ultra testing of samples at DH level, including samples processing for stools sent from PHCs implementing the PHC-focused decentralisation strategy.

Clinicians, who may be nurses, clinical assistants/officers or equivalent, or MDs will be in charge of the clinical evaluation and diagnosis decision. Clinicians and possibly radiographers will be involved in CXR interpretation.

The study may build some capacity with logistic support and small equipment supply based on needs to be able to implement the study intervention. All staff involved in the implementation of the intervention at DH and PHC level will receive support from the study country coordination team and the district study team to cope with the extra burden of work and potential challenges experienced by the staff.

The different component of the innovative childhood TB diagnostic approach will be integrated in routine care and settings as follows:

- ***Screening***

Depending on the local settings, screening of children will be done at the triage level in the OPD of the health facility by existing staff, designated staff or CHWs. Most participating countries at this stage do not recommend systematic TB screening at triage. If systematic screening is already in place, the TB-Speed screening criteria will replace existing criteria in agreement of the NTP. Data on screening will be captured within existing tools (triage registers or logs) if feasible or using a separate data collection tool.

- ***Sample collection***

Sample collection will be performed in OPD of DH and PHCs and in IPD of DH for hospitalized children by existing trained nurses. The study will ensure that all sample collection will be done in a ventilated area to limit the risk of airborne transmission and that basic infection control measures will be followed by the nurse.

- ***Ultra-testing***

In PHCs, Ultra testing will be performed for NPA, other routine respiratory samples and stool for facilities that have capacity to process samples onsite or when the stool kit for use is available for PHCs not processing stool samples onsite., on G1 Edge or Omni or G4 set up in the usual PHC laboratory if existing, or in another room fit for this purpose, as assessed by the study country laboratory coordinator or a trained delegated staff. A specific SOP for GXpert devices installation will be provided by the study. Ultra-testing will be performed either by the identified laboratory person or by a trained nurse or health worker (See section 8). Stools will be sent to DH for Ultra testing until when stool kit for use at PHC is available.

At DH level, Ultra testing will be performed for routine respiratory samples as well as NPA and stools on G4 or G1 (Edge) or Omni machines at DH laboratory, by usual laboratory staff. NPA and routine respiratory sample testing with Ultra will be performed on the spot or the next morning, while stool might be performed until 3 days after collection depending on the sample flow and burden.

- ***Clinical evaluation and diagnostic process***

The clinical evaluation will be performed in the routine consultation areas by the routine staff. The intervention, in agreement with the NTP, will include standard minimum evaluation and diagnostic processes. The diagnostic decision will be made following the TB-Speed TB diagnostic algorithm adapted from the Union desk guide (see Figure1 & 2). All presumptive TB children will be clinically evaluated for TB. All children will receive the same level of clinical assessment regardless of whether their parent(s)/guardian(s) consent or not to their enrolment in the study.

After triage the children will be evaluated as routine during their waiting time for NPA or expectorated sputum results. Any other comorbidities will be identified by the same clinicians evaluating for TB. As much as possible a single sample shall be obtained for both routine and study procedures.

- ***CXR***

Digital CXR will be obtained by using DR plates on standard analogue CXR machines already in place in DH or from digital radiography CXR machine if already in place. CXR images will be accessible to clinicians for interpretation on tablets serving as monitors. Radiographers will be in charge of uploading CXR on the study website, for children enrolled. Where feasible, radiographers will be trained to interpret CXR using the simplified reading tool.

- ***TB treatment***

In PHCs that are involved in the PHC-focused decentralization strategy and that were previously not initiating TB treatment for children and adults, permission will be sought from NTPs to implement TB treatment initiation in these health facilities. The study together with NTPs will provide additional training to CHWs/TB focal person on anti-TB drug management, TB treatment initiation and follow-up procedures, registration and reporting. These PHCs will receive registers and logs from the NTP and will be included in the network of TB treatment units of the NTP at district level. Mentoring activities, conducted in collaboration with NTPs, will include TB treatment management.

Routine NTP registers will be used and filled for all children diagnosed with TB and receiving TB treatment. The district study coordination team will actively monitor that children in the study are registered at the NTP level.

- ***Job aids, guidelines, tools for patient follow up***

The study will provide a set of procedures and algorithms in the selected centres and support tools such as pocket guides, clinician desk references, cohort monitoring charts, and wall charts. Clinical data helpful to both follow patients and supervise diagnostic process and follow-up of patients will be collected in study specific registers. These will serve as source documents for individual data collection in the eCRF.

7.2.4. SAMPLE STORAGE, REFERRAL AND TRANSFER

- ***Sample storage***

Stools and NPAs will be stored at room temperature in PHCs before testing or transfer to DH laboratory.

At DH, NPA samples will be tested on the spot or the next morning; storage at room temperature will be allowed before testing. Stool samples will be tested on the spot or as soon as possible upon arrival at DH; they will be kept in a specific compartment/box in the refrigerator before testing. Bacteriological samples leftovers and whole blood samples for biobank will be kept at 2-8°C, while plasma samples will be stored at -20°C after aliquot preparation by the DH laboratory.

- ***Sample transfers from PHC to DH***

The following samples will be transferred from PHC to DH during the study:

- Stools will be systematically transferred to the DH for stool processing and Ultra testing until the stool kit for use at PHC is available except for PHCs that have capacity to process stool samples onsite.
- NPA may be transferred during the first few months of the intervention period in some countries where there are feasibility issues for implementing Ultra at PHC level
- Blood collected for biobanking at M2

Stools and NPA will be transferred twice a week, as samples cannot be stored without refrigeration for more than 3 days. The samples will be transported in cool boxes (optionally with ice pack to maintain at 2-8°C during transfer).

Each sample will be referred with a sample tracking form implemented by the study. The samples will be transferred using local transportation systems if existing or a dedicated transportation system implemented for the study.

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- ***Samples transfer from DH to central laboratory***

For study reasons, the following samples will be transferred from DH to central laboratory for biobanking:

- Plasma
- Whole blood
- NPA and stools leftovers for only participants in the Cohort study

Samples will be transferred to the central laboratory once a week in cool boxes (with ice pack to maintain at 2-8°C during transfer). Each sample will be referred with a sample tracking form implemented by the study. The samples will be transferred using a dedicated transportation system implemented for the study.

For TB care and intervention implementation reasons, additional bacteriological samples could also be collected and transferred to central laboratory for culture and drug susceptibility testing in case of rifampicin resistance detected on Ultra tests. These transfers will either be done using routine NTP procedures or facilitated by the study specific transportation system.

7.2.5. CHILD REFERRAL

A child may be referred from PHC to DH in the following situations or needs:

- PHC-focused decentralisation strategy
    - TB diagnosis CXR or M6 follow-up CXR in cohort
    - Blood sample collection for M0 visit in cohort
    - Severe TB requiring hospitalisation
    - RIF-resistant Ultra result requiring further MDR-TB assessment
    - SAM or HIV care if not implemented at PHC level
- DH-focused decentralisation strategy
    - Childhood TB diagnostic approach as part of the diagnostic strategy
    - TB diagnosis CXR or M6 follow-up CXR in cohort for children diagnosed at PHC level in PHCs with already partly decentralized diagnosis

The following rules will be applied to subsidize transfer costs:

- No transfer subsidy for referral of presumptive TB to DH for childhood TB diagnostic approach in the DH-focused decentralization strategy
- Transfer subsidy or ambulance system use for referral of severe cases to DH. Referral will be to DH where the study will have provided oxygen concentrators.
- Transfer subsidy or ambulance system use for referral of cases with adverse events (AEs) due to NPA requiring referral to DH. Referral will be to DH where the study has provided oxygen concentrators. The parent/guardian will be given reasonable financial support during the admission. Transfer subsidy for referral to DH for CXR in the PHC-focused decentralization strategy
- Transfer subsidy for follow-up in the nested cohort; includes transfers from home to DH or PHC

Transmission of information (documents, reports): HCWs from PHCs will inform by SMS or phone call the TB focal person of the DH or equivalent before they refer a patient. In addition, a tracking log will be implemented at PHC and DH to document the referrals and the patients will be referred with a referral form. Local referral tracking system and forms will be used when they exist or will be implemented by the study. This will also allow the TB focal person to inform the health staff of the PHC in case the child did not reach the DH. The health staff of the PHC could then contact by phone or visit the child's parent/guardian if necessary.

7.2.6. MONITORING OF ACTIVITIES

Monitoring of activities implemented in the study will be done with a structured supportive supervision approach. Study coordination teams will regularly visit the implementing facilities to oversee the study activities, identify challenges and discuss with teams how to overcome them. This will be done every one to two weeks by study field workers (research assistant employed by the country CTU based at district level) and monthly by the country central coordination team (i.e. project managers, laboratory coordinator, CRAs and other relevant CTU staffs plus country PIs and senior study nurses if relevant), using specific study procedures, during the first 3 months. Frequency of visits will be adapted secondarily based on performance and challenges. During these visits the teams will look generally into how the study is progressing, management of logistics and supply issues, clinical mentoring and CME, issues arising in referral of children and samples, challenges in the processes of implementing for example NPA sample collection. Performance indicators will be established and supervisory check lists/grids will be used by field workers and coordination teams. This will allow to monitor progress in the implementation of different components of the intervention and to identify areas that would require additional support or monitoring. Specific aspects will be covered by different study staffs and at different intervals:

- Screening, referral of samples and patients, infection control procedures, routine implementation of activities will be monitored once a week by field workers
- Quality of implementation of specimen collection methods, adequacy of performance and difficulties faced in NPA collection will be addressed by project coordinators/clinical coordinators with the support of senior study nurses if relevant
- Ultra-testing procedures and biobank management will be supervised monthly by laboratory coordinators
- Monitoring of clinical evaluation, CXR review, TB diagnostic decision making process will be monitored specifically through clinical mentoring visits (See section 7.2.7).
- Monitoring of CXR data transfer will be performed mostly remotely at country level by IT persons and/or data managers from CTUs
- Data collection and research monitoring (e.g. informed consent and regulatory aspects) will be ensured by separate specific routine monitoring visits (see section 16).

7.2.7. CLINICAL MENTORING VISITS

Clinical mentoring visits will be done monthly during the first 3 months and quarterly thereafter for all health facilities. The aim of the mentoring visit is to: 1) empower clinicians (nurses, clinical assistant or equivalent, or MDs) to diagnose TB in children, 2) provide clinical training (practical skills building), 3) perform clinical checks on the clinical decisions.

The mentoring visits will be performed by the study team together with the NTP or their representatives. The purpose of the mentoring visit will be to support site teams in implementing the childhood TB diagnostic approach, especially in systematic TB screening, clinical evaluation and CXR interpretation, treatment management to consolidate HCWs' knowledge and to ensure that standard TB treatment and care is provided to all children in accordance with the NTP guidelines.

The visits will be organised by the project managers based on a standardised and optimised study mentoring tool depending on the level of health facility visited. During the mentorship visit the mentors will work with staff at their work stations. The mentors will observe, assess and provide guidance on any gap areas. They will utilise a standardised mentoring tool at each visit. Selected child TB cases in the study will be discussed with the site clinicians and a few CXRs will be jointly reviewed, especially those submitted for quality control coming with a different re-reading interpretation. As part of the mentoring process, onsite continuous medical education on childhood TB conducted by the site clinicians at health facility level will be supported using the local NTP training curriculum with adaptations for the study.

7.3. INTERVENTION PHASE –INNOVATIVE CHILDHOOD TB DIAGNOSTIC APPROACH

7.3.1. PATIENT STUDY SCHEDULE

Table 4: Patient schedule including specimen collection in children

Study component	**Systematic TB Screening**	**Patient care level diagnostic**			**Nested prospective cohort**			
Population	**Sick children <15 years attending OPD**	**Children with presumptive TB**			**1/10 of presumptive TB and all those diagnosed with TB**			
Timing		**D0**	**D7**	**Optional visit**	**M0 (+/-7)**	**M2 (+/-7)**	**M4 (+/-7)**	**M6 (+/-7)**
Screening	X							
Eligibility criteria		X			X			
Informed consent		X			(X[1])			
Clinical evaluation for TB[2]		X	X					
Follow on clinical examination and history			X			X	X	X
Nasopharyngeal aspirate		X						
Stool sample or expectorated sputum for children above 5 years		X				(X)[10]	(X)[10]	(X)[10]
Ultra		X						
Chest X-Ray		X[3]	X[4]		X[5,6]			X
TB treatment if needed[7]		X	X		X[6]	X	X	X
TB drug adherence assessment						X	X	X
TB treatment response								X
HIV diagnosis (serology or dry spot)[8]					X[8]			
Biobank (frozen samples):								
Plasma sample					X	X		
Whole blood sample					X			
Number of tubes collected					3	1		
Volume of blood collected (mL)[9]					5	2		
Biobank: NPA and stool leftovers					X[11]			

[1]Verify that informed consent for nested cohort including storage was obtained at enrolment
[2]Clinical evaluation defined in the project as detailed history, physical examination and conducting relevant investigations as a second step in TB diagnosis.
[3]In the DH focused - CXR will be done immediately if indicated,
[4]In PHC focused done at 7 days if CXR is indicated at review
[5]For children from PHCs in the PHC focused decentralization if not done prior
[6]For children enrolled after diagnosis
[7]If HIV positive or SAM child will be referred for appropriate care but not treated by the study
[8]Routine HIV testing will be done for the presumptive cases
[9]2.0 ml for plasma and 2.5 ml whole blood for storage, 0.5 ml for HIV testing.
[10]If indicated
[11]Only for cohort

7.3.2. SYSTEMATIC TB SCREENING AND IDENTIFICATION OF PRESUMPTIVE TB

During the intervention phase, all sick children entering the selected health facilities (PHC or DH) OPD will be screened for TB using standardized screening questions based on WHO presumptive

TB case definition (37, 38). This simple TB screening at triage will be undertaken by a HCW or lay provider.

The screening questions will assess presence of:

- Cough for >2 weeks
- Fever for >2 weeks
- Documented weight loss
- History of TB contact with any duration of cough

Presumptive TB at screening will be defined as a positive answer to any of the screening questions listed above. The result of the screening from the questions either positive (presumptive TB case) or negative will be documented in the routine registers (usually HMIS Health Management Information System recording tools) and information captured in the study database as aggregated data.

In addition to this systematic screening, presumptive TB cases may be identified through routine clinical care at the OPD or later at IPD if hospitalized by the clinician irrespective of the above criteria, especially presumed extra-pulmonary TB cases. For example, symptomatic children screened negative at triage could still be identified as presumptive TB during consultation.

7.3.3. ENROLMENT AND OBTAINING INFORMED CONSENT

Parents/guardians of all children with presumptive TB and those diagnosed with TB at the partially decentralized PHCs in DH focused strategy, will be informed about the study and will be offered the opportunity to have their child participating in the study. Informed assent for children above 7 years will be sought; if not obtained, the child will not be enrolled in the study.

Parents/guardians will be informed that participation is voluntary and that they will be free, without justification, to withdraw their child from the study at any moment without consequence on the quality of care and follow-up provided to their children. They will also be informed that whether or not they consent for the study they will receive the best available standard of care for TB diagnosis at the health facility. They will also be given information about the purpose, procedures and duration of the nested cohort (see section 7.4). In addition to oral explanations, a written information sheet will be provided.

Informed consent from parents/ guardians and child assent (if aged >7) should be obtained before performing any study specific procedures (i.e. collecting individual data, NPA, stool, blood draws) for children eligible for patient care level diagnostic approach.

- **Consent at PHCs in DH-focused decentralisation strategy**

Parents/guardians of children with presumptive TB at PHCs in the DH-focused strategy will be asked to consent to allow individual data to be collected for purposes of follow up and tracking the referral process. The children will be referred to DH for TB evaluation.

Those that do not provide consent for their referral to be tracked will still be referred according to routine practice but individual data will not be collected and the referral process will not be tracked.

In PHCs that have already partially decentralized TB diagnosis only parents/guardians of children with presumptive TB who will NOT have been diagnosed with TB through the routine care services will be asked to consent for referral to DH for additional TB evaluation using the TB-Speed innovative diagnostic approach. Likewise the consent will be intended to allow individual data to be collected for purposes of follow up and tracking the referral process. The children will be referred to DH for TB evaluation.

The children with presumptive TB who will be diagnosed with TB at the partially decentralised PHCs in DH-focused strategy will be asked to consent to participate in the nested prospective cohort under the TB-Speed Decentralisation study.

- **Consent at DH in both strategies or at PHC in PHC-focused decentralisation strategy**

The informed consent sought will include information on the prospective cohort and consent for storage of samples for future TB related studies including genetic studies. The purpose of the prospective cohort study will be explained to the parents/guardians of all children in order to enrol later children who will be diagnosed as TB; furthermore, 1 out of 10 children will be consecutively offered to participate in the prospective cohort regardless of the TB diagnosis. If the parent/guardian refuses enrolment, the next child with presumptive TB will be offered to participate in the prospective cohort.

As part of the acceptability assessment a purposive sub-sample of parents/guardians agreeing or refusing to provide informed consent will be invited for an individual interview to explore their reasons for accepting/refusing this diagnosis procedure.

The informed consent and assent process will be implemented by trained delegated health facility staff or CHW. They will ensure that parents/guardians and children if over 7 years old have understood the content of the information sheet, and that they have received answers to all their questions before signing the informed consent/assent. If one of the parents/guardians does not agree with the child's participation, the child will not be enrolled. If the consent to participate is given by the parents/guardians, the consent/assent form will be completed, signed and dated by the parents/guardians/older child and the designated study staff. Thumbprint signature of consent form in the presence of a witness (not from the medical team) is acceptable in the case of illiteracy.

An original copy of the signed consent/assent will be given to parent(s)/guardian(s). The original consent/assent form will be retained in the investigator site file in a safe place throughout the study period. The consents/assents will be stored for at least 15 years after study end in sealed envelopes at the country research coordination center (consortium member).

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

7.3.4. CHILDHOOD TB DIAGNOSTIC APPROACH FOR CHILDREN WITH PRESUMPTIVE TB

All presumptive childhood TB cases identified, whether enrolled or not, will receive the different components of the innovative childhood TB diagnostic approach following study specific procedures except for NPA/stool. Specific flow charts for DH and PHC level childhood TB diagnostic approaches as well as a complementary DH level childhood TB diagnostic approaches in the DH-focused decentralisation strategy when PHC already apply local clinical/sputum sample based diagnosis are available in Appendix 5.

- **Microbiological sample collection**

Microbiological sample collection will be initiated immediately after positive screening. NPA and stool will only be collected in children enrolled in the study after informed consent. Microbiological specimen collection will include:

- 1 NPA will be performed immediately in a dedicated part/room of the OPD using mucus aspirator and a suction machine without prior nasal instillation. Age adapted catheter size (French 6 or 8), low aspiration pressure (80-120 mmHg), and short aspiration time (10 seconds per nostril) will be used/applied to maximize sample collection success and avoid nasal mucosal trauma. The NPA will be performed in supine position or with the child sitting on his/her caregiver's lap.

AND

- 1 stool sample collected if possible on the spot or on the next day; if not collected on the spot parent(s)/guardian(s) will be given a stool container and instructions on how to collect and bring the sample back on the next day.

OR

- 1 expectorated sputum in children aged above 5 years able to expectorate sputum or any other sample recommended by the NTP for children not enrolled. If this sample cannot be collected on the spot, a sample container will be provided as explained above.

NPA will be performed by delegated health facility staff (nurses). Ultra will be the only test performed on these samples. Children without informed consent from parents/guardians will be assessed according to the guidelines of the country NTP.

- **Clinical evaluation**

After undergoing sample collection, children will undergo a detailed clinical evaluation by the clinician, as they wait for results, including:

  - Interview of parent/guardian and child on:
    - History of household TB contact history and past TB
    - History of past and current medication, including TB treatment and ART if HIV+
    - History of chronic diseases
    - Assessment of TB symptoms including presence and duration of: cough and its characteristics (non-remitting, productive, presence of blood in sputum), weight loss or failure to gain weight, fever and/or night sweats, fatigue, reduced playfulness/activity
  - General physical examination of the children including at the minimum:
    - Vital signs: respiratory rate (presence of tachypnea), heart rate, temperature
    - Anthropometric measures: weight, height/length, Mid Upper Arm Circumference (MUAC)
    - Physical signs:
      - TB suggestive signs: lymphadenopathy, gibbus,
      - Signs of pneumonia: wheezing, stridor, chest-indrawing, nasal flaring

- **CXR for TB diagnosis**

In the DH-focused decentralisation strategy, all children will be assessed for TB diagnosis at DH where they will have either sought outpatient care or have been referred from PHCs, and will benefit from systematic CXR on the initial visit.

In the PHC-focused decentralisation strategy, CXR will not be done systematically in children seeking care in PHCs. Children will only be referred to the DH for CXR in the following situations:

- Children still symptomatic and Ultra-negative at the 1-week review visit (2nd visit)
- High risk groups like HIV and SAM
- Clinician deems it is clinically indicated

Additionally, all presumptive TB case and all children diagnosed with TB, clinically or microbiologically without CXR done, enrolled in the prospective cohort study at the PHC, will be referred for a CXR.

For all children in whom a CXR is indicated, standard postero-anterior in children aged ≥5 years or antero-posterior and lateral view (in children below 5 years of age) will be performed.

The CXR interpretation will be based on a simplified CXR reading tool collecting information on presence of:

- Enlarged lymph nodes
- Alveolar opacity of the lung tissue
- Airways compression
- Miliary
- Cavitation
- Pleural or pericardial effusion

The clinician requesting the CXR will also interpret the CXR whether at PHC or DH. Radiographers may be trained and involved in CXR interpretation.

For the purpose of evaluating the added value of the CXR, clinicians will document a TB treatment decision in the CRF before requesting a CXR. The clinician will secondarily access CXRs on the tablet for interpretation and will take final decision regarding TB treatment.

- **TB diagnosis visits**

Children with presumptive TB will have 1 to 2 additional visits following the initial visit when samples are collected and children get a clinical evaluation (See Appendix 5 Detailed patient flow chart). The visits for the diagnosis process will thus be:

- Initial Visit: initial sample collection (NPA & stool or expectorated sputum or other respiratory samples) and clinical evaluation visit (on the day of + screening), as well as CXR in DH. Same day NPA results could be available. An antibiotic course could be provided if the child has not received any treatment for pneumonia in case of cough.

The parent/guardian should also return on the next day to bring stool or sputum sample if not collected on the spot at initial visit.

If Ultra results are positive, the family will be contacted to return to the PHC as soon as possible before day 7 (2nd visit).

- 2nd visit (systematic) at 1 week for clinical re-evaluation, possibly after antibiotic course (5 days amoxicillin), for those not diagnosed with TB at initial visit.

Children with persistent symptoms should be reassessed clinically (for TB as well as asthma, chronic obstructive airway diseases such as bronchiectasis, cardiopathy), sent for CXR if not done initially because they are managed at PHC (in DH-focused decentralization).

- 3rd visit (optional) to interpret CXR results in children from PHC sent for CXR at DH.

TB diagnosis may be made at these 3 different time points:

- At Initial sample collection and clinical evaluation visit:

The clinician may make an immediate treatment decision for clinical TB (e.g. in a child with typical symptoms and history of contact with a smear positive TB case, following the TB-Speed diagnostic algorithm adapted from the Union desk guide, and the specific algorithm if the child is known to be HIV-infected (Figure 1 and 2) and start treatment, or may delay the decision pending NPA + Stool Ultra results or other respiratory samples.

If results are quickly available on NPA or other respiratory samples, children with Ultra positive will immediately initiate TB treatment. The children will still see the clinician for completion of baseline clinical assessment.

In the DH focused decentralisation strategy, for the purpose of evaluating the added value of the CXR, clinicians will document a TB treatment decision in the eCRF before requesting CXR.

- As soon as possible following the reception of Ultra-positive results a TB diagnosis could be made and the family contacted to return to the PHC if possible before day 7 (2nd visit)
- On the 2nd visit at 1 week: a clinical TB diagnosis treatment decision could be made; in Ultra-negative children from PHCs still symptomatic referred for CXR, a TB diagnosis could be made once CXR is interpreted.

Additionally, a TB diagnosis could be made at M2, M4, or possibly M6 cohort follow-up visits for cohort participants not initially diagnosed with TB (see section 7.4.1 and 7.4.2).

7.3.5. TB TREATMENT

Children diagnosed with TB will be registered and treated by the NTPs. Staff from the PHC or DH will ensure initiation of TB treatment for the children. Health workers will initiate TB treatment using standard regimens and dosing schedules following WHO and national guidelines and will use the new paediatric FDCs provided by the NTP. The study will not provide TB medicines. Management of drug toxicity will be performed according to National guidelines. HCWs from PHCs not previously initiating TB treatment will receive specific initial and continuous training through mentoring visits and regular NTP supervision (See section 7.2.2 and 7.2.6, and 7.2.7).

Mentoring activities, conducted in collaboration with NTPs, will include TB treatment management.

Cases identified with rifampin resistant TB using the Ultra will be referred to MDR treatment centres for continued care. Sites will be encouraged to collaborate with their NTPs to ensure prompt initiation of treatment.

7.3.6. HIV AND MALNUTRITION

Newly diagnosed HIV-infected children through the study and HIV-infected children not already of ART will be referred for HIV care to HIV referral clinics of the PHC and DH.

In addition to MUAC, the weight for height Z-score (WHZ) will be determined using current standard WHO tools for children. Children with Severe Acute Malnutrition (SAM) or any other severity sign will be treated or appropriately referred for care.

7.3.7. MANAGEMENT IN THE CASE OF SAMPLE COLLECTION ADVERSE EVENTS (AES)

NPA collection in children is generally safe and well tolerated. Expected adverse events occurring from NPA collection procedure include, in order of reducing frequency: cough (this induced cough reflex is expected as it is the mechanism by which sample is obtained), local trauma/nose bleeding, sneezing, vomiting, and in rare cases dyspnoea/low O2 saturation (33). Children will be clinically assessed for nose bleeding, vomiting and respiratory distress and those with history of bleeding tendency or heavy nose bleeds, ongoing vomiting or reduced level of conscience will not have NPA sample collection due the risk of complication.

In case of acute respiratory distress occurring during sample collection at PHC level, the procedure will be immediately stopped and the child assessed/referred to the DH where the study will have provided an oxygen concentrator. The criteria for referral for oxygen include: persistent respiratory distress and oxygen saturation measured by pulse oximetry of <90% in air. The study will subsidise the transfer costs and provide reasonable financial support to parent(s)/guardian(s). Management in case of AEs will be detailed in the study clinical SOP.

7.4. INTERVENTION PHASE – COHORT

7.4.1. INITIAL VISIT FOR COHORT FOLLOW UP (M0)

The initial cohort follow-up visit (M0) will be performed:

- During the initial diagnosis visit for presumptive TB cases systematically enrolled regardless of TB diagnosis i.e every tenth presumptive TB case (1/10)
- During the visit when TB is diagnosed for TB cases

It will include:

- Verification of informed consent and child assent (>7 years) for cohort follow-up and blood samples
- CXR if not yet done at DH level, or request and referral for a CXR in children seen at PHC level in the PHC-focused decentralization strategy
- Blood sample for HIV test and frozen samples, or request and referral for blood sample at DH level in children seen at PHC level in the PHC-focused decentralization strategy
- There will be no additional clinical evaluation compared to those performed in the diagnostic visits

7.4.2. COHORT FOLLOW-UP VISITS

Children enrolled in the prospective cohort will have additional follow up visits at 2, 4, and 6 months from the time of enrolment. The visit window will be 7 days. The follow-up visits procedures will include clinical assessment for TB symptoms, weight changes, sputum sample collection if indicated and CXR at month 6 (See Table 4, patient schedule in section 7.3.1). The follow up visits can be physical or via the phone depending the situation within the implementing sites

The children will be followed up at the PHC or DH where they were enrolled in the study. However for the PHCs in the DH strategy already implementing partial decentralization, cohort enrolment will be at the DH but the cohort follow up visits will be done at the PHCs for both children diagnosed as TB through the routine system and the referred presumptive enrolled into the study from the DH.

7.4.3. FINAL STUDY VISIT

For the presumptive TB children not diagnosed with TB or not in the prospective cohort study, the final visit shall be when the clinician decides they do not have TB. This shall be at D0, D7 or the optional visit.

In the prospective cohort study, the last visit will occur at the month 6 of follow up for each child enrolled in the cohort.

7.4.4. END OF THE RESEARCH FOR THE COHORT FOLLOW UP

- ***Definition***

Each child in the prospective cohort is followed-up for 6 months. The official end of the research for the cohort follow-up, except in case of premature termination, is defined by the last visit of the last patient included in the study.

- ***Withdrawal of consent***

Withdrawal of the participant from the study may be at the initiative of the investigator or the participant/parents/guardians/children (withdrawal of consent or premature exit). The parents/guardians may decide to withdraw the child from the study at any time if they wish to, without any consequence on the quality of subsequent health care.

When parents/guardians/children withdraw their consent/assent for participation in the study, as they have the right to do at any time, no new information must be collected and recorded in the database after the date of withdrawal. Similarly, no samples should be collected after that date in the context of the study.

When parents/guardians/children who withdraw consent explicitly express that the child's data be removed from the database and the stored samples be destroyed, the study team will carry out such will. When parents/guardians/children who withdraw consent do not express such will, data and samples collected prior to the date of the withdrawal will be used for the analysis.

Withdrawals of consent/ assent to participate in the study must be reported to the country CTU as soon as possible. The principal investigator or designee must document in the patient's study records the date, the reason for withdrawal if possible, and any answers given in response to the child and/or child's parents/guardians.

- ***Loss to follow-up***

When a child for whom parents/guardians have not explicitly withdrawn consent does not show up for routine clinic visits, with their prior agreement, the study team will contact the parents/guardians via telephone or any other feasible means available. The principal investigator or designee 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 6 month visit is considered definitely lost to follow-up in the study, 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 study team (either at the hospital or via telephone, or other feasible means).

- ***Post-study care conditions***

This study will be implemented within the framework of the MOH facilities. Once they leave the study, children will continue to receive care and treatment in the routine health services according to the conditions defined by their country authorities. Once patients complete 6 months of study follow-up and their TB treatment will be completed past the end of their last study visit, they will benefit from the regular care provided by National TB Programs of their respective countries. .

7.5. ASSESSMENT OF FEASIBILITY, ACCEPTABILITY, FIDELITY

7.5.1. OVERVIEW OF ASSESSMENT METHODS

We will evaluate the feasibility, acceptability and fidelity of the intervention guided by the Proctor *et al* conceptual framework on implementation outcome (43). In each study country, a team composed of a doctoral social scientist, social sciences research assistants and clinical mentors, supervised by country PIs and coordinating investigators/associated investigators in implementation research, will collect quantitative and qualitative data from various informants. This assessment will be conducted during the intervention phase and as follows (Table 5).

Table 5. Overview of data collection methods assessing the feasibility, acceptability and fidelity of the intervention

Endpoint	Methods & data collection	Example of indicators
Feasibility	- Clinic routine/process data - KAP questionnaire - Individual interviews with HCWs, key informants and beneficiaries; self-administered intervention questionnaire and observations of HCWs	- Uptake of the diagnosis approach components - Process indicators (for example days without operational devices or available staff)
Acceptability	- KAP questionnaire - Individual interviews with HCWs, key informants and beneficiaries	- Individual acceptability - Individual experience - Individual costs

		- Cultural acceptability - Perceptions of generalizability/scalability and sustainability
Fidelity	- Observation, self-report, checklists - Structured supervision and clinical mentoring reports and other activity logs - Situation analysis	- Adherence to implementation procedures - Quality of delivery - Barriers and facilitators of delivery

- ***KAP questionnaire***

A KAP questionnaire (which will be developed based on a literature review and assessment of existing and validated KAP questionnaires) will be self-administered by HCWs involved in delivering the innovative childhood TB diagnostic approach, and in implementing the model of care (decentralisation strategy). It will be used during the observation phase and repeated towards the end of the intervention period.

- ***Intervention questionnaire***

A self-administered intervention questionnaire will be completed towards the end of the intervention period by HCWs involved in delivering the innovative childhood TB diagnostic approach, and in implementing the model of care (decentralisation strategy), in order to describe the conditions of delivery of the intervention, to document the overall barriers and facilitators of delivery of interventions. This intervention questionnaire will be administered together with the KAP questionnaire.

- ***Individual interviews***

Semi-structured interviews will be conducted with HCWs and parents/guardians towards the end of the intervention period, during M9-M11 after intervention start. If face-to-face interviews are not possible (travel to sites constrained due to sanitary or political reasons), interviews may be conducted on the phone / via video call. In case of interviews via phone, the interviewee's phone number will be provided to TB-Speed staff to allow the staff calling the interviewee.

- HCWs will be interviewed on their experience of delivering the intervention (both the innovative childhood TB diagnostic approach and the model of care – decentralisation strategy): barriers and facilitators of delivery of the intervention, experience of delivery, complexity or ease of delivery of interventions, perceived usefulness, intervention climate, unexpected outcomes, perceptions of generalizability
- Beneficiaries (parents/guardians) will be interviewed on their perceptions, experience and decision-making process regarding the innovative childhood TB diagnostic approach, and the model of care (decentralisation strategy).
- Parents/guardians refusing study participation at enrolment stage will be interviewed on their reasons for refusal.

- ***Key informants' interviews***

Health care managers and decision-makers, national and local health authorities' representatives, will be purposively sampled for individual interviews. Interviews will be conducted face to face or, if that is not possible, by phone / video call. In case of interviews via phone, the interviewee's phone number will be provided to TB-Speed staff to allow the staff calling the interviewee. Interviews will be conducted towards the end of the intervention phase. Key informants will be asked to reflect on their perception of the intervention, perceived usefulness, intervention climate, and perceptions of generalizability.

- ***Observations***

Within each study health facility, a sample of screening processes and diagnostic visits will be observed to assess the feasibility, quality and fidelity of the delivery of the innovative childhood TB diagnostic approach and of the decentralisation strategy. These observations will take place after 2 months and during the last trimester of the intervention implementation. The observations will be conducted after consent by the HCWs and the parent/guardian and guided by a checklist based on the project SOPs.

- ***Situation analysis***

Throughout the course of the interventions delivery, country project managers and PIs will conduct a regular scrutiny of national, provincial and local policies, organisational changes (including staff), media or other campaigns/initiatives that may influence the delivery and reception of the intervention.

7.5.2. POPULATIONS INVOLVED IN ASSESSMENT

- ***Health care workers***

HCWs involved in the delivery of the innovative childhood TB diagnostic approach and of the decentralisation strategy will be identified by the study coordination team. They will be invited and consented to participate in this assessment, for observations of their practice and/or for a self-administered questionnaire and/or an interview (conducted in their location of choice: at the clinic, in the community, or at home). Consent from HCWs will be sought by social sciences research assistants (SSRAs) independent from the clinical team if the interview is to be conducted face-to-face; or by FRA if the interviews is conducted by phone/video call. We will target a sample of 2-3 staff per health facility, per country, as relevant according to the local context.

- ***Key informants***

Health care managers and decision-makers, national and local health authority representatives will be identified by the country principal investigators, then expanded using the snowball technique. Each person will then be invited and consented by SSRAs, by email and/or telephone, to be interviewed (verbal consent). Interviews with key informants will be conducted face-to-face or my phone/video call, as per respondent preference.

- ***Parents/guardians***

- For individual interviews:
    - Parents/guardians who accept all or part of the innovative childhood TB diagnosis approach: We will recruit a convenient sample of 6 to 10 parents per country and per decentralization strategy (to be adapted according to the local context and to preliminary quantitative feasibility data). Parents will be selected based on specific socio-demographic criteria (in order to maximise diversity). They will be invited to participate during the systematic screening visit or during routine follow-up visits. They will be consented either by SSRAs or by the site clinical team depending on the presence onsite of SSRAs.
    - Parents/guardians who refuse all or part of the innovative childhood TB diagnosis approach: We will recruit a convenient sample of 3 to 5 participants per country. Parents will be selected based on specific socio-demographic criteria (in order to maximise diversity); however, depending on refusal rates during the last 6 months of intervention we may revert to exhaustive sampling. They will be invited to participate by the clinical team immediately after the systematic screening visit. They will consented either by SSRAs or by the site clinical team, depending on the presence onsite of SSRAs.
- For observation of consultation:
    - Parents/guardians who accept all or part of the innovative childhood TB diagnosis approach: We will recruit a maximum 10 participants per selected study health facility.

Parents will be selected and invited for observation of consultation based on specific socio-demographic criteria (in order to maximise diversity). They will be invited to participate by the clinical team during the systematic screening visit or during routine follow-up visits. They will be consented either by SSRAs or by the site clinical team, depending on the presence onsite of SSRAs

7.6. END OF THE STUDY/ RESEARCH

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

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

8. LABORATORY AND RADIOLOGICAL EVALUATIONS

8.1. BIOLOGICAL SPECIMEN COLLECTION

For children with informed consent, NPA, and stool or sputum samples are collected at enrolment (see Table 4) for Ultra tests performed at PHC or the DH laboratory. Blood samples are collected at M0 visit in the cohort for HIV test, if not yet performed, as per recommended local and WHO standard of care as well as for biobanking in children in the prospective cohort follow-up who specifically consented to storage. Blood samples will be collected at the DH at enrolment or when the children from PHCs are referred for CXRs if not yet performed.

All specimen collection methods and biological examination procedures will be detailed in a manual of standard operating procedures (MOPs) which will be translated in the language used by the staff on site (English, French, Khmer, 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 lying or seated (See appendix 2). The NPA will be performed using a battery-operated suction device.

- **Stool sample**

Stools cannot be tested with Xpert assay without prior processing to avoid invalid 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 (See Appendix 3). Stool processing optimization is developed as a separate work-package of the TB-Speed project. It will assess centrifugation-free methods for stool processing hence generating evidence on the diagnostic value of stools as alternative specimen for TB diagnosis in resource-limited settings. Once validated in an in vivo study, the optimised stool processing method could be used before Ultra testing in the present study.

- **Blood sample**

For children in the cohort follow-up, 0.5 mL of blood will be collected at baseline for HIV testing if HIV status is unknown. Collection of blood samples for biobank will be performed at the DH laboratory level when children will be referred for CXR. However PHCs in the PHC focused strategy that have capacity to collect the blood samples for biobanking as per the SOP will be allowed to collect the samples onsite then transport them to the DH laboratory. It will include 2 mL EDTA tube for 1.0 mL plasma storage and additional 2.5 mL of whole blood in an appropriate RNA tube for biobanking for future use. At 2 months, 2.0 ml of blood will be collected for plasma storage at DH.

8.2. LABORATORY TESTS AND QUALITY CONTROL

8.2.1. TB BACTERIOLOGICAL TESTS

This will include Ultra performed on 2 samples per child: 1 NPA, 1 stool sample or expectorated sputum in children able to expectorate. Ultra on stool will be performed at the DH laboratory using the flotation method based on the Sheather's solution, until availability of the stool processing optimization method for testing at PHC level.

8.2.2. FROZEN SAMPLES AND BIOBANK

- **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 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 bio-signatures 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]. CRP may have value as a point-of-care screening test, as shown in HIV-infected adults, but data is lacking in children.

- **Type of samples and purpose**

For children in the prospective cohort follow-up, the following samples will be frozen:

  - *NPA, stool or expectorated sputum,*

  Leftover samples from NPA, stool or sputum will be frozen and stored at the Central country level repository.

  Bacteriological examinations such as culture and mycobacterial antigens assays (commercial Lipoarabinomannan assays or other in-house antigens assays) could be performed in the future on NPA, stool or sputum leftovers.

  - *Whole blood*

  2.5 mL of whole blood will be collected in an appropriate Blood RNA tube for potential future genomic analyses.

  - *Plasma*

  A 1.0 mL plasma aliquot will be prepared at baseline and at month 2 from whole blood collected in EDTA tubes. Plasma samples will be collected for exploratory retrospective CRP testing, as well future metabolic, immunologic or pharmacokinetics sub-studies.

- **Storage of samples**

During the study, biological samples will be stored at -80°C at the country level repository. 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 study. 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. This will be subject to country specific IRB approvals. Cameroon will biobank NPA and Stool left over samples but not whole blood or plasma. Sierra Leone will not biobank any samples.

8.2.3. LABORATORY QUALITY CONTROL

- **Ultra testing**

An internal control is built-in the cartridge. The assay includes a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. A Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

An external quality assessment (EQA) using proficiency testing for Ultra testing will be implemented by the study. Every 3 months during the 19 months of implementing phase, a panel of dry culture spots consisting of a combination of MTB (RIF sensitive, RIF resistant), non-tuberculosis mycobacteria (NTM) and/or MTB negative material will be sent to each site (PHC and DH) and processed by the dedicated staff in place. An EQA analysis report will be then generated and sent to the international laboratory coordinator. This report will highlight overall program performance as well as detailing which sites need improvement. Following the report, recommendations including where appropriate the type of training /improvement required, will be communicated to the international and country laboratory coordinator.

Maintenance of GX-1 Edge, Omni and GX-4 that is mainly cleaning, will be done by lab worker or health worker on a regular basis following the SOPs provided by the study. Maintenance log will be monitored by country laboratory coordinator (see section 16).

- **Storage of samples and biobank**

Maintenance report and temperature log of fridges and freezers installed at DH and or central lab will be kept and monitored on a regular basis. Maintenance report, temperature log of the deep freezer and biobank database management will be monitored on a regular basis using a standardized checklist provided by the study.

8.3. RADIOLOGICAL ASSESSMENT

8.3.1. CHEST RADIOGRAPHY (CXR)

CXR is part of the evaluation tests for TB to be done during clinical evaluation and at 6 months for those in the prospective cohort study, as well as if indicated.

A 2-view chest radiography (postero-anterior or antero-posterior and lateral in children < 5 years old) will be performed using standard analogue X-ray machines with digital plates, or digitalized radiography machines where possible. Interpretation at DH and PHC level will be done using a simplified CXR reading tool developed as part of the capacity building component of the TB-Speed Approach. CXR will not be printed; they will be accessible on the patient eCRF and could be downloaded for reading and interpretation.

Digitalized CXRs will be archived on a centralized database accessible through a secured website (the MEREVA tool).

As part of Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children, CXRs will be reviewed independently by two readers blinded to clinical and biological data to identify CXR lesions consistent with TB (29). Discordant opinions will be resolved by a third reader.

A quality control program for the CXRs will be established where selected random 10% of the CXRs in each district will be evaluated by experienced paediatricians or paediatric/ordinary radiologists at the regional or national level. This will be done both in DH focused and PHC focused decentralization strategies and the CXRs will be reviewed for quality (both of the image and the interpretation).

8.3.2. CXR QUALITY CONTROL

CXRs will be randomly selected and centrally read at the national level to assess for quality of interpretation. Reviewers will access CXR without clinical data on the centralized database through a restricted secured access to the MEREVA tool website. At international level, an external consultant will be contracted to look at CXRs to assess for the quality and interpretation across all the countries.

9. STUDY VIGILANCE

9.1. DEFINITIONS

- **Adverse events**

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

- **Serious adverse events**

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

- Results in death;
- Is life-threatening (means 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 modify significantly the evaluation of the benefit/risk ratio of the research or the study product, likely to affect the safety of participants or that could modify the study product administration, the study documentation or the conduct of the study, or to suspend or interrupt or modify the protocol or similar studies.

Examples: a SAE which could be associated with the study procedures and which could modify the conduct of the study, recommendations of the Independent Data Monitoring Committee, 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 [53].

- **Causality**

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

9.2. ADVERSE EVENTS

a) *Expected adverse events related to the study intervention*

Expected adverse events occurring from NPA collection procedure could include nose bleeding, vomiting, cough, nausea, sneezing, dyspnea and respiratory distress. 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 (35).

AEs occurring during NPA will be monitored by study nurses and reported in the CRF in case of severe or life-threatening AE. Management of AEs will be detailed in a SOP.

b) *Reporting of adverse events*

Severe or life-threatening AEs occurring as a consequence of NPA collection, death and cause of death and main diagnosis in case of hospitalisation will be reported in the CRF.

c) *Notification of serious adverse events*

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

- Deaths
- SAEs related to NPA collection

9.3. 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.

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 SAE must be reported if it occurs in a participant:

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

9.4. RESPONSIBILITIES OF THE SPONSOR

9.4.1. SAE RECORDING AND ASSESSMENT

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

The sponsor is responsible for the assessment of the causality of the SAE in relation to the NPA collection. In the absence of information on causality from the reporting investigator, the sponsor should consult the reporting investigator and encourage him to express an opinion on this aspect.

The causality assessment given by the investigator should not be downgraded by the sponsor. If the sponsor disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor should be provided in the report to the National Competent Authority.

All SAE for which the investigator or the sponsor considers that a causal relationship is a reasonable possibility are considered as suspected Serious Adverse Reaction (34).

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

9.4.2. NEW FACT REPORTING

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

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

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

10. DATA COLLECTION AND PROCESSING

10.1. DESCRIPTION OF DATA COLLECTION

- **Aggregated data**

In the observation and intervention phase of the study, the study team will collect aggregated data extracted from daily/monthly produced from routine registers by health facility staff which include OPD/triage attendance register and TB treatment register. In some countries, TB presumptive register may also be used. TB treatment register may be located in other health centers if children are referred for TB diagnosis/treatment. Details on the referral process will be collected during the observation phase.

During the observation phase, only aggregated data will be collected to document: 1) the number of sick children attending the OPD of the PHC or DH facility, 2) those who are TB presumptive if registers are available and 3) those who are treated for TB.

- **Individual patient data**

For all children with presumptive TB enrolled in the study and those who participate to the nested cohort, the following data will be collected for each patient by designated study staff, on remote data capture devices:

- Individual identifiers: month and year of birth, sex
- Anthropometric and clinical data
- Radiological interpretation
- Laboratory data
- Samples collected for biobanking
- TB treatment if initiated

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

Radiological data will also be collected as digital imaging and communications files (.dicom files) and transferred to a FTPS in a server hosted at UBx (Figure 6). CXRs interpretation will be directly reported in the patient's CRF using standardized forms developed as part of the capacity building component of the study.

In the same way, Xpert Ultra test result files (.gxx files) will be extracted directly from the GeneXpert software on monthly basis and transferred by the lab coordinator to each CTU on encrypted CD-ROM. Then, each country CTU will monthly transferred gxx files to a FTPS in a server hosted at UBx (Figure 6).

- **Diagnosis and referral practices quantitative data**

This data will be collected using a KAP questionnaire tool, a self-administered intervention questionnaire, as well as observation checklists. The KAP data will include knowledge indicators on childhood TB epidemiology, symptoms and diagnostic test and treatment; attitudes indicators related to the management of infection control and stigma; practice indicators regarding diagnostic and referral processes. The intervention questionnaire data will include data on conditions of delivery of the intervention, overall barriers and facilitators of delivery of interventions. The observation data will include general logistical, human resources and infrastructure data, as well as information on the childhood TB diagnosis steps/tests; it will contain no patient personal identifier.

- **Qualitative data**

Qualitative data, collected during individual interviews and key informants interviews, will consist in individual narration of personal experience and perceptions in supervising, delivering, or receiving the intervention.

The key informants and individual interviews will be conducted in English, or French, or in the local language. Notes will be taken in English, or French, or in the local language. Interviews will be recorded if permission provided by the interviewees. Any audio recording will be transcribed, and/or translated in English or French if relevant, by the doctoral social scientist and/or the social science research assistants involved in data collection.

- **Cost data**

We will collect data to estimate the TB diagnostic approaches cost that will include direct cost of TB diagnostic and public health resource utilization. TB diagnostic cost will include test and sampling costs. In the frame of the ingredient costing methods, the material cost will be derived from the national Ministry of Health existing data or during interviews with health officials.

We will measure and record resource utilization for the innovative childhood TB diagnostic approach and for both decentralisation strategies. Public-sector unit costs will be collected from relevant sources including clinic sites and the national Ministry of Health.

To estimate human labour cost, we will conduct a time and motion study survey to estimate quantities of public-sector resource use. To do so, we will measure during 5-clinic days, at 3 time points, the time nurses and other health workers use executing each tasks.

Costs will be expressed in U.S. dollars. For those countries that do not have the U.S. dollars, costs will be converted into U.S. dollars using purchasing power parity exchange rates, i.e. market exchange rates adjusted for differences in purchasing power between countries .The price year is 2019.

10.2. DEFINITION OF SOURCE DATA

Source data will be available to document the existence of patients enrolled in the study and should substantiate integrity of the data collected.

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

- Patient’s demographic data (month and year of birth, sex)
- Details related to the study eligibility criteria
- Laboratory results
- Adverse events occurring during NPA

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

During the intervention phase, site staff will collect individual data on a dedicated paper register. This register will be part of the intervention and will reinforce the TB diagnostic implementation at PHC level.

10.3. ELECTRONIC DATA ENTRY

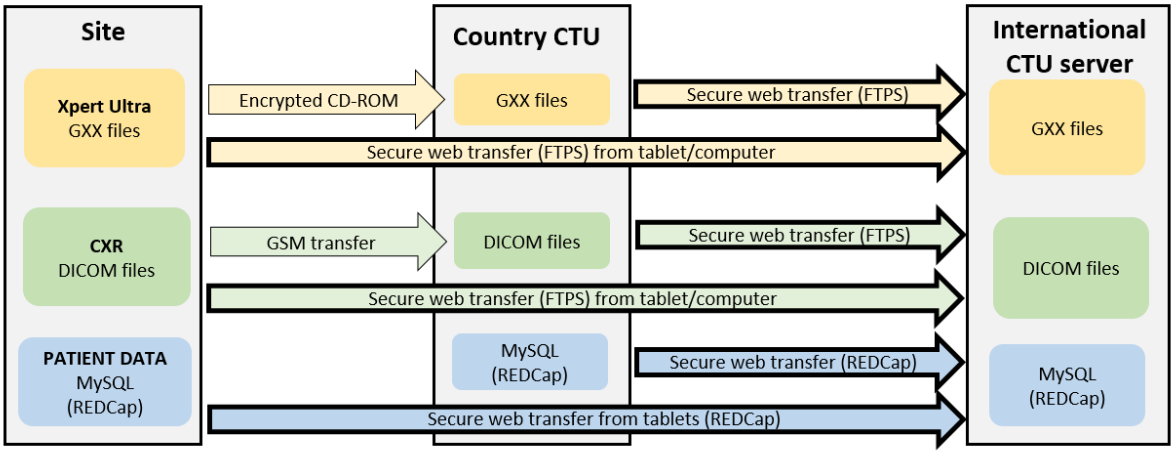
Individual data will be collected by designated study staff through single data entry on remote data capture devices into a database located on a server hosted by the CREDIM (*Centre de Recherche et Développement en Informatique Médicale*) at UBx.

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.

Additionally, quantitative data from KAP questionnaires and observation checklists will be entered in dedicated databases developed on REDCap.

The REDCap MySQL database server will be hosted by the international CTU at the University of Bordeaux (UBx), France. Field-based users will be able to access REDCap either through a classical Internet-connected on computer, or through the REDCap mobile App application on tablet. The REDCap Mobile App enables offline data entry through a tablet or an Android mobile phone: data will be entered locally on the mobile device, and further synchronized to the central database once connected to the Internet (Figure 6). Internet data transfer will use a secure file transfer protocol (ftps).

Figure 6: Secure data flow



The eCRF will be accessible 24/24h 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 and rights levels will be granted and managed by the international data manager (international CTU).

CXRs (.dicom files) and Xpert test result files (.gxx files) will be transferred to the international CTU central server using a secure web transfer (ftps). In case of issues with web access in implementing sites, the transfer of the CXRs files will be done by GSM with a tablet (equipped with a SIM card) from the implementing sites to the country CTU and then from the country CTU to the international CTU central server via a secure web transfer (ftps).

Project Managers from country CTUs will be in charge of training the relevant study staff for data collection and issuing of electronic data queries for quality control. The principal investigator/designee is responsible for ensuring that all sections in the eCRF are completed correctly.

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

10.4. DATA VERIFICATION, VALIDATION AND PROCESSING (DATA MANAGEMENT)

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

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

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

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

10.5. QUALITATIVE DATA ARCHIVING AND MANAGEMENT

Management of the qualitative data collected within this study is the responsibility of the PIs and of the doctoral social scientist. Qualitative data will be anonymised using numerical identification codes.

Audio recordings, transcripts, translations and any analytics documents (coding frameworks, thematic summaries) will be stored on a secure data repository hosted by the CREDIM together with the quantitative data.

10.6. 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 and will be declared to the French data protection authority CNIL (*Commission Nationale de l'Informatique et des Libertés*) in accordance with European General Data Protection Regulation (https://eugdpr.org/) on personal data protection.

The server is located in a secure computer room. The network is protected by uninterrupted power supply firewalls and up-to-date viruses and malwares scanning softwares. 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. Electronic database will be retained at UBx for fifteen years. Essential study documents will be retained at the country CTU for fifteen years after study completion.

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

10.7. STUDY DOCUMENTS ARCHIVING CONDITIONS AND MANAGEMENT

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

Informed Consent form and Assent will be kept at country CTU in a sealed envelope. Study documents constituting the 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 study website. Country CTUs will be responsible for routinely updating national documentation on the study website.

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. DATA ANALYSIS

11.1. STATISTICAL ANALYSIS MANAGER

The statistical analysis manager will be the study statistician, based at UBx. The statistical analysis plan for the primary endpoint and secondary endpoints assessed using individual data will be written by the study statistician and validated by the coordinating investigators. Separate analysis plans will be written for the cost-effectiveness analysis and qualitative and diagnostic and referral practices quantitative data analysis. These will be respectively under the responsibility of a post-doctorate in health economics supervised by the cost-effectiveness specialist, and of a doctoral social scientist supervised by the associated investigator on implementation science.

11.2. PLANNED STUDY ENROLMENT AND POST-HOC POWER CALCULATIONS

Because this study is operational research, we will enrol consecutive children attending the OPD of the selected facilities over 12 consecutive months in the innovative childhood TB diagnostic approach evaluation.

Based on data collected during the baseline assessment, we estimate that the number of sick children attending OPDs in the selected health facilities in 12 districts (2 districts in 6 country) during the 12-month observation phase (3-month observation + 9-month retrospective data) and 12-month implementation period will be 260,000 (See Table 3 and Table 6). Hypothesizing a 10% presumptive TB rate among sick children, and a 20% TB disease rate among those, we expect that a total of 26,000 children per 12-month period will have presumptive TB, including 5200 with TB disease.

Assuming a baseline 20% detection rate prior to study intervention and an increase to a 50% rate during the intervention, the proportion of TB detected in sick children, i.e. the study primary endpoint, will increase from 0.4% to 1.0%. With a significance level of 0.05, and the expected study size, we will have a >0.99 power to show the expected difference between the proportions of TB detected in sick children before and after the intervention using overall 12-month retrospective data.

Table 6: Expected OPD attendance and TB detection rate planned during observation and implementation phases.

	Duration (months)	Sick children attending OPD	Presumptive TB (10%)	Active TB disease (20%)	TB detection rate	TB detected	Proportion of TB detected in sick children*
Observation phase	3	65 000	6 500†	1 300	20%	260	0.4%
Total retrospective data	12	260 000	26 000†	5 200	20%	1 040	0.4%
Lead in phase**	3	65 000	6 500†	1 300	35%	4 55	0.7%
Intervention phase	12	260 000	26 000	5 200	50%	2 600	1.0%

*Study primary endpoint
†Not formally identified as systematic screening is not implemented before intervention
**Preparatory and transitory phase not used in analysis

Considering that 95% of parent(s)/guardian(s) will accept the enrolment of the child in the study, we expect that about 25,000 children with presumptive TB will be assessed with the innovative childhood TB diagnostic approach during the intervention period, including about 5000 active TB diseases, evenly distributed between both decentralisation strategies. Assuming a TB-detection rate of 45% in the DH-focused decentralisation strategy and 55% in the PHC-decentralized strategy, the number of TB detected will be 1125 and 1375 in the DH-focused and the PHC-focused decentralization strategy, respectively, for corresponding estimated proportions of TB detected among children with presumptive TB enrolled in the study of 9.0 and 11.0% (1125/12500 and 1375/12500).

With a significance level of 0.05, and the expected study size, we will have a >0.99 power to show the expected difference between the proportions of TB detected in presumptive TB enrolled in the study during the intervention phase.

These numbers will be enough to answer the study objectives.

11.3. STATISTICAL ANALYSIS PLAN

11.3.1. ANALYSIS OF THE PRIMARY ENDPOINT

The final analysis will be conducted on data collected after 19 months of enrollment of intervention assuming that it will require 3 months to have both decentralisation strategies fully operational in each district.

With our first hypothesis, we aim to test whether a decentralized innovative childhood tuberculosis diagnosis approach will increase childhood TB case detection. This hypothesis concerns the main effect of the intervention (independently of the decentralization level – DH or PHC). To test this hypothesis, we will use aggregated data to compare TB detection (proportion of children with presumptive TB & TB among all sick children attending) before / after the intervention. Mixed models will be used to control for the nested design and the potential fixed and random effects of Countries /Districts. If the Country/District effects are important, interactions and random slopes will be checked and incorporated if significant.

The primary analysis will be conducted by comparing the proportion of children detected with TB among those entering the health system OPDs during the observational and interventional phases.

The primary analysis will not differentiate between DH and PHC levels. An analysis per country and per decentralization strategy will be conducted secondarily.

A detailed statistical analysis plan will be written before the start of the intervention phase. Analysis will be carried out with version 9.4 or higher of the SAS® software (SAS Institute Inc., Cary, NC, USA).

11.3.2. ANALYSIS OF SECONDARY ENDPOINTS

Secondary analyses will include the comparison between the two decentralization strategies in terms of the different study endpoint as listed section 3.2 and will be performed using appropriate statistical methods with the latest SAS version or other appropriate software.

The second main hypothesis aims to test whether the decentralization to PHC level could enhance TB detection compared to the decentralization to DH level only. The first step will be to check the feasibility of the decentralization to this level (e.g. feasibility of samples collected compared to the planned samples collected and acceptability of the various procedures). Then, we will test the benefits of decentralization to the PHC level using the individual data collected during the intervention phase (after) and comparing the two strategies. Then, we will compare TB detection (proportion of children with presumptive TB among all sick children attending) after the intervention between PHC and DH districts. To take into account the nested design, mixed models will be used to control for countries and districts as potential fixed and random effects.

11.3.3. INTERMEDIATE ANALYSES: SCHEDULE

This project will perform an interim analysis at 3 months in the intervention phase to adapt the strategy if needed.

11.4. COST-EFFECTIVENESS ANALYSES

The adoption and implementation at national or international level of TB-speed diagnostic approaches in children will require health-economic evaluation. We will develop a mathematical model in collaboration with University of Sheffield and the TB-CAP project to capture the health system levels and referral patterns between levels regarding TB diagnostic and care interventions in children. The model will track children since their first contact with the health system and follow them during all the process from screening to treatment and outcomes. The developed model will be used to compare the following strategies: 1) The standard of care (SOC) define as current TB diagnostic procedures in children combining interventions at District Hospital (DH) level and Peripheral Health Center (PHC); 2) the DH focused decentralized strategy; 3) the PHC-focused decentralized strategy.

The data gathered during the sites assessment survey about health system description and structures will help design the model. The data during the observational phase and the approaches implementation will be used to project health outcomes for each strategy included number TB diagnosed cases, number children initiation TB treated and life years saved (LYS). The cost data will be derived from national health data and specific surveys (See section 10.1). Health outcomes and costs will be use to estimate Incremental-Cost Effectiveness Ratio (ICER) expressed in Cost per LYS comparing the different approaches.

A global ICER will be estimated to measure the overall project impact and also specifically for each of the country included in the project. The willingness to pay depending on the level of resources of countries and the TB burden will play a key role in the decision to implement the TB-speed new approaches. Extensive sensitivity analyses including TB prevalence variation and referral patterns scenario will be held to better account for various health contexts.

11.5. QUALITATIVE DATA ANALYSIS

A coding framework will be developed, based on themes which were pre-defined by the semi-structured interview guides and also which emerge from the data. The research assistants involved in collecting the data will work with the doctoral social scientist and associated investigator in implementation research to agree on the coding framework and code the data. Thematic summaries will be prepared from the data, and discussed with the whole study team. These summaries will form the building blocks for reporting the findings. The thematic analysis will be led by the doctoral social scientist, in collaboration with the research assistants, and supervised by the associated investigator in implementation research and PIs.

12. COMMUNICATION AND PUBLICATION POLICY

12.1. FINDINGS PUBLICATION PROCEDURE

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

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

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.

The mention of the origin of the funding, authorizations from competent authorities, and consent of participants must appear in the acknowledgments according to the model suggested below:

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

12.2. PROCEDURE FOR WRITING UP THE FINAL REPORT

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

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

12.3. PROCEDURE FOR INFORMING THE STUDY PARTICIPANTS OF THE OVERALL RESEARCH FINDINGS

The final study 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 study. 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.

12.4. PROCEDURE FOR INFORMING THE PARTICIPANTS OF THEIR HEALTH DATA DURING AND AFTER THE RESEARCH

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

During the study, any clinically significant abnormality detected in the examination or test results will be communicated to 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.

12.5. PRESS COMMUNICATION PROCEDURE

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

13. STUDY OVERSIGHT

13.1. OUTPUT STEERING COMMITTEE

The study (Output) Steering Committee (25) is the operational team that will undertake the day-to-day decisions related to study implementation in each country, based on the model applied in all clinical trials currently managed by the IDLIC team at UBx.

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

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

Members of OSC will interact once a month. Every 6 months, the OSC meeting will be opened to Protocol Writing Committee external members for a review of study progresses and results.

13.2. SCIENTIFIC ADVISORY BOARD

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

- **Role**

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

- The relevance of the project objectives within the context of the paediatric TB research landscape;
- The appropriateness of designs and methods of the proposed studies (outputs) to the research questions;
- The scientific strength, safety and feasibility to meet the stated objectives of the project;
- The complementarity of the project with other ongoing or planned external studies;

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

13.3. COUNTRY PROJECT COMMITTEE

At country level, the TB-Speed Country Project Committee (CPC) without any steering role will consist of major TB and child health stakeholders in the country (e.g. implementers, political supports, local NGOs). The CPC shall be chaired by the Country Principal Investigator and will act as facilitators for national operations as well as for dissemination and communication activities.

13.4. ENDPOINT REVIEW COMMITTEE

In each country, an endpoint review committee will consist of: (i) the country principal investigator; (32) one or several other adequately trained physicians, selected by the country principal investigator; (iii) a representative of the country CTU.

The endpoint review committee will make a criterion-related validation of differential diagnoses. In addition to differential diagnoses, the expert committee will review criteria for the reference diagnosis.

The Coordinating Investigators and members of the international CTU will review all event validation forms and uploaded documents, as well as any relevant information in the database, in order to verify that the criterion-related validation is applied homogeneously across participating countries, and ask the country principal investigators for additional information whenever needed.

13.5. 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). Study implementation, monitoring and data management activities will be coordinated by an international CRA.

The study 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 study sponsor. Writing of the SOPs is coordinated by the International CTU.

In each country where the study 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, Epicentre and MUJHU (Technical Partner) in Uganda, Solthis in Sierra Leone, Instituto Nacional de Saude (INS) in Mozambique, and Institut Pasteur in Cambodia. The country CTU will be in charge of study coordination, monitoring and data management in the country. Study activities will be coordinated by a country project manager, who will work in close collaboration with the international project (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.

14. CONFIDENTIALITY

14.1. PROCEDURE FOR RESPECTING THE CONFIDENTIALITY OF PARTICIPANTS

Each study participant will be assigned a unique study identification number. This number will be the only participant identifier on any document, record, report or laboratory specimen related to the study, as well as in the electronic study database.

The participant ID assignment log (only in paper form) will be kept shut-away on site under the responsibility of the site's principal investigator. Direct personal identifiers (including names, dates, demographic and contact information) will only be made available to those whose job within the operational activities of the study makes having such information absolutely essential, subject to signature of a confidential agreement. This includes routine DH or PHC 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' and other participants names will be kept in a locked cabinet under the responsibility of the site's principal investigator.

Parent(s)/guardian(s) who want to access or withdraw their child's data will approach the site study staff or site investigator and request in writing or verbally to withdraw information. The site study representative or the investigator will inform the Principal investigator who will contact the Data Protection Officer at University of Bordeaux to allow access or remove data.

14.2. PROCEDURE FOR KEEPING THE NECESSARY STUDY DATA CONFIDENTIAL

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

The data recorded during this study will be the subject of computer processing on behalf of the Sponsor. The sponsor will declare the database to the French CNIL, in compliance with the

provisions of the French Law No. 78-17, dated January 6, 1978, and amended by Law No. 2004-80, dated August 8, 2004.

15. PROTECTING RESEARCH PARTICIPANTS

15.1. ETHICAL JUSTIFICATION OF THE PROTOCOL

- **Risks**

Risks due to para-clinical investigations will be explained to the participants. The potential risks of NPA and blood draw will be limited by ensuring 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 the facilities will ensure that sample collection will be done with access to salbutamol and oxygen within the routine systems.
- 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 adverse events (paradoxical reactions and IRIS).

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

- **Benefits**

This study is providing the following direct opportunities for eligible children:

- an improved and early diagnosis of TB, especially by optimizing bacteriological specimen collection and processing for young children;
- an enhanced prognosis due to timely and appropriate TB treatment initiation;
- in case of rifampicin resistance detected on Xpert Ultra, access to rapid drug susceptibility testing for *Mycobacterium tuberculosis* and the opportunity to receive individualised MDR-TB treatment regimen based on to these results;
- 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 decentralisation efforts to increase TB case detection in high TB burden countries.

The risk benefit ratio for this study for individual child participants is seen to be favourable with low risk and reasonable additional benefits due to study participation.

15.2. REGULATORY PROVISIONS

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

- the French regulations in force, including provisions relating to research involving the human person provided for in Articles L 1121-1 *et seq.* of the Public Health Code, the Bioethics Laws, the Data Protection Act;
- Participating countries' laws and regulations relating to biomedical research on human participants;
- The Declaration of Helsinki (approved by the World Health Association on June 1964, lastly amended at the 64th WMA General Assembly, Fortaleza, October 2013);
- The Good Clinical Practice (ICH Harmonized Tripartite Guidelines for Good Clinical Practice E6 step 4 - 2016) 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 study will be registered at the ClinicalTrials.gov registry and The Pan African Clinical Trials Registry (PACTR).

15.3. ETHICAL APPROVALS

Before carrying out the research, the protocol, the information sheet, the consent form and any other relevant documents will be submitted to the approbation of each implementing country's National Ethics Committee, 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 all the necessary ethical and administrative clearance has been received. The research can only start when Inserm has been informed of the favourable opinion delivered by the different ERCs concerning the submitted protocol. This notice will include the title and protocol number assigned by the proponent, the documents reviewed, as well as the date of review and the list of ERC members who participated.

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

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

15.4. ADDITIONAL APPROVALS

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

In Cameroon, 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.

15.5. DATA PROTECTION

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

15.6. INSURANCE

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

Inserm has taken out a civil liability insurance for the entire duration of the study under number <country insurance contract number>, in accordance with the French legal provisions and regulations on research.

The certificate of insurance relating to this Protocol constitutes Appendix 6.

15.7. PARTICIPANTS AMENITIES

Study investigators will ensure that each subject receives the following benefits throughout the study: reimbursement of transportation fees to the study visits, TB related medical examinations and tests. TB medications will be obtained from the NTPs.

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.

16. QUALITY ASSURANCE AND MONITORING

16.1. DESCRIPTION OF THE QUALITY ASSURANCE SYSTEM

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

Research monitoring will be conducted according to the Good Clinical Practices (ICH Harmonized Tripartite Guidelines for Good Clinical Practice 2016) to guarantee the quality of the research and safeguard the health and the rights of the patient. The site's principal investigator is above all the guarantor of the quality of the study progress.

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

16.2. MONITORING (QUALITY CONTROL OF THE STUDY)

16.2.1. GENERAL ORGANIZATION

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

16.2.2. MONITORING BY THE COUNTRY CTU

A clinical research associate (26) will regularly visit each implementing site during the all study period. During these visits, the CRA will check the following, according to the monitoring plan:

- check adherence to the protocol, SOPs and Good Clinical Practice, including patient's screening, eligibility criteria, informed consent;

- establish and maintain the investigator's Trial Master Files (TMF) up-to-date (paper or access to electronic TMF);
- check the completeness and the accuracy of patient key data on the eCRF (electronic source data verification) not for all patients (percentage defined in monitoring plan);
- verify that confidentiality of data is fully respected;
- verify SAEs reporting, documentation and follow-up, and send the forms to the sponsor's pharmacovigilance and to the international coordinating CTU;
- Follow up with site teams centralized correction requests sent by the national and international coordinating CTU.

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

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

- check the quality management of samples and biobank;
- Ensure that quality controls and quality management for laboratory assessments are implemented.

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

16.2.3. MONITORING BY THE INTERNATIONAL COORDINATING CTU

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

A member of the international coordinating CTU will visit each country at least once a year, and each study site at least once during the study period if feasible. In the context of the COVID 19 pandemic, virtual site monitoring visits might be conducted by the international CTU where needed due to travel restrictions. Anonymized source documents will be sent by the country CRA through FTPS files for monitoring purpose only, source documents will be destroyed afterwards.

The purpose of these visits will be to review with the country CTU advances and issues with the study implementation, 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 for a subset of patients (percentage defined in monitoring plan)
- Compliance with the study protocol, SOPs and Good Clinical Practices, including eligibility criteria and reporting of SAEs
- Consistency with the source documents for key data for a subset of patients (percentage defined in monitoring plan)
- Management of samples and bio banking
- Laboratory quality controls

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

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

A closing visit will be carried out at the end of the study by the international coordinating CTU virtually or face to face.

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16.2.4. DIRECT ACCESS TO SOURCE DATA

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

16.2.5. AUDITS/INSPECTIONS

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

17. ACCESS TO DATA AND FROZEN SAMPLES

17.1. 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.

17.2. FROZEN SAMPLES

17.2.1. BIOBANK GOVERNANCE

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. Specific SOPs will describe methods and procedures for the collection of biological samples, as well as the Laboratory Quality Assurance system put in place. 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.

Biological samples will be retained for 10 years after study completion, unless objection expressed by parent(s)/guardian(s). Unless the investigators can contact participants who turn 18 during the period of storage and obtain renewed consent, their samples may not be used in any way. 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.

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.

17.2.2. 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 unless it is a requirement by the National Ethics committee.

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 benefiting from a 6-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.

18. SUBSTANTIAL AMENDMENTS TO THE PROTOCOL

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

These requirements for approval should in no way prevent any immediate action from being taken by the investigators or by the sponsor in the interests of preserving the safety of all study participants. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented by him/her for safety reasons, the Inserm should be notified and each country's National Ethics Committee should be informed within 10 working days.

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20. APPENDICES

20.1. APPENDIX 1: PROTOCOL SYNOPSIS

Clinical trial ID number: NCT04038632

Title: IMPACT OF AN INNOVATIVE CHILDHOOD TB DIAGNOSTIC APPROACH DECENTRALIZED TO DISTRICT HOSPITAL AND PRIMARY HEALTH CARE LEVEL ON CHILDHOOD TUBERCULOSIS CASE DETECTION AND CASE MANAGEMENT IN HIGH TUBERCULOSIS INCIDENCE COUNTRIES.

Short title: TB-Speed Decentralisation

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Participating countries: Cambodia, Cameroon, Cote d'Ivoire, Mozambique, Sierra Leone, and Uganda

Primary Objective: To assess the impact of implementing decentralized diagnostic approaches on childhood TB case detection at district hospital and PHC levels compared to the pre-intervention status

Secondary Objectives:

- To compare impact of the DH focused and the PHC focused decentralization strategies of an innovative childhood TB diagnostic approach in terms of:
    - TB case detection

- TB screening in outpatient children
- Feasibility of implementing the different diagnostic approach components
- TB treatment uptake and time to TB treatment initiation
- Cost-effectiveness from the health services perspective
- Acceptability by health care providers, national TB program and health authorities, and beneficiaries
- Fidelity of the implementation of the diagnostic approach as compared to the protocol and study procedures

- To determine, within a nested cohort of children with presumptive and diagnosed TB, and compare between the decentralization strategies:
  - Performance of the diagnostic approach at patient level
  - TB treatment outcome

- To assess the CXR component of the intervention in terms of:
  - Diagnostic performance of CXR reading by clinicians at DH and PHC levels
  - Added value of CXR in the diagnosis of TB in children as compared to microbiology and clinical evaluation only
  - Uptake of the quality control of the CXR reading

METHODS

The study will comprise an observation phase followed by an intervention phase in participating districts. During the last month of the observation phase, each district will be randomly assigned to implement either DH or PHC-focused decentralisation. There will be no patient level randomisation.

During this 3-month observation phase, we will i) describe the childhood TB diagnosis data and practices; ii) describe the referral processes and outcomes of referrals for TB diagnosis and treatment where feasible and iii) assess existing challenges in childhood TB diagnosis and treatment, as well as readiness (including potential challenges) for the study intervention implementation There will be no interference with the routine TB childhood diagnosis processes.
Mixed-methods (quantitative and qualitative) will be used including the collection of retrospective and prospective aggregated data by study nurses from facility registers, the implementation of a self-administered questionnaire among all HCWs, the observation of consultations and care provided, and the conduct of individual interviews with HCWs and key informants.

At the beginning of the intervention phase, a 3months preparation period will set up the health facilities for decentralization by providing equipment, materials, and reagents, training health workers in childhood TB care, in NPA and stool collection and testing on Ultra, setting up G1 (Edge) or Omni or G4 at DH and PHC if not already available and digital CXR and CXR quality control. Existing health care workers will be trained in childhood TB care according to the NTP guidelines, and also in NPA and stool collection and testing on Ultra for study purposes.

Implementation of the innovative childhood TB diagnostic approach at the selected DH and PHC will start as soon as sites are equipped and HCWs trained in childhood TB care and NPA and stool collection, and will implement continued capacity building

at sites, regular clinical mentoring visits with NTP or their representative, and continued CXR quality control.
The 6-month prospective cohort follow-up study will be initiated immediately and will consecutively include every tenth child with presumptive TB and all children diagnosed with TB.
Individual data collection will be initiated as soon as the innovative childhood TB diagnostic approach including NPA and stool sample collection and Ultra testing is implemented in the site and will be conducted throughout the intervention phase to document secondary endpoints. Aggregated data for TB screening will be collected throughout the study.
Feasibility, acceptability, and compliance to the intervention protocol will be assessed by mixed methods including a repeat self-administered questionnaire among all HCWs, observations of consultations and care provided, and individual interviews with HCWs, National TB program & local health authorities representatives, and beneficiaries i.e. parents/guardians.

Planned study enrolment post-hoc power calculations: Because this study is operational research, we will enrol consecutive children attending the facilities over 12 consecutive months in the innovative childhood TB diagnostic approach evaluation.
We estimated that the expected number of children entering the health system in 12 districts (2 districts in 6 country) over the 12-month implementation period will be 260000.
Hypothesizing a 10% presumptive TB rate among sick children, and a 20% TB disease rate among those, we expect that a total of 26,000 children per 12-month period will have presumptive TB, including 5200 with TB disease.

Primary endpoint:
Proportion of TB cases detected among the sick children attending outpatient services before and after the intervention.

Secondary endpoints:

1. Decentralisation strategy specific secondary endpoints
   a. Proportion of TB cases (confirmed and unconfirmed) detected among children identified as presumptive TB
   b. i) Proportion of children screened for TB among sick children attending outpatient services
      ii) Proportion of children identified with presumptive TB among children screened.
   c. i) Proportion of children with presumptive TB receiving the different components of the innovative childhood TB diagnostic approach (NPA and stool or sputum sampling attempt and success, sample testing with Ultra and results, clinical evaluation, CXR and interpretation, full diagnostic package)
      ii) Time to sample test and results delivery to clinician
      iii) Number of visits to the health facility until final diagnosis
   d. i) Proportion of children initiating TB treatment among those diagnosed as TB
      ii) Time from positive TB screening to TB treatment initiation
   e. Incremental-Cost Effectiveness Ratio (ICER) of the diagnostic approach
   f. Acceptability endpoints:

i) Perceptions and experience of the intervention by healthcare workers (HCWs), the NTP and health authorities, and the beneficiaries (parents/guardians).

g. Fidelity endpoints:
   i) Changes in the intervention implementation as compared to 1) study standard implementation procedures and 2) country implementation procedures. These changes could be related to NTP guidelines dispositions, adaptation to local context and constraints not initially planned per standard and country implementation procedures.
   ii) Proportion of clinical mentoring visits performed per study procedures; proportion of health facilities implementing NPA and stool sample collection and performing sample processing and Ultra testing per study procedures.

2. Nested cohort specific secondary endpoints
   a. Sensitivity and specificity of the diagnostic approach as compared to the reference diagnosis based on the Case Definitions for Classification of Intrathoracic Tuberculosis in Children (see section 3.3)
   b. TB treatment outcome as defined by WHO

3. CXR secondary endpoints
   a. Sensitivity and specificity of CXR reading by clinicians at DH and PHC to detect lesions suggestive of TB as compared to the reference reading (independent reading by external radiologist experts)
   b. Proportion of children diagnosed with TB based on CXR and incremental yield of TB detection with CXR results as compared to microbiological (Ultra on NPA and stool or sputum) and clinical evaluation, respectively
   c. Proportion of CXR selected for quality review assessed by the reference reviewer and time to results of the quality control to the clinic

Selection criteria:

For the primary endpoint and systematic TB screening

- Inclusion criteria
  - Sick children seeking care at OPD of DH or PHC
  - Age <15 years
- Non-inclusion criteria for this group
  - None

Generally, no informed consent will be sought because only aggregated data will be collected from routine reports and registers. However, for those children whose consultation will be observed during observation phase, parent(s)/guardian(s) informed consent will be sought.

For the secondary endpoints comparing the DH and PHC-focused strategies

- Inclusion criteria
  - Age <15 years
  - Presumptive TB defined as children presenting ≥1 systematic screening criteria among the following:
    - Cough with a duration of >2 weeks
    - Fever with a duration of >2 weeks
    - Documented weight loss

- History of TB contact with any duration of cough

- Presumptive TB identified by the site clinician for TB assessment irrespective of the above criteria, especially presumed extra-pulmonary TB cases
- Informed consent signed by parent/guardian
- Child's assent obtained in those aged >7 years

- Non-inclusion criteria
  - Children who have received TB treatment in the past 6 months

For the secondary endpoints from the nested cohort

- Inclusion criteria
  - Presumptive TB as defined above and identified as 1/10 by selection process

    OR
  - Diagnosed TB

    AND
  - Informed consent signed by parent/guardian to participate to the prospective cohort follow-up. Child's assent to participate to the prospective cohort follow-up obtained in those aged >7 years

- Non-inclusion criteria
  - Children who have received TB treatment in the past 6 months

For the secondary endpoints to assess feasibility, acceptability and compliance to the protocol diagnostic approaches

These non-patient study population groups invited to participate will be as follows:

- Parents/guardians of the children with presumptive TB
- Health care providers delivering the innovative childhood TB diagnostic approach
- Health care managers such as National TB program and health authorities

All non-patient participants will provide consent to participate in the study.

Innovative childhood TB diagnostic approach

The intervention will consist of the following 4 components:

- Systematic TB screening: refers to asking simple specific questions at health care OPD entry point to identify children with symptoms suggestive of TB.
- Clinical evaluation: refers to detailed history taking, relevant physical examination, severity of illness. Clinical evaluation will be for all children with presumptive TB.
- Xpert Ultra testing of NPA and stool (or expectorated sputum) for microbiological diagnosis (genotypic detection of *M.tb* and rifampicin resistance).
- Optimised CXR reading: using digital radiography, improvement of reading skills, simplified reading tool, and quality control of CXR reading.

These components will provide guidance to the clinician for the decision whether or not to treat for TB, and when to start treatment (based on individual clinical judgement of urgency and certainty).

All components of this innovative childhood TB diagnostic package are recommended by the WHO and national guidelines for diagnosis of childhood TB, at the exception of NPA and stool sample collections. The intervention will therefore replace/reinforce standard of care TB diagnostic procedures in implementing health facilities, at the exception of NPA and stool, that will be tested in the context of the research. All children, regardless of whether or not they are enrolled in the study, will therefore be given the best diagnostic approach for TB offered in the context of the project as "standard of care", i.e. systematic screening, Ultra testing on any sample collected, chest X-ray, and reinforced clinical diagnosis. The parent(s)/guardian(s) will therefore provide consent for NPA collection and stool collection for TB testing as well as access to personal clinical records if they exist and use of the routine clinical data collected for study purposes. Children whose parent(s)/guardian(s) do not consent for enrolment to the study, will have access to the abovementioned improved diagnostic approach – with exceptions of NPA and stool sampling – and to the same standards of care, but will not be included in data collection or analysis as per specific study protocol.

Decentralisation strategies:

DH-focused decentralisation strategy

In this strategy, the patient care level innovative childhood TB diagnostic approach will be implemented at the DH level. PHCs in this district, will only conduct systematic TB screening. In facilities not already implementing childhood TB diagnosis and treatment when the study starts, all children will be screened and those identified as presumptive TB cases will be referred to the DH for clinical evaluation, Ultra testing on NPA and stool or expectorated sputum, and CXR if indicated.

In participating countries who have already decentralized TB treatment initiation in children at PHC level using clinical evaluation and sputum collection (for children able to expectorate), these PHCs will continue to provide these services. Only children in whom clinicians decide NOT to treat for TB according to national guidelines shall be referred to the DH for further investigations and for those cases the decision to initiate treatment will be taken at the DH. All the referrals will be documented and tracked. Children that are diagnosed with TB at PHC level will be offered to participate in the nested-cohort.

PHC-focused decentralization strategy

In this strategy, the patient care level innovative childhood TB diagnostic approach will be done at the PHC. This will include systematic TB screening, clinical evaluation, and testing of NPA and stool or expectorated sputum with Xpert Ultra. NPA or sputum samples will be collected at the PHC and tested using G1 (Edge) or Omni or G4 where possible. The stool samples will be referred to the DH for testing until a simplified stool testing kit becomes available for use at the PHC level. Children will be referred to the DH for CXR only, when indicated.

The DH in the districts implementing the PHC decentralization strategy will also implement the patient care level diagnostic approach for children screened with presumptive TB at DH level and will be supported with digital radiography plates and G1 (Edge) or Omni or G4 as well as training in child TB diagnosis and treatment.

Statistical analysis:

Analysis of the primary endpoint

The final analysis will be conducted on data collected after 19 months of enrollment of intervention assuming that it will require 3 months to have both decentralisation strategies fully operational in each district.
The primary analysis will be conducted by comparing the proportion of children detected with TB among those entering the health system OPDs during the observational and interventional phases. The primary analysis will not differentiate between DH and PHC levels. An analysis per country and per decentralization strategy will be conducted secondarily.
Analysis of secondary endpoints
Secondary analyses will include the comparison between the two decentralization strategies in terms of the defined secondary endpoints.
All analysis will be performed using appropriate statistical software with the latest SAS version.

Expected results: Increase childhood TB case detection at the PHC level and feasible decentralised paediatric sample collection and microbiological diagnosis can be decentralized to PHC level. Increased childhood TB diagnostic capacities at district hospital (DH) and PHC levels using adapted and child-friendly specimen collection methods, sensitive detection tests (Xpert Ultra) close to the point-of-care (GeneXpert G1 (Edge) or Omni or G4 and digitized chest radiography.

20.2. APPENDIX 2: SUMMARY PROCEDURE FOR NASOPHARYNGEAL ASPIRATES COLLECTION

Nasopharyngeal aspirates consisted in the collection of 2-5 ml of throat contents through a catheter tube connected to a mucus aspirator. Nasopharyngeal aspirate is done for all children.

The procedure is performed in a child in a supine position on his/her back or side, or sitting on his family member/guardian's lap, after re-explaining to the child and the accompanying person the reason for sample collection and the steps of the procedure. In order to avoid child injury due to movement, young children are wrapped in a piece of cloth, and an assistant nurse asked to hold the child's head throughout the procedure.

After connecting a mucus extractor to the suction pump and catheter, the suction pressure was adjusted. The pressure and catheter size was recommended 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; and in children aged >10 years, 10 CH catheter with 120-150 mmHg (0.20 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 induced cough and sputum expectoration that could 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 with nasopharyngeal lavage by inserting 5ml of normal saline in nostril and 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 are transported to the laboratory within 4 hours.

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

20.3. APPENDIX 3: SUMMARY PROCEDURE FOR THE PREPARATION OF STOOL SAMPLES FOR XPERT MTB/RIF ULTRA

Stool samples will be prepared for Xpert MTB/RIF 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 the centrifuged at 100 x g for 1 minute (no brake). After careful removal of the tube from the centrifuge to avoid disturbing the suspension, 0.5 mL of suspension was retrieved from the top of the specimen and added to 1.8 mL of Xpert MTB/RIF Sample Reagent, shaken vigorously 10 - 20 times, and incubated for 15 minutes at room temperature. After 5 to 10 minutes of incubation, the specimen was shaken again vigorously 10 to 20 times. The specimen obtained was then directly tested with the Xpert MTB/RIF.

20.4. APPENDIX 4: DEMOGRAPHIC AND TB STATISTICS OF IMPLEMENTATION DISTRICTS

CAMBODIA

The districts to implement in Cambodia will be Batheay and Angroka, a summary of their demographics and TB statistics is shown below

	CAMBODIA	
District name	**BATHEAY**	**ANGROKA**
Population	125 299	144 723
Adult TB cases 2017	175	210
Pediatric TB cases 2017 (Children [0-15y[)	14	24
Number of Health centers in the District	8	12
Presence of regional hospital in the District or nearby	1	1

	BATHEAY					ANGROKA				
	BATHEAY RH	TUMNUB HC	PHAAV HC	SAMBOUR HC	CHOEUNG CHNOK HC	ANGROKA RH	TAPHEM HC	NHAENG NHANG HC	KUS HC	TROPANG ANDERT HC
Pediatric TB cases 2017(Children[0-15y[)	0	4	7	0	0	0	0	0	1	0
Pediatric OPD number in 2017 (<15 years)	2680	5027	6439	4153	4890	2660	415	3699	4341	6224
Implementing sample collection	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Gastric aspirate	No	No	No	No	No	No	No	No	No	No
Expectorated sputum	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Induced sputum	No	No	No	No	No	Yes	No	No	No	No
Nasopharyngeal aspirate	No	No	No	No	No	No	No	No	No	No
TB laboratory	Yes	No	No	No	No	Yes	No	No	No	No
Smear microscopy	Yes	No	No	No	No	Yes	No	No	No	No
Gene Xpert	Yes	No	No	No	No	Yes	No	No	No	No
Implementing radiography	Yes	No	No	No	No	Yes	No	No	No	No

CAMEROON

The districts to implement in Cameroon will be Obala and Bafia, a summary of their demographics and TB statistics is shown below

	CAMEROON									
District name	**OBALA**					**BAFIA**				
Population	138 843					161 401				
Adult TB cases 2017	176					244				
Pediatric TB cases 2017 (Children [0-15y[)	4					2				
Number of Health centers in the District	27					47				
Presence of regional hospital in the District or nearby	0					0				
	OBALA HOSP	CSI NGOGO	CMA BATCHENGA	CMA FOMAKAP	CSI ESSONG	HD BAFIA	CMA BOKITO BAFIA	CSI MESSNGSSANG	CSI BALAMBA BAFIA	CMA KIIKI BAFIA
Pediatric TB cases 2017(Children[0-15y[)	4	NA	NA	NA	NA	2	NA	2	NA	NA
Pediatric OPD number in 2017 (<15 years)	1118	279	214	330	214	3518	455	460	118	549
Implementing sample collection	Yes	No	No	No	No	Yes	Yes	Yes	No	No
Gastric aspirate	Yes	No	No	No	No	Yes	Yes	Yes	No	No
Expectorated sputum	Yes	No	No	No	No	No	No	No		
Induced sputum	Yes	No	No	No	No	Yes	Yes	Yes		
Nasopharyngeal aspirate	No	No	No	No	No	No	No	No	No	No
TB laboratory	No	No	No	No	No	No	No	No	No	No
Smear microscopy	Yes	No	No	No	No	Yes	Yes	Yes	No	No
Gene Xpert	Yes	No	No	No	No	No	No	Yes	No	No
Implementing radiography	No	No	No	No	No	No	No	No	No	No

IVORY COAST

The districts to implement in Ivory coast will be Danane and Sassandra, a summary of their demographics and TB statistics is shown below

	IVORY COAST	
District name	**DANANE**	**SASSANDRA**
Population	289 258	329 043
Adult TB cases 2017	146	116
Pediatric TB cases 2017 (Children [0-15y[)	11	9
Number of Health centers in the District	23	27
Presence of regional hospital in the District or nearby	0	0

	H G DE DANANE	DR BANTEAPLEU	CSU KOUAN-HOULE	CSU MAHAPLEU	CSR DALEIU	H G SASSANDRA	CSU DAKPADOU	CMS SAGO	CSR MEDON	DR DE SAHOUA
Pediatric TB cases 2017(Children[0-15y[)	9	0	0	0	0	3	1	6	0	0
Pediatric OPD number in 2017 (<15 years)	16111	3609	1442	3342	4077	7888	3080	3133	3100	1337
Implementing sample collection					YES					
Gastric aspirate	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Expectorated sputum	Yes	No	No	No	Yes	Yes	No	Yes	No	No
Induced sputum	Yes	No	No	Yes	No	Yes	Yes	Yes	No	No
Nasopharyngeal aspirate	No	No	No	No	No	Yes	No	No	No	No
TB laboratory	No	No	No	No	No	No	No	No	No	No
Smear microscopy	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gene Xpert	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes
Implementing radiography	Yes	No	No	No	No	No	No	No	No	No

MOZAMBIQUE

The districts to implement in Mozambique will be Manjacaze and Chokwe, a summary of their demographics and TB statistics is shown below

	MOZAMBIQUE									
District name	**MANJACAZE**					**CHOKWE**				
Population	145 231					248 178				
Adult TB cases 2017	999					1643				
Pediatric TB cases 2017 (Children [0-15y[)	115					272				
Number of Health centers in the District	16					26				
Presence of regional hospital in the District or nearby	1					1				
	HOSPITAL RURAL DE MANJACAZE	**LARANJEIRA**	**MACUACUA**	**CHIBONZANE**	**CHIDENGUELE**	**HOSP RURAL CHOKWE**		**HOKWE**	**CHALOCUANE**	**CHIAQUELANE**
Pediatric TB cases 2017(Children[0-15y[)	81	NA	11	NA	NA	75		NA	20	10
Pediatric OPD number in 2017 (<15 years)	13525	2058	5645	3851	3971	4058		2985	17081	2292
Implementing sample collection	Yes	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes
Gastric aspirate	Yes	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes
Expectorated sputum	Yes	No	No	No	No	No		No	Yes	No
Induced sputum	Yes	Yes	Yes	Yes	Yes	Yes		Yes	Yes	Yes
Nasopharyngeal aspirate	Yes	No	No	No	No	Yes		No	Yes	No
TB laboratory	No	No	No	No	No	No		No	No	No
Smear microscopy	Yes	No	Yes	No	Yes	Yes		Yes	Yes	Yes
Gene Xpert	Yes	No	Yes	No	Yes	Yes		Yes	Yes	Yes
Implementing radiography	Yes	No	No	No	No	Yes		No	No	No

SIERRA LEONE

The districts to implement in Sierra Leone will be Port loko and Bo, a summary of their demographics and TB statistics is shown below

	SIERRA LEONE									
District name	**PORT LOKO**					**BO**				
Population	678 331					616 106				
Adult TB cases 2017	1323					1281				
Pediatric TB cases 2017 (Children [0-15y[)	69					109				
Number of Health centers in the District	109					134				
Presence of regional hospital in the District or nearby	0					0				
	PORT LOKO GOVT HOSP	**MANGE CHC**	**GBINTI CHC**	**PETIFU CHC**	**BABARA CHC**	**BO GOVT HOSP**	**New Police barracks**	**GERIHUN CHC**	**KORIBONDO CHC**	**NJALA UNIVERSITY CHC**
Pediatric TB cases 2017(Children[0-15y[)	18	11	4	0	1	49	0	0	0	3
Pediatric OPD number in 2017 (<15 years)	2554	5919	4155	4928	2947	7509	5892	5360	5097	4114
Implementing sample collection	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gastric aspirate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Expectorated sputum	Yes	No	No	No	No	No	No	No	No	No
Induced sputum	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nasopharyngeal aspirate	No	No	No	No	No	No	No	No	No	No
TB laboratory	No	No	No	No	No	No	No	No	No	No
Smear microscopy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gene Xpert	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Implementing radiography	Yes	No	No	No	No	Yes	No	No	No	No

UGANDA

The districts to implement in Uganda will be Kanungu and Rakai, a summary of their demographics and TB statistics is shown below

	UGANDA									
District name	**KANUNGU**					**RAKAI**				
Population	263 180					553 300				
Adult TB cases 2017	328					503				
Pediatric TB cases 2017 (Children [0-15y[)	20					37				
Number of Health centers in the District	52					33				
Presence of regional hospital in the District or nearby	0					0				
	KANUNGU HCIV	**KAMBUGA HOSPITAL**	**NYAMIRAMA HCIII**	**KANYANTOROGO HCIII**	**MATANDA HCIII**	**RAKAI HOSPITAL**	**BUYAMBA HC III**	**LWANDA HC III**	**ST BERNARDS MANYA HC III**	**LWAMAGGWA HC III**
Pediatric TB cases 2017(Children[0-15y[)	0	2	0	NA	6	9	1	0	0	0
Pediatric OPD number in 2017 (<15 years)	4063	5904	2656	5609	5630	10302	2130	4653	3428	5151
Implementing sample collection	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Gastric aspirate	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Expectorated sputum	No	No	No	No	No	No	No	No	No	No
Induced sputum	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nasopharyngeal aspirate	No	No	No	No	No	No	No	No	No	No
TB laboratory	No	No	No	No		No	No	No	No	No
Smear microscopy	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Gene Xpert	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Implementing radiography	No	Yes	No	No	No	Yes	No	No	No	No

20.5. APPENDIX 5: DETAILED PATIENT FLOW CHARTS

Figure A1. Patient flow chart at PHC in PHC focused

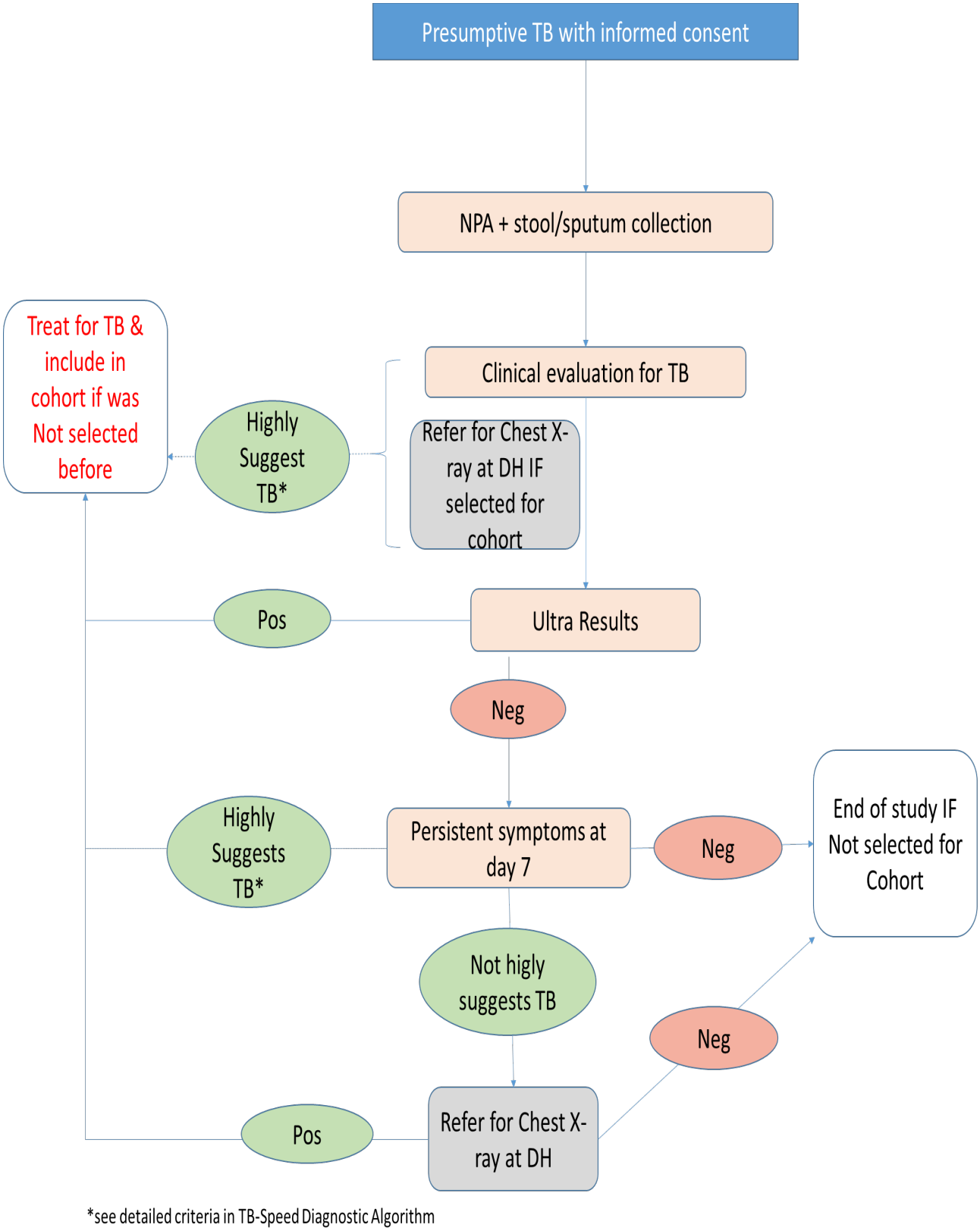


Figure 4: Patient flow chart at PHC in DH focused

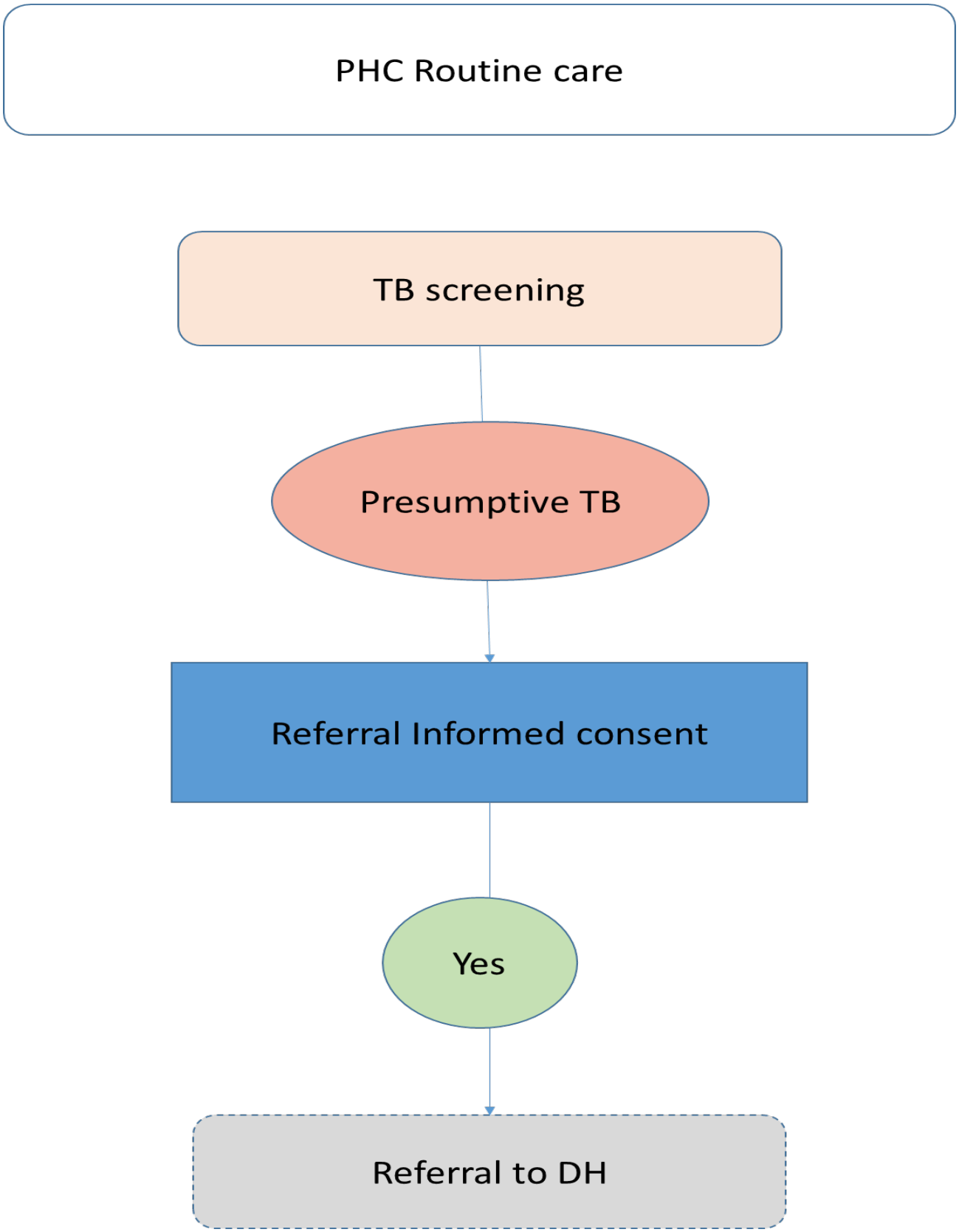
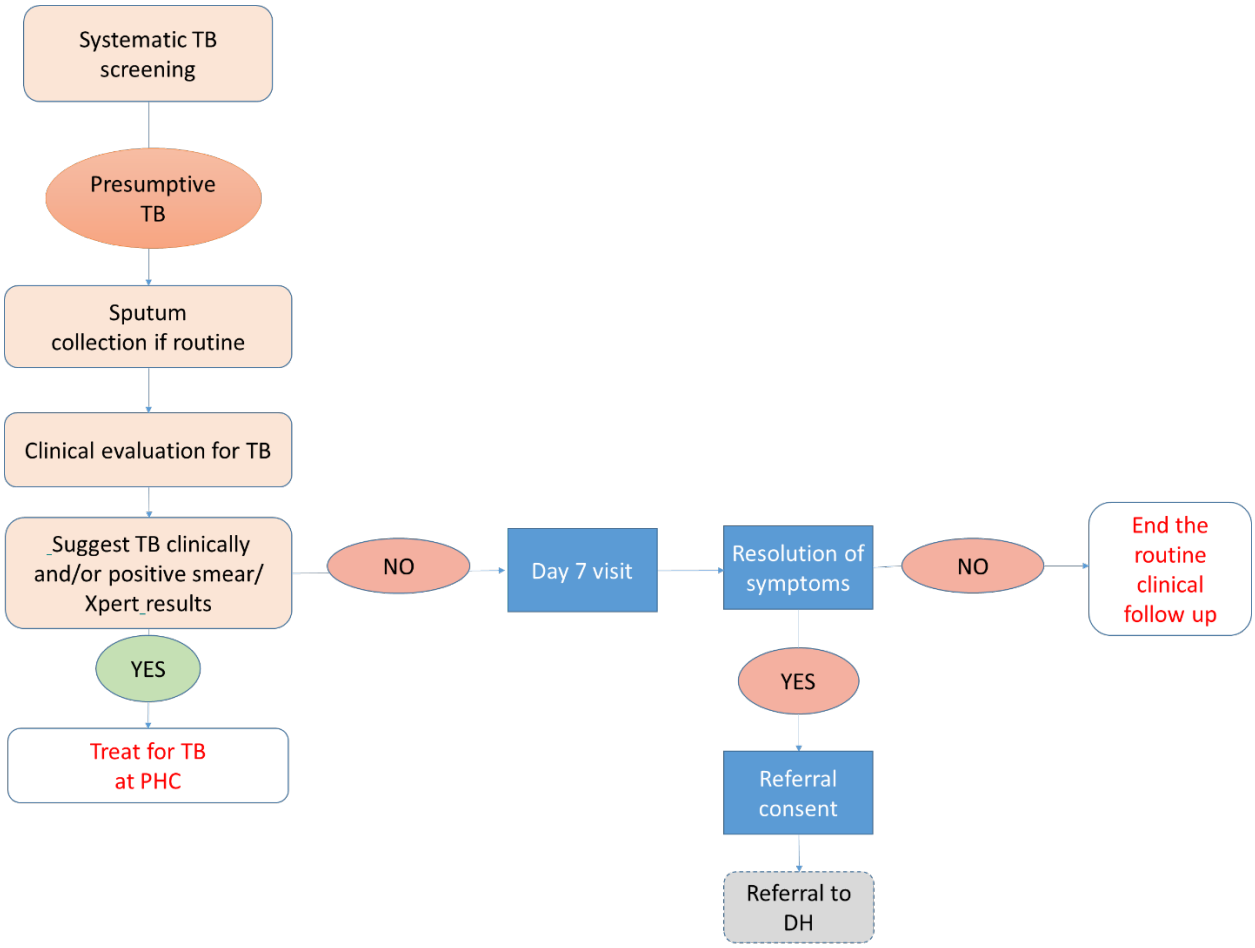


Figure 5: Patient flow chart at already decentralised PHC in DH focused approach



20.6. APPENDIX 6: COPY OF THE INSURANCE POLICY

20.7. APPENDIX 7: COPY OF THE WHO-ERC APPROVAL

20.8. APPENDIX 8: COPY OF THE INSERM CEEI (ERC) APPROVAL

20.9. APPENDIX 9: COPY OF THE ERC APPROVAL (FOR COUNTRY PROTOCOL VERSION ONLY)

20.10. APPENDIX 10: COPY OF THE COMPETENT AUTHORITY'S AUTHORIZATION (FOR COUNTRY PROTOCOL VERSION ONLY)