VALIDATION OF A TUBERCULOSIS TREATMENT DECISION ALGORITHM IN HIV-INFECTED CHILDREN

- TB-Speed HIV -

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CONFIDENTIAL

RESEARCH PROTOCOL INVOLVING HUMAN PERSON

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☐ **Monocentric study**
☐ **French multicentre study**
☐ **European multicentre study**
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HISTORY OF PROTOCOL VERSION

TB-Speed HIV

Version NO.	Version Date	Amendment Summary
1.0	18/12/2018	
2.0	17/05/2109	- Modification of the study title - Clarification and details added on the external validation process - Reformulation of the primary objective - Details added on the screening process - Clarification on the statisitical analysis plan (primary and secondary endpoints)
3.0	10/12/2019	- Recruitment sites: addition of one study site in Côte d'Ivoire
4.0	23/09/2020	- Update of the study timelines - Addition of the cost-effectiveness ancillary study: objective, endpoint, data collection and analysis - Recruitment sites: removal of Yopougon UTH in Côte d'Ivoire
4.1	07/07/2021	- Study adaptation to the Covid pandemic - Extension of the study schedule - Addition of details on the safety measures taken for infection prevention and control in the context of the Covid pandemic (in the protocol and the Information Notice) - Mention made that site moniroting could be conducted remotely in the context of the Covid pandemic

LIST OF ABBREVIATIONS

ANRS	French National Agency for Research on HIV/AIDS and Hepatitis
AE	Adverse Event
ART	Antiretroviral therapy
CPC	Country Project Committees
CRA	Clinical Research Assistant
CREDIM	Centre de Recherche et Développement en Informatique Médicale
CRF	Case Report Form
CRP	C-reactive protein
CXR	Chest radiography or chest X-ray
CTU	Clinical Trials Unit
DMP	Data Management Plan
DR	Digital Radiography
DST	Drug sensibility testing
eCRF	Electronic Case Report Form
ERC	Ethical Review Committee
GA	Gastric Aspirate
GCP	Good Clinical Practices
HIV	Human Immunodeficiency Virus
ICER	Incremental Cost-Effectiveness Ratio
IDLIC	Infection Diseases in Low Income Countries
IDMC	Independent Data Monitoring Committee
IPC	Infection Prevention and Control
IPT	Isoniazid Preventive Therapy
IRD	Institut de Recherche pour le Développement (Research Institute for Development)
IRIS	Immune Reconstitution Inflammatory Syndrome
IS	Induced sputum
LAM	Lipoarabinomannan
LTBI	Latent TB infection
MLR	Monocyte-to-lymphocyte ratio
MTB	Mycobacterium tuberculosis
NPA	Nasopharyngeal aspirate
NTP	National Tuberculosis Program
NPV	Negative Predictive Value
OSC	Output Steering Committee
PCC	Project Coordination Committee
PI	Principal Investigator
PPV	Positive Predictive Value
QC	Quality control
RIF	Rifampicin
SAB	Scientific Advisory Board
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
TB	Tuberculosis
TMF	Trial Master File
TST	Tuberculin skin test
UBx	University of Bordeaux
WHO	World Health Organization

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1. INTRODUCTION: BACKGROUND, RATIONALE AND HYPOTHESIS

The burden of childhood tuberculosis

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

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

TB and HIV infection in young children

HIV-infected children are a highly vulnerable population with regards to TB morbidity and mortality. A mathematical modelling study estimated that in 2015, 17% of paediatric TB deaths worldwide were in children with HIV, most of them in sub-Saharan Africa where the case-fatality rate in HIV-infected children reaches 36% [1]. These findings are consistent with hospital-based studies conducted in sub-Saharan Africa, reporting case fatality ratios of 14–41% in HIV-infected children receiving TB treatment [6], [7]. A retrospective study in the city of Cape Town including more than 3,000 HIV-infected children treated for TB showed that this group had increased mortality compared to HIV-negative children and twice the odds of unfavourable outcome [8]. Overall, TB is the leading cause of death in HIV-infected children worldwide, accounting for 1/3 of all deaths in this group [1], [9]. A recent meta-analysis confirmed that HIV-infected children who have access to antiretroviral therapy (ART) have an increased mortality risk compared to children without HIV, even when receiving tuberculosis treatment [10].

Challenges of TB diagnosis in HIV-infected children

Besides the usual difficulties in obtaining bacteriological confirmation in children, TB diagnosis in HIV-infected children is even more challenging. Complementary clinical and radiological approaches for empirical treatment decision lack specificity, in a context of immunodeficiency and overlapping clinical features not addressed by current TB diagnostic algorithms [11]. Thus, a symptom-based approach which performs well in HIV-uninfected children may perform poorly in the HIV-infected population [12]. Furthermore, immunodeficiency also reduces the sensitivity of immunological tests used to detect latent TB infection [13]. These difficulties occur in a context of more severe clinical presentations, co-treatment issues and higher TB mortality, which further justifies rapid TB diagnosis and treatment [11], [14].

Weaknesses of current diagnostic approaches

WHO criteria for the diagnosis of TB in children employ simple clinical features and chest X-ray (CXR). These include prolonged duration of cough and/or fever, failure to thrive, results of tuberculin skin test (TST), suggestive physical findings, and positive findings on CXR [15]. However no definite cut-offs, such as duration of symptoms, have been validated and its accuracy will depend on the clinical context. Strict symptom criteria have lower sensitivity and specificity in very young children, and in immunocompromised children. Cough, fever and poor

weight gain may have multiple confounding etiologies in HIV-infected children, given their high prevalence of other co-morbidiites.

CXR and TST, although recommended by WHO, have limitations in young children and particularly in those immunosuppressed. Though HIV infection is recognized as a cause of false-negative TST, it may be used in conjunction with other diagnostic tests [15]. Limited data are available from children in TB-endemic settings regarding interferon-gamma release assays (IGRAs), especially from populations with diagnostic challenges such as HIV-infected children, and IGRAs are not recommend by WHO to diagnose TB, including in high-burden settings.

The gold standard of diagnosis, mycobacterial culture, although the most sensitive in children, is expensive and has long turnaround time (typically at least 2 weeks), implying limited immediate benefit to clinical decisions to initiate antituberculosis therapy in children, especially in high-burden and resource-limited settings.

The automated nucleic acid amplification test (NAAT) Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale CA, USA) was endorsed by WHO in 2010 for the diagnosis of HIV-associated and drug-resistant tuberculosis in adults [16], [17]. A meta-analysis confirmed its good performance for rapid diagnosis of culture-confirmed pulmonary tuberculosis in children [18]. These findings supported the revised WHO recommendations on scaling-up the use of Xpert in children in 2014 [15]. Xpert remains nevertheless less sensitive in children than in adults to detect culture-confirmed TB [19]. Coupled to the low sensitivity of culture in children, Xpert yield in children is therefore much lower than in adults and a negative result does not exclude TB disease. The increased detection yield of the recently released Xpert MTB/RIF Ultra (Ultra) will potentially benefit most patients with paucibacillary disease as seen in HIV-infected children [5]. A recently published study however reveals a lower specificity of Ultra in adults, particularly in those with a previous history of TB, potentially resulting in false diagnoses and overtreatment of TB [20]. Though further prospective studies are needed, the risk of false-positive results could be less significant in children [21]. The estimated clinical impact of Ultra is therefore likely to vary depending on the settings, with a recent modelling in favour of a larger mortality benefit in patient populations with high TB prevalence, high HIV prevalence, and high case fatality ratios for untreated TB [22]. An update of the current guidelines for the use of Ultra is planned for late 2019/early 2020 [23].

Alternative specimen collection methods adapted to children

Furthermore, in children, specimen collection methods and their ability to retrieve bacilli are key factors of the microbiological diagnosis of TB. Young children are frequently unable to spontaneously expectorate sputum and there is still no clear evidence and guidance on which specimens or combination of specimens, and the number of specimens, which should be used in order to maximize the bacteriological confirmation of TB in children, while considering the feasibility of collecting multiple specimens. Standard collection methods recommended by WHO include expectorated sputum, which is not feasible in young children, gastric aspirates (GAs) which require fasting and most often hospitalization, and induced sputum (IS) [15], [24]. At the programmatic level, in community-based primary healthcare centres, where children are typicaly evaluated for TB, implementation of GAs can be poorly accepted and IS could be challenging [25], [26]. So far, the implementation of methods to collect samples in children unable to expectorate remains very limited [25].

Our research group and other groups in Africa and Asia have shown that alternative specimen collection methods to GA and IS such as nasopharyngeal aspirates (NPAs) and stools are easier to implement in resource-limited settings and are better tolerated in young and HIV-infected children [27]–[32]. These methods do not require a child to fast (as for GAs) and are more suitable than IS in children with severe respiratory deficit [33]. In children with presumptive TB, Xpert MTB/RIF has a sensitivity on NPAs close to the one achieved with IS [27], [34]. Two recent studies have shown similar sensitivity of Xpert MTB/RIF on the combination of one stool and one NPA (75.0% and 68.0%) [35], [36], as compared to two IS samples (71% and 75·9%) [27], [37] or two NPAs (65.1%) [27]. Stool testing by Xpert shows

results close to respiratory samples in terms of sensitivity but requires simplified specimen processing method for further field use [29], [30], [38]–[41]. The flotation method based on Sheather's sucrose solution used in the PAANTHER study showed promising results but relies on centrifugation and a labour-intensive process [35]. This method will be further optimized in Output 4 of the TB-Speed project to enable its implementation at lower level of care.

However, use of Xpert for paediatric TB diagnosis is still scarce in resource-limited settings, particularly at lower levels of health care. In practice, even when laboratory and radiological diagnostic resources for TB are available, treatment is frequently initiated empirically based on clinical features for most young children.

Potential biomarkers of interest in the diagnosis of TB in children

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 [42]. 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 or urine would be of great advantage. However, to date few biomarkers have proven to be of value in discriminating childhood TB from other diseases, as well as active TB from latent TB infection. Measurement of immune response molecule concentrations, such as interferon gamma and C-reactive protein, is a complementary strategy to the direct detection of *M. tuberculosis*. However, to this end IGRAs are not useful as they are unable to discriminate between latent and active TB. Findings from metabolomics studies have provided useful information on the host metabolic response to *M. tuberculosis* infection, but their potential as a TB diagnostic has yet to be confirmed. Emerging research using transcriptional biosignatures in whole blood has been the most promising (accuracy >80%). Studies in cohorts of children from South-Eastern Africa, South America and India have identified mRNA signatures and gene sets capable of distinguishing active disease from latent TB infection [43]–[46], as well as TB from non-TB pneumonia [47]. 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 [44].

Lipoarabinomannan (LAM), a mycobacterial antigen and virulence factor which is found in the urine of many TB patients, has been the most studied TB biomarker for the development of a low-cost point-of-care assay. However, the adoption of LAM tests has been limited due to their relatively poor clinical sensitivity across the spectrum of incident TB cases. The observation that LAM levels in urine tend to be higher in HIV-positive TB patients than in HIV-negative TB patients led to the WHO recommendation to use the Alere Determine™ LAM assay (only commercially available test) to diagnose TB only in HIV-positive individuals with CD4 counts <100 cells/µl who have TB symptoms [48]. Urinary LAM assays have an interest as a complement to Xpert for people living with HIV, given the relatively low sensitivity of Xpert in this population, and could potentially improve the PAANTHER TB treatment decision algorithm. A novel urine-based assay co-developed by FIND and Fujifilm, The Fujifilm SILVAMP TB LAM, has shown a higher sensitivity and specificity compared to the Alere LAM test based on initial results on frozen biobanked urine samples of almost 1000 HIV-infected patients ([49]; non-peer-reviewed manuscript). Further prospective and operational studies are nevertheless needed in paediatric populations.

The blood Monocyte-to-Lymphocyte ratio (MLR) correlates with TB disease in HIV-infected adults, but very few data are available on its validity in children. Findings from a cohort of 183 hospitalized HIV-infected children, presented at the 2017 Union Conference, showed that the MLR distinguished HIV-infected children with microbiologically-confirmed TB from those with unconfirmed or unlikely TB, possibly reflecting bacterial burden [50], [51].

Improved TB diagnostic algorithms are urgently needed for HIV-infected children

Systematic reviews on clinical scoring systems for the diagnosis of TB in children reveal that a minority of systems are relevant to developing countries with a high burden of HIV/AIDS. Few studies have examined the validity of these systems in HIV-infected children. Specificity was generally found to be lower, leading to a concern for over-diagnosis of TB in HIV-infected children [52], [53].

Although these scoring systems and diagnostic criteria are commonly used, they are currently not recommended by WHO. Their reliability and validity remain unclear due to the lack of an established and practicable gold standard. Different diagnostic criteria are used in different settings, and they may or may not have been validated for those locations [52].

There is strong evidence that undiagnosed and untreated TB increases the risk of death in children, especially those HIV-infected, who are highly vulnerable to TB disease [6]. Specific decision-making tools are therefore urgently needed to guide clinicians from high-TB burden and low-income countries to initiate treatment quickly in HIV-infected children with suspected TB, without substantial overdiagnosis and treatment.

The PAANTHER TB treatment decision algorithm

The ANRS 12229 PAANTHER 01 (Pediatric Asian African Network for Tuberculosis and HIV Research) study, which enrolled 438 HIV-infected children of median age 7.3 (IQR: 3.3 – 9.7) years with presumptive TB in four countries (Burkina Faso, Cameroon, Cambodia, Vietnam) from 2011 to 2014, aimed at developing a diagnostic prediction score and algorithm for TB treatment decision in HIV-infected children. This was the first study developing a TB diagnostic prediction score exclusively in HIV-infected children, using methods recommended for diagnostic prediction models. Based on microbiological, clinical, abdominal ultrasonography and radiological features, the best scoring system obtained had a sensitivity of 88.6% and specificity of 61.2% when Xpert MTB/RIF was included in the algorithm 3 (see Appendix 2). Abdominal ultrasonography has shown promise for the diagnosis of TB in both HIV-infected adults and children [54]–[56]. In the PAANTHER study, it detected abdominal lymphadenopathy in 50% of culture confirmed TB cases and 35% of all confirmed and unconfirmed cases, with a specificity of 85%.

We showed previously that mortality is high in children with both confirmed and unconfirmed TB and that initiation of anti-TB treatment, that occurred at a median time of 7 (IQR: 5 – 11) days in the study, led to delayed ART initiation which is associated to significantly increased mortality [57]. Based on its diagnostic accuracy measures, the score could enable faster TB treatment decision in children with a high risk of mortality, despite a rather poor specificity. It was thus incorporated into a TB treatment decision algorithm, as it constitutes a consequentialist approach to tuberculosis in HIV-infected children considering the need to initiate treatment to reduce mortality, rather than an essentialist approach considering the trueness of tuberculosis diagnosis [58].

Developed in tertiary research-experienced health facilities, the PAANTHER score integrated in a stepwise TB treatment decision algorithm could enable standardized treatment decision in HIV-infected children with presumptive TB, and could be recommended for extensive use in secondary and primary healthcare settings where most of these children are seeking care. However, external validation studies are now needed to assess the predictive performance of the PAANTHER diagnostic score on independent datasets.

Expected benefits and risks of the PAANTHER TB treatment decision algorithm

The main expected benefit of the PAANTHER TB treatment decision algorithm is a quick TB treatment decision. Rapid initiation of TB treatment in children with both confirmed and unconfirmed TB should lead to improved health outcomes including reduced risk of death.

In those who are ART-naïve and initiated on TB treatment as per the PAANTHER TB treatment decision algorithm, shortening the delay to TB treatment should also enable earlier access to

ART. In 266 ART-naïve children from the PAANTHER 01 study, 212 (79.7%) were initiated on ART at a median time of 24 (IQR: 14 – 41) days. Children treated for TB started ART later than children not treated for TB [30.5 (IQR 17.0 – 41.5) vs. 14.0 (IQR 11.0 – 32.5) days; p<0.0001]. 26 and 24 deaths occurred before and after ART initiation, respectively. Initiation of ART during follow-up, whatever the timing, was associated with a strong decrease in mortality (p<0.00001). In the multivariate analysis, ART was associated with an estimated 91.6% reduction in the risk of death over the first month of the study [hazard ratio (HR) 0.084; 95%CI 0.010-0.670]. Reduction in time to TB treatment initiation and subsequent earlier ART introduction should lead to improved survival, especially in this population at very high risk of death.

Children not initiated on TB treatment as per the PAANTHER algorithm could immediately benefit from ART initiation. Isoniazid Preventive Therapy (IPT) will be proposed to prevent incident TB but time to initiation of IPT may depend on the level of immune suppression. In children recently initiated on ART, the possible occurrence of unmasking immune reconstitution inflammatory syndrome (IRIS) will be managed cautiously with adjunctive steroids if needed. In those presenting with TB in a context of ART failure, ART should be modified quickly for improved immunologic and clinical outcomes.

Given the high sensitivity of the algorithm, it is expected that only a small proportion of children with TB will be missed in the group not initiated on treatment. Therefore, since true TB cases not recognized by the algorithm (false negative cases) are expected to be a low proportion, the risk of unmasking TB IRIS under ART due to missed TB case at ART initiation is expected to be low.

Given the relatively low specificity of the algorithm, it is expected that there will be a significant proportion of children later classified as unlikely TB in those initiated on TB treatment as per the PAANTHER TB treatment decision algorithm. Despite the better safety profile of first-line TB treatment (very low risk) and ART in children compared to adults, some children with unlikely TB could therefore be exposed to unnecessary cumulative drug toxicity, drug-drug interactions requiring additional safety monitoring, and high pill burden, eventually hampering adherence to ART and TB treatment.

Validation of the PAANTHER TB-treatment decision algorithm

External validation is essential to support the general applicability of developed scores. External validation studies may address aspects of geographical and clinical spectrum transportability. Ideally this should be done using a reference standard, but this is practically lacking in childhood TB (the gold standard, mycobacterial culture, being inadequately sensitive). To classify children as unconfirmed or unlikely TB cases, the recently updated international consensus Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children is highly dependent upon the clinician's decision to treat [59]. This reference standard would therefore not be independent from the PAANTHER TB treatment decision algorithm, which would lead to major incorporation bias in the estimation of the algorithm diagnostic accuracy. The option of a diagnostic clinical trial in primary and secondary healthcare facilities would bring high quality evidence but raises major logistical issues.

We will therefore proceed to an external validation of the PAANTHER prediction model in new populations of HIV-infected children from four high TB burden countries in Africa to examine its robustness and transportability to other geographical, epidemiological and health care settings, using a "management study" design. This type of design has been largely used in pulmonary embolism diagnostic studies, where the potentially iatrogenic reference standard cannot be applied to all patients [60], [61].

With such a design, our study will focus on the evaluation of the safety of withholding TB treatment in HIV-infected children with presumptive TB based on a negative result for the PAANTHER treatment decision algorithm, which will be substituted to routine clinical care at baseline, except in children with severe/life threatening conditions for whom TB treatment decision could be made at the discretion of the site clinician. All children will be prospectively followed-up and monitored, especially those not initiated on treatment as per the PAANTHER

TB treatment decision algorithm. Careful repeated assessment at follow-up visits, initiation of TB treatment if needed, TB case validation by local and international clinical review committees, as well as regular review of safety reports by the Independent Data Monitoring Committee (IDMC) will ensure a thorough and careful safety monitoring. The study will also assess the feasibility of the algorithm, especially with regards to specimen collection, the accuracy of some components of the algorithm and the outcome of TB in HIV-infected children.

If validated, the PAANTHER TB treatment decision algorithm could be systematically implemented at the primary or secondary health care level, where screening recommended by the WHO Three I's strategy is implemented and HIV-infected children may present with signs of presumptive TB.

Participating countries and added value of the multi-country aspect

To assess the model's predictive performance outside of settings where the development study was implemented, the study will be implemented in 4 countries across Africa where TB incidence, HIV prevalence, patient mix and healthcare practices are likely to vary: Côte d'Ivoire, Uganda, Mozambique, and Zambia (Table 1).

Zambia is among the 30 high TB burden countries according to the WHO classification, based on TB incidence rate per 100 000 population, while Uganda is in the top 20 high TB/HIV burden countries based on estimated numbers of incident TB cases among people living with HIV. For the purpose of this study, we will differentiate between 'high' and 'very high' TB incidence countries using a cut-off annual incidence rate of 300 cases/100,000 population.

Table 1: TB incidence and paediatric HIV prevalence in participating countries

Region	Country	TB incidence rate /100,000 pop (2016)	Paediatric HIV prevalence	HIV prevalence in incident paediatric TB cases (2015)
Western Africa	**Côte d'Ivoire**	153	0.3%	16.2%
Eastern Africa	**Uganda**	201	0.5%	17.1%
Southern Africa	**Mozambique**	551	0.9%	22.6%
	Zambia	376	1.1%	32.6%

Sources: WHO Global TB report, 2017 [2]*; Dodd et al., 2017* [1]

National TB Programs (NTPs) from these countries support the TB-Speed project and are members of Country Project Committees (see Chapter 13.6). NTPs will play an instrumental role in the scale up of the TB-Speed strategy, taking the opportunity of the 2020 country dialogue for funding request to the Global Fund. This will be further supported by the WHO-Unitaid TB enabler grant, through which the project will receive support from WHO to ensure country preparedness for accelerated uptake and integration of the TB-Speed approach into national and international guidance for the management of childhood TB.

2. OBJECTIVES

2.1. Primary Objective

1. To evaluate the proportion of missed TB cases in HIV-infected children with presumptive TB not initiated on treatment as per the PAANTHER TB treatment decision algorithm (false negative cases)

2.2. Secondary objectives

1. To evaluate the feasibility of the PAANTHER TB treatment decision algorithm in HIV-infected children with presumptive TB
2. To assess the proportion of HIV-infected children with unlikely TB among those initiated on treatment as per the PAANTHER TB treatment decision algorithm
3. In HIV-infected children with presumptive TB, including comparison of those initiated and those not initiated on TB treatment as per the PAANTHER TB treatment decision algorithm, to assess:
    a. Incidence of morbidity (drug toxicity, opportunistic infections, IRIS) and mortality during 6 months after enrolment
    b. Time to ART initiation in those who are ART-naïve
    c. Immunologic evolution at 6 months after enrolment
4. To assess TB treatment outcomes in HIV-infected children initiated on treatment as per the PAANTHER TB treatment decision algorithm
5. To assess the feasibility of IPT initiation in HIV-infected children not initiated on treatment as per the PAANTHER TB treatment decision algorithm
6. To assess the performance of the MLR and the C-reactive protein (CRP) and their potential added value in the PAANTHER score and algorithm to detect TB
7. To evaluate the diagnostic performance of Ultra performed on one NPA and one stool sample against mycobacterial culture performed on standard samples (GA in younger children or expectorated sputum in older children) in HIV-infected children
8. To assess the feasibility of stool sample collection, and the feasibility, safety, and tolerability of NPA collection in HIV-infected children
9. To assess the performance of the PAANTHER algorithm for TB diagnosis across various ages, CD4 counts, nutritional statuses, and timings of ART initiation
10. To evaluate the cost effectiveness of implementing the PAANTHER TB treatment decision algorithm compared to the estimated effect of the standard of care in children with HIV

3. STUDY ENDPOINTS

3.1. Primary study endpoint

1. Proportion of missed TB cases (false negative cases) in children not initiated on treatment as per the PAANTHER TB treatment decision algorithm

3.2. Secondary study endpoints

1. Time to final TB treatment decision and proportion of children with presumptive TB having completed the PAANTHER TB-treatment decision algorithm

    The algorithm will be considered completed if a decision to initiate TB treatment has been taken at any step of the algorithm or if TB has been excluded after systematic evaluation, and all steps planned in the algorithm have been implemented.

2. Proportion of cases considered as unlikely TB by the Expert Committee in those initiated on treatment as per the PAANTHER TB treatment decision algorithm (false positive)

3.
    a. Morbidity (drug-induced toxicity – ART and TB treatment-related, opportunistic infections, IRIS), with or without TB treatment, and mortality at 6 months
    b. Time to ART initiation in ART-naïve children
    c. CD4 (absolute count and %) gain
4. Weight gain at 6 months (absolute value and percentage of body weight), TB symptoms resolution and outcomes in children on TB treatment
5. Time to IPT initiation and proportion of children initiated on IPT in those not initiated on treatment as per PAANTHER TB treatment decision algorithm
6. Discrimination (area under the receiver-operating-characteristic curves [AUROC]) and calibration measures of the PAANTHER prediction model including or not MLR and CRP, against the TB composite reference standard as defined by the Expert Committee
7. Proportion of NPAs (or sputum) and stool samples with *mycobacterium tuberculosis* (MTB) detected using Ultra
8. Feasibility defined as proportion of children with NPA and stool samples collected as per study protocol

    Safety defined as proportion of NPA with AEs (vomiting, nose bleeding, low oxygen saturation, respiratory distress) occurring during NPA and

    Tolerability defined as discomfort/pain/distress experienced by the child during NPA as assessed by the child (Wong-Baker face scale), by the parents (visual analog scale), by the nurses (FLACC behavioral scale) (quantitative assessment) in a subset of children
9. Diagnostic accuracy (false negative and false positive rates i.e. NPV and PPV and corresponding sensitivity and positivity) of the PAANTHER algorithm in the different subgroups in terms of age, CD4 counts, nutritional statuses, and timings of ART initiation (on/off ART)
10. Incremental cost-effectiveness ratio (ICER)

3.3. Reference diagnosis/case definitions and validation by the Endpoint Review Committee and the international Expert Committee

An Endpoint Review Committee will be set up at the national level for the purpose of case review and validation of TB diagnosis. The national Endpoint Review Committee will review and validate all new TB cases in children initially non-treated for TB, using the updated Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children (see Table 2). The Endpoint Review Committee will also validate differential clinical and other diagnoses on opportunistic infections and adverse events. All reviews will be performed following Standard Operating Procedures (SOPs) specifically developed for the study.

Additionally, at the international level, an Expert Committee will be set up for the purpose of clinical validation of TB diagnosis, including identification of children with unlikely TB in those initiated on treatment as per the PAANTHER TB treatment decision algorithm. The International Expert Committee will validate final TB diagnosis and will determine, based on timing, clinical features, and any other relevant information whether missed TB cases are false negatives, potential TB unmasking IRIS or new TB infections. The use of the updated Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children will not be possible as treatment decision is not clinician based but algorithm-guided. The Expert Committee will also review part of all cases reviewed at country level to ensure homogeneity of classification across countries (See figure 2).

The Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children were initially developed for children with suspected TB and may not be fully relevant for children with HIV infection [59]. They may be revised by the Scientific Advisory Board (SAB) based on current state of the art on time of final case review.

For the purpose of reference diagnosis and case definition, CXRs will be read by 2 independent experts experienced in reviewing CXRs in children, blinded to all of the clinical information and to each other's interpretation. In case of disagreement, advice from a third expert will be sought. For routine care, CXR will be read by the site clinician.

Table 2. Updated Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children (adapted from Graham et al, 2015 [59])

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

[1] Clinical signs/symptoms suggestive of tuberculosis include: (a) Persistent cough: persistent (>2 wk), unremitting cough. (b) Weight loss/failure to thrive: (b1) Unexplained weight loss: >5% reduction in weight compared with the highest weight recorded in last 3 mo OR (b2) Failure to thrive defined as (i) Clear deviation from a previous growth trajectory, and/or (ii) Documented crossing of percentile lines in the preceding 3 mo, and/or (iii) Weight-for-age z score of ≤−2 in the absence of information on previous/recent growth trajectory, and/or (iv) Weight-for-height z score of ≤−2 in the absence of information on previous/recent growth trajectory AND (b3) Not responding to nutritional rehabilitation (or antiretroviral therapy if HIV infected). (c) Persistent unexplained fever: Persistent (>1 wk) and unexplained fever (>38°C) reported by a guardian or objectively recorded at least once. (d) Persistent, unexplained lethargy or reduced playfulness: persistent, unexplained lethargy or decrease in playfulness/activity reported by the parent/caregiver. (e) Infants 0–60 d (or neonate): additional signs and symptoms suggestive of tuberculosis include: (e1) neonatal pneumonia or (e2) unexplained hepatosplenomegaly or (e3) sepsis-like illness.

[2] CXR will be considered consistent with tuberculosis if reviewers agree on the presence and location (right/left) of ≥ 1 lesion among the following: alveolar opacity, bronchial compression, excavation, ghon focus, gibbus, miliary, nodular infiltrates, paratracheal nodes, peri-hilar nodes, pleural effusion, tracheal compression (as suggested by Graham et al. 2012 [62]*).*

4. STUDY DESIGN

4.1. Study type

TB-Speed HIV is a prospective multicentre management study evaluating the safety and feasibility of the recently proposed PAANTHER TB treatment decision algorithm for HIV-infected children with presumptive TB. It will be conducted in four countries with high and very high TB incidence (Côte d'Ivoire, Uganda, Mozambique, and Zambia) which have not participated in the study that developed the PAANTHER algorithm.

4.2. Methodology

The PAANTHER algorithm was initially developed in two countries from South-East Asia (Cambodia, Vietnam) and two West African countries (Burkina Faso, Cameroon). To assess the algorithm's performance outside of its development settings, the study will be purposefully implemented in 4 different countries across Africa where TB incidence, HIV prevalence, patient mix and healthcare practices are likely to vary: Côte d'Ivoire, Uganda, Mozambique, and Zambia (Table 1).

The PAANTHER TB treatment decision algorithm will be used for a TB treatment decision by site clinicians in all children enrolled in the study. Validation of the algorithm will be performed by evaluating the proportion of missed TB cases in children not initiated on treatment as per the PAANTHER TB treatment decision algorithm. The safety of this strategy will be carefully assessed through review of safety reports every 4 to 6 months during study conduct by the IDMC.

Occurrence of algorithm failures in terms of missed TB cases (TB cases subsequently detected among untreated cases, i.e. false negatives) and cases with unlikely TB among those initiated on TB treatment as per the algorithm (cases not secondarily validated as confirmed or unconfirmed TB cases by the Expert Committee, i.e. false positives) will enable to estimate the negative and positive predictive values of the algorithm.

A centralized international Expert Committee will clinically review and validate TB diagnosis in children. This will enable assessment of the added value of new markers (MLR and CRP) and, if need be, to propose a new version of the score based on an optimised predicted probability.

- Study adaptation to the Covid-19 pandemic

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

Enrolments resumed progressively at sites from June 2021, depending on country situations and appropriate safety measures (see section 7.14). However, the arrangements described above may be reapplied if the epidemiological context and government restrictions require so. These also include remote site monitoring (see section 16.2.3).

4.3. Sample size

Data obtained from the PAANTHER study showed that the TB prevalence was 57.3% in HIV-infected children with presumptive TB as defined per standard algorithm entry criteria, with a proportion of 39.3% of children being on ART. We expect a higher proportion of children on ART in this new study, reaching 50%, and a resulting lower prevalence of TB, around 50%, based on improved accesss to HIV care in our study settings.

With an algorithm sensitivity and specificity of 89% and 61%, respectively, the proportion of missed TB cases among children not started on treatment based on the PAANTHER algorithm (assessed by the TB incident cases in the 6 months period after applying the algorithm) is estimated to 15%, which corresponds to a negative predictive value (NPV) of 85%. For sample size calculations, we used this expected NPV of 85% and an unacceptable NPV of 75% (minimal acceptable 95% lower confidence interval limit), a one-sided test of level 5% and a probability β=5%, which results in a sample of 176 children not-initiated on treatment as per

the PAANTHER TB treatment decision algorithm and a total sample of 550 children, taking into account an expected 10% of missing data [63].

4.4. Provisional study timeline

- First inclusion: 4th quarter 2019
- Inclusion period: 27 months
- Duration of follow-up for each participant once enrolled: 6 months
- Enrolment stop due to Covid-19 pandemic: April 1st, 2020
- Progressive enrolment restart: June-December 2020
- Last visit of the last patient: 2nd quarter 2022
- Overall duration of the study (from the first inclusion to the last visit): 33 months

4.5. Cost-effectiveness ancillary study

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

A mathematical model will be developed to project health-economic outcomes including TB cases and mortality in children with HIV. The model will be developed in collaboration with ScHARR of the University of Sheffield (UK) and the CaP-TB project (see Chapter 11.2.4).

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

A separate analysis plan will be written for the cost-effectiveness analysis. Data collection methods for cost data are outlined in Chapter 10.1.2.

5. STUDY ENROLLMENT

5.1. Study population

5.1.1. Inclusion criteria

1. Children aged 1 month to 14 years
2. Documented HIV-infection (i.e., confirmed before entry into the study)
3. Presumptive TB based on at least one criteria among the following[1]:
    a. Persistent cough for more than 2 weeks
    b. Persistent fever for more than 2 weeks
    c. Recent failure to thrive (documented clear deviation from a previous growth trajectory in the last 3 months or Z score weight/age < 2)
    d. Failure of broad spectrum antibiotics for treatment of pneumonia
    e. Suggestive CXR features

    OR

    History of contact with a TB case and any of the symptoms listed under point 3 with a shorter duration (< 2 weeks)

[1] PAANTHER inclusion criteria

4. Informed consent signed by parent/guardian

5.1.2. Non-inclusion criteria

1. Ongoing TB treatment or history of intake of anti-TB drugs in the last 3 months (isoniazid alone or rifampin/isoniazid for preventive therapy is not an exclusion criteria)

5.2. Recruitment sites

The study will be implemented at HIV inpatient/outpatient centres from 7 selected hospitals in four African countries where TB incidence and paediatric HIV prevalence are likely to vary: Côte d'Ivoire, Mozambique, Uganda, and Zambia (see Table 1). All participating hospitals are University Teaching Hospitals (UTH) or equivalent in terms of level of care, including national or regional reference hospitals collaborating with the TB-Speed Pneumonia and TB-Speed HIV studies (see Appendix 3).

Table 3 presents indicative recruitment capacities of participating hospitals, based on a capacity assessment questionnaire including the number of children <15 years followed up in HIV treatment and care centres in 2016 and 2017.

Table 3: Study sites from participating countries

Country	Nb sites	Hospitals	Paediatric HIV active file
Côte d'Ivoire	2	Cocody UTH, Abidjan	347
		Treichville UTH, Abidjan	460
Uganda	1	Regional reference Hospital, Mbarara	800
Mozambique	2	Maputo Central Hospital, Maputo	700
		José Macamo General Hospital, Maputo	240
Zambia	2	Lusaka University Teaching Hospital, Lusaka	2 413
		Arthur Davidson Children Hospital, Ndola	2 863

6. STUDY INTERVENTIONS

6.1. Diagnostic procedure evaluated

The PAANTHER algorithm and prediction score was designed as a guiding tool for empirical TB treatment decision in HIV-infected children with presumptive TB (see Figure 1 below and Appendix 2). The proposed score includes:

- History of close contact with a smear+ TB case
- Suggestive TB symptoms (prolonged fever, unremitting cough, hemoptysis, weight loss in the past 4 weeks)
- Tachycardia
- Chest radiography features (miliary, alveolar opacities, lymph nodes)
- Abdominal ultrasound features (abdominal lymphadenopathy)
- Xpert MTB/RIF assay which will be replaced by Ultra

Points scored by each sign or symptom is shown in the table below.

Table 4: PAANTHER TB treatment decision algorithm components and point scoring

Exam/assessment	Features/items	Points
Contact history	History of contact with a smear + TB case	118
Symptoms	Fever lasting > 2 weeks	66
	Unremitting cough	39
	Hemoptysis in previous 4 weeks	79
	Weight loss in previous 4 weeks	24
Clinical signs	Tachyardia	54
Xpert MTB/RIF Ultra	Positive Xpert MTB/RIF Ultra result	241
Chest radiography	Miliary	90
	Alveolar opacity	74
	Lymph nodes	100
Abdominal ultrasound	Abdominal lymph nodes	73

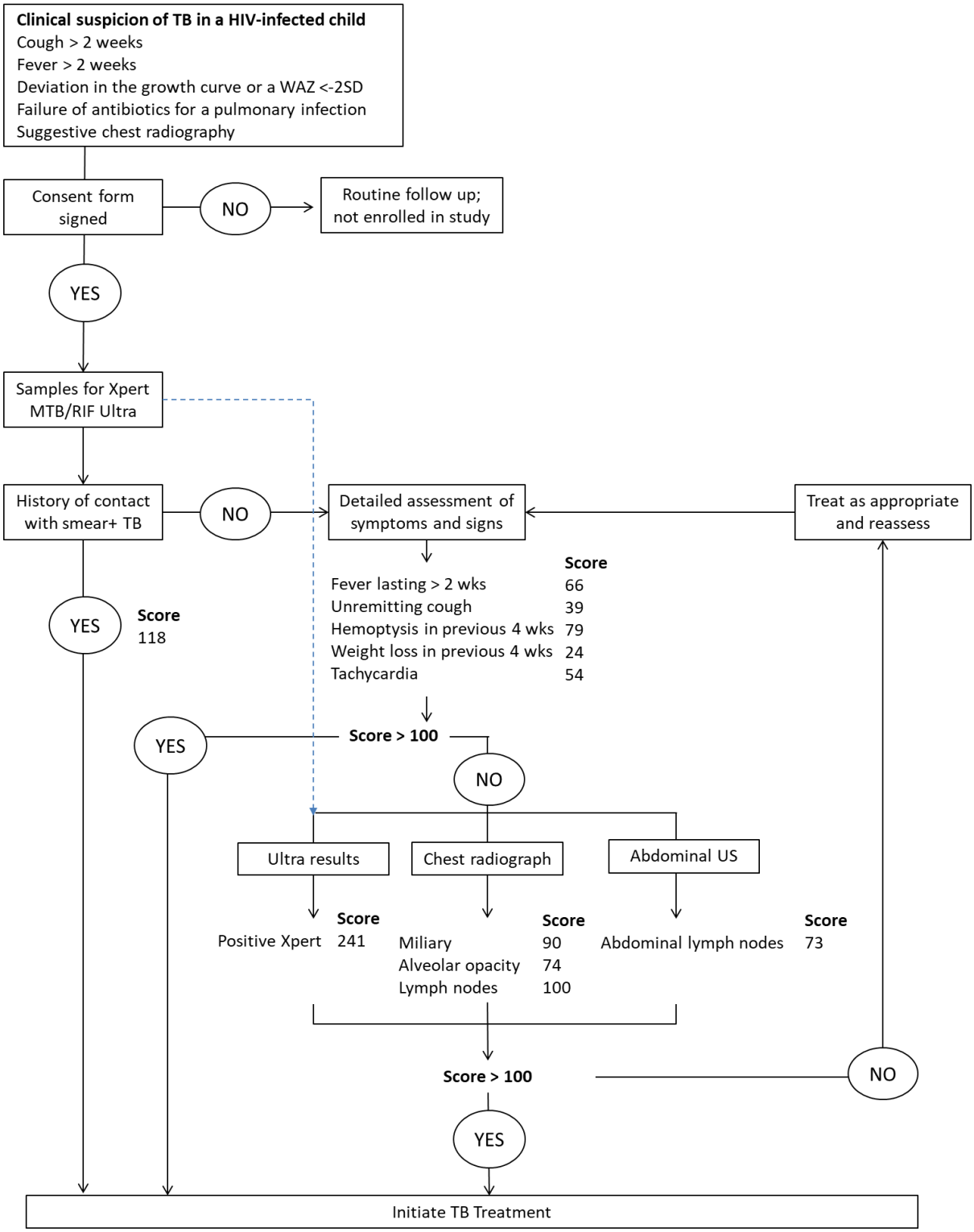


Figure 1: Implementation of the PAANTHER TB treatment decision algorithm in the context of the TB-Speed study

A score of >100 is highly predictive of TB. In children with a score >100, anti-TB treatment will be initiated immediately. In children with a score of <100, TB treatment will not be initiated except in those with severe/life-threatening conditions for whom TB treatment decision could be made at the discretion of the site clinician. TB could be definitely ruled out after further assessment and decision made as to what treatment the children should receive in accordance with existing national protocols, along with clinical follow-up.

6.2. Capacity building

Implementation of the TB-Speed HIV study will include a preparation and capacity building phase conducted at participating hospitals, which are tertiary level reference and university teaching hospitals also involved in the TB-Speed Pneumonia study in Zambia and Uganda. Sites which are not already equipped will be provided with digital radiography (DR) plates for digital CXR, ultrasound machine, and sample collection material (including battery-operated suction machines for NPA collection). Laboratories will be equipped with GeneXpert devices if not available through National Programs as well as -80°C freezers for biobanking.

Specific training will be provided to site investigators and hospital staff in HIV outpatient/inpatients centres (doctors and nurses) on TB diagnosis, study procedures, and Good Clinical Practice (GCP). Initial training will be provided onsite by the study central coordinating team. Based on didactic and practical presentation, trainings will include the following topics:

- Childhood TB diagnosis and management
- Clinical follow-up (eligibility criteria, informed consent process, patient schedule, TB assessment, management in the case of AE/SAEs)
- Biological samples collection (NPA) and laboratory procedures including biobanking
- Use of DR plates
- Non-routine TB assessment procedures and their interpretation (MLR, CRP, abdominal echography)
- Use of the PAANTHER treatment decision score
- Data collection and use of electronic Case Report Form (eCRF)
- Data management and monitoring

6.3. Implementation at site level

The TB-Speed HIV study will be implemented in 7 tertiary healthcare level hospitals in main cities of Côte d'Ivoire, Uganda, Mozambique, and Zambia (see Table 1 and Table 3). A total of 550 HIV-infected children (aged < 15 years) with clinically suspected TB (presumptive TB) will be enrolled, using standard inclusion criteria. The inclusion period will last until the expected number of children is reached, but recruitment by study site will however not be capped. This could be achieved in about 27 months based on estimated recruitment capacities of participating sites (see Chapter 5.2) and the overall delay incurred by the study, in particular due to the Covid-19 pandemic.

6.4. Study flow chart

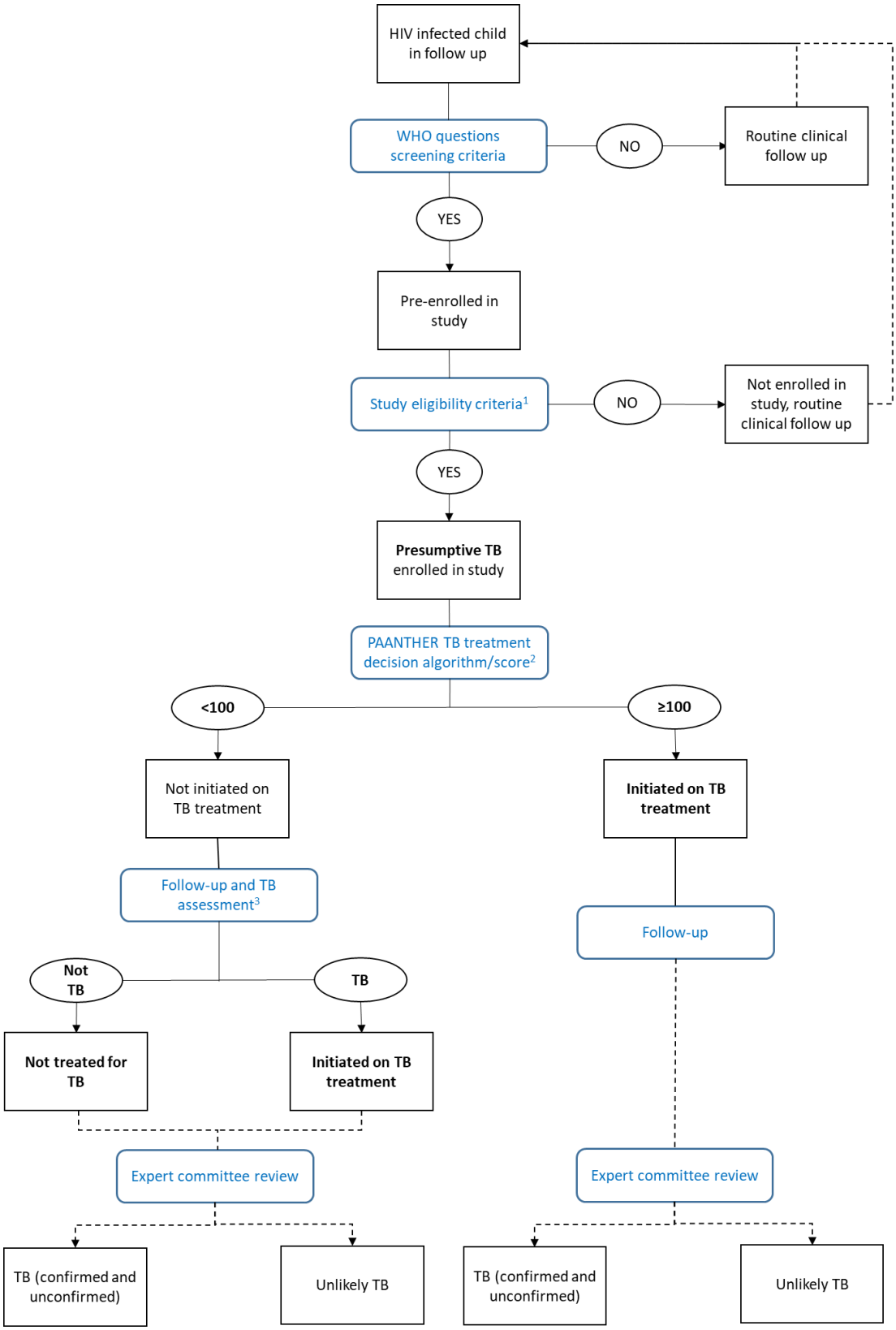


Figure 2: TB-Speed HIV study flow chart

[1]Includes CXR features on CXR possibly performed in previous assessment of acute cough or fever
[2]Includes CXR features on CXR performed previously or specifically for the score
[3]Includes children with score <100 initiated on TB treatment by the clinician on the basis of severe/life-threatening condition at D0

7. STUDY PROCEDURES

Recruitment will be performed at entry in HIV outpatient/inpatient centres of participating hospitals, as well as during the follow up of HIV-infected children on ART.

7.1. Selection process

Children will be first pre-screened for TB by attending clinicians and nurses in the context of routine care based on WHO criteria, i.e. with any one of the following symptoms: poor weight gain, fever, current cough and contact with a TB case [15]. If not routinely performed, this pre-screening step will be reinforced and a screening logbook will be set up (if not already in place) to collect aggregated data on the eligibility criteria assessment process. Children pre-screened positive for TB will be further subjected to the study selection criteria by the study nurses.

Assessment for eligibility will be detailed in specific clinical SOPs. Children assessed as not having eligibility (presumptive TB) criteria may undergo a CXR and receive a broad spectrum antibiotic course as part of routine care and be reassessed later.

7.2. Informed consent and assent from children

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

The purpose, the nature of constraints and the foreseeable risks and benefits of the trial will be fully explained to the parent(s)/guardian(s) of eligible children. Importance of follow-up, especially in the case of children not initiated on treatment as per the PAANTHER TB treatment decision algorithm, will be emphasized. Parent(s)/guardian(s) will be informed that participation is voluntary and that they will be free to withdraw from the trial at any moment without justification or without any negative consequences for the quality of care and follow-up provided to their child. In addition to oral explanations, a written information sheet will be systematically provided (see Appendix 6). Documents with pictures will be available to help the parents and children to better understand the exams proposed in the study.

Children aged over 7 years are unable to consent but their assent should be obtained using age-appropriate information [64]. Separate information sheets and assent forms will be used to explain the purpose, the risks and benefits of the study. Whenever possible, a written confirmation will be obtained from the child. Consent should be obtained from the parent(s)/guardian(s) before assent is sought from the child [64]. If the child's assent is not collected, this will be recorded in the consent form with the reasons.

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

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

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

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

7.3. Patient schedule

Table 5: Patient schedule including specimen collection in children

	Protocol visit							
	D0	**D1-D2**	**D7**[1] *(+/- 1 day)*	**D15** *(+/- 2 days)*	**Month 1** *(+/- 2 weeks)*	**Month 2** *(+/- 2 weeks)*	**Month 3** *(+/- 2 weeks)*	**Month 6** *(+/- 2 weeks)*
Eligibility criteria	X							
Informed consent(s)	X							
Clinical evaluation	X		X	X	X	X	X	X
Medical history	X		X	X	X	X	X	X
Abdominal Ultrasonography	X							
CXR	X		(X)[4]	(X)[4]	(X)[4]	X	(X)[4]	X
TB treatment decision algorithm scoring[2]	X	X						
TB treatment (if required)	X	X	X	X	X	X	X	X
TB drug adherence assessment			X	X	X	X	X	X
TB treatment response			X	X	X	X	X	X
LTBI treatment (IPT or RH 3 Mo)[3]		X	X	X	X	X	X	X
Treatment and care of co-morbidities if needed	X	X	X	X				
Lab assessment:								
- Complete blood count (CBC)	X					X		X
- Transaminases and CRP	X					X		X
- CD4 count	X							X
NPA	X							
Stool sample	X							
Two GAs or expectorated sputum		X	(X)[4]	(X)[4]	(X)[4]	(X)[4]	(X)[4]	
Xpert MTB/RIF Ultra	X	X	(X)[4]	(X)[4]	(X)[4]	(X)[4]	(X)[4]	
Mycobacterial culture[5]		X	(X)[4]	(X)[4]	(X)[4]	(X)[4]	(X)[4]	
NPA safety and tolerability	X							
Biobank (frozen samples):								
- Whole blood	X							
- Plasma	X							
- Urine	X							
- NPA, sputum and stool leftovers	X							
Maximum number of tubes collected[6]	5					2		3
Maximum volume of blood collected[7]	10.5 mL					4 mL		6 mL

(1) Day 7 visit is performed only in children not initiated on TB treatment as per the PAANTHER treatment decision algorithm
(2) TB treatment decision using the algorithm is taken as soon as the individual score is >100, or once the final score remains <100 and all tests have been performed
(3) In those not initiated on treatment as per PAANTHER TB treatment decision algorithm
(4) Performed in children with TB excluded by the algorithm, not improving or deteriorating clinically during follow-up
(5) Performed on two GAs or 2 expectorated sputum
(6) 2 mL of whole blood collected on heparin tubes for transaminases and CRP; 3 x 2 mL collected on EDTA tubes for CBC, CD4 count, and plasma biobank; 2.5 mL collected on PAXgene Blood RNA tubes for whole blood biobank. In children <18 months weighing <5 kg or children presenting with signs of severe anaemia (conjunctival or palmar pallor), plasma sample for biobank will not be collected
(7) Volume of blood draw must not exceed 3 ml/kg/visit and 7 ml/kg/6 weeks

7.4. Inclusion visit

The following procedures will be performed during the inclusion visit (Day 0 to Day 2):

- **Complete clinical evaluation**
  - Demographic information
  - Interview of parent/guardian and child on:
    - History and duration of symptoms including fever, cough, weight loss
    - History of household TB contact
    - History of past and current medication, including TB treatment and ART
    - History of chronic diseases and concomitant opportunistic infections
    - BCG immunization
  - Vital signs: respiratory rate, heart rate, temperature, weight, height, oxygen saturation
  - Physical examination including respiratory system exam, adenopathy, hepatosplenomegaly, assessment of nutritional status
  - Evaluation of the WHO clinical stage of HIV infection
- **Blood samples**
  - For full blood count for MLR calculation
  - For transaminases and C-reactive protein
  - For CD4 cells count
  - For biobanking (storage of frozen samples)
- **Imaging exams**
  - Abdominal ultrasonography to assess the presence of intra-abdominal lymphadenopathies included as one item of the PAANTHER TB treatment decision algorithm, and additional signs such as hepatomegaly, splenic or hepatic micro-abscesses, pericardial/pleural effusion, and/or ascites
  - Chest X-ray: standard anteroposterior and lateral view in children aged ≤ 5 years; standard anteroposterior view in children aged > 5 years
- **Bacteriological specimen collection**:

Initial bacteriological specimen collection will be done as soon as possible and within 3 days of inclusion, including:

- 1 NPA, collected by the nurse on the day of admission without prior nasal instillation (see Appendix 4)
- 1 stool sample, collected as soon as the child is able to produce stool
- 2 GAs or 2 expectorated sputum samples in children who are able to expectorate

The tolerability, feasibility, and adverse events linked to NPA specimen collection will be assessed by study nurses using qualitative and quantitative tools (Cf § 7.8 and 9.2).

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

Additionally, a urine sample will be collected for biobanking.

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

7.5. TB treatment decision using the algorithm

As soon as possible and during clinical, biological and radiological investigations, the clinician will make a diagnosis according to the PAANTHER TB-treatment decision algorithm (see Figure 1). Alternative diagnoses will be collected. The diagnosis visit is expected at the latest at day 1 or 2.

A flexibility to start TB treatment in children with a score <100 will be allowed in case of severe/life threatening condition. Children without such conditions not started on TB treatment will be reevaluated at the day 7 visit.

7.6. Treatments

- **TB treatment initiation**

TB treatment using 2HRZ(E)/4RH with new paediatric formulations according to National Guidelines will be initiated in children with a score≥100. Children with rifampicin resistance detected by Ultra will have culture and phenotypic drug sensibility testing (DST) performed on leftovers from NPAs and stool (and additional samples if needed), and will be started on empirical MDR-TB treatment. Arrangements will be made with NTPs for fast-track MDR-TB treatment initiation.

- **Isoniazid Preventive Therapy**

As recommended per WHO 2011 “Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings”, children not initiated on treatment as per PAANTHER TB-treatment decision algorithm will be initiated as soon as possible on IPT or an alternative treatment for latent TB infection (LTBI), such as 3RH [65], as locally available in-country.

- **Evaluation of adherence**

Adherence to the TB treatment will be evaluated by pill count except in case of Directly Observed Therapy.

- **Antiretroviral treatment**

ART will be started following national recommendations. As recommended by WHO, this should be done within 2 to 8 weeks of TB treatment initiation in children diagnosed with TB, and not later than 2 weeks in children with CD4 count below 50 cells/µL [66]. In children already on ART, a change of antiretroviral regimen will be considered if appropriate (e.g. switch from nevirapine or lopinavir to efavirenz, or double boosting of lopinavir if possible in accordance with National Guidelines).

- **Associated treatments**

Prophylactic treatments for opportunistic infections (Cotrimoxazole +/- Fluconazole) may be prescribed according to the recommendations of national programs, as well as Isoniazid prophylaxis in case TB diagnosis is ruled out by the clinician.

- **Treatment dispensing**

Anti-tuberculosis and antiretroviral drugs will be provided by national authorities. They will be dispensed in accordance with each hospital’s procedures. Their access will be free of charge for patients in accordance with the recommendations of national protocols.

7.7. Follow-up

All children will be followed-up for 6 months upon enrolment, with systematic study visits at day 15, month 1, 2, 3, and 6. In children not initiated on TB treatment as per the PAANTHER treatment decision algorithm, an extra visit will be performed at day 7 for an early detection of missed TB cases. The dates of each visit must comply with the provisional patient schedule generated from the date of inclusion of the child. For month 1 to month 6 visits, a flexibility of +/- 2 weeks around theoretical dates of protocol visits will be allowed to enable study visit to coincide with routine ART and HIV follow-up visits. If a child fails to attend a study follow-up visit, the clinical team on site will confidentially contact the parent(s)/guardian(s) and encourage/assist them to bring back the children for follow-up.

Each visit will comprise:

- Clinical evaluation: complete physical examination, vital signs, nutritional status
- Medical history since the last visit: any new clinical and AE with special attention to drug tolerance and TB paradoxical reaction
- CXR to detect new or aggravated lesions (at M2 and M6 final visit)
- Laboratory assessment: CBC, transaminases and CRP at M2 and M6 visit, CD4 count at M6 visit
- Evaluation of adherence to TB treatment, if initiated
- Any change of ART occurring during the study follow-up
- Study drug prescription and dispensation to cover time to the next follow-up visit

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

In children not initially diagnosed with TB, who show no clinical improvement or present additional signs of presumptive TB at day 7, 15, M1, M2, or M3, bacteriological specimen collection and Ultra testing will be repeated. These will include standard samples, i.e. GA or expectorated sputum. CXR could also be repeated, in addition to M2 and M6 visits, for children with clinical signs suggestive of TB at other scheduled or unscheduled study visit.

During the last follow-up visit at month 6, an assessment of the TB disease evolution and response to treatment will be made (cured, treatment failure, continuation of TB treatment, death, lost to follow-up).

7.8. Feasibility and tolerability assessment of NPA and stool collection procedures

- **Feasibility**

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

- **Tolerability**

Children's tolerability will be measured according to the level of discomfort/distress/pain felt assessed by the child, the parents/guardians and the nurses, using a set of validated tools. This evaluation will include: at child level, the Wong-Baker Face scale; at parent(s)/guardian(s) level, the Visual Analog Scale (evaluating child's tolerability); at nurse level, the FLACC (Face Legs Activity Cry Consolability) behavioural scale. These assessments will be conducted in a subset of children in all participating countries.

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

Whenever enrolled children become ill, they will have access to medical personnel during the business hours of their respective study site. Children will receive care in the form of consultations, inpatient day care and inpatient hospitalizations, depending on the severity of

disease. Care will be provided in accordance with national guidelines and study clinical SOPs. Data will be collected similarly to routine protocol visits.

7.10. Management in the case of selected adverse events

7.10.1. Management in the case of sample collection adverse events

Expected AEs occurring from NPA collection procedure include, by decreasing order of frequency: cough (this induced cough reflex is expected as it is the mechanism by which sample is obtained), nausea, local trauma/nose bleeding, sneezing, vomiting, and in rare cases dyspnea/low O2 saturation and heart rate deceleration <60/mm [32].

NPA will be performed by study nurses under SpO2 monitoring. In case of acute O_2 desaturation or respiratory distress occurring during sample collection, the procedure will be immediately interrupted and the child will be started on O_2 therapy. If a sample could not be obtained, a new attempt will be performed as soon as the child respiratory status allows for it.

Management in case of AEs will be detailed in specific SOPs.

7.10.2. Management in the case of treatment-limiting adverse events

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

7.11. Final study visit

For each enrolled child, the last protocol visit will occur 6 months after inclusion. Once completed the 6-month follow-up period, children will continue to benefit from regular treatment and care provided by National Programs.

7.12. End of the research

7.12.1. Definition

Each child will be followed up for 6 months after inclusion. The official end of the research, except in case of premature termination, is defined by the last visit of the last patient included in the study. At the end of the study, children will benefit from regular treatment and care provided by National Programs.

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 from the SAB, the IDMC, 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.

7.12.1. Withdrawal from the study

Withdrawal of the participant from the study may be at the initiative of the investigator or the participant himself (withdrawal of consent or premature exit).

An investigator may withdraw a subject from some or all study components at any time, at his/her discretion. Circumstances can include: when the child's safety may be compromised such as when experiencing adverse events, when the child's participation to the study prevents him/her to participate in another research which would be more beneficial, when the study is being closed by the sponsor related to increased risk to participants, or when the subject is

non-compliant with required study regimens or procedures. In such a case, the investigator will inform parent(s)/guardian(s) that the child has been withdrawn from participating in the study, and the reasons therefor.

The parent(s)/guardian(s) may decide to withdraw the child from the study at any time if they wish to, without any consequence on the quality of subsequent follow-up and care.

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

When parent(s)/guardian(s) who withdraw consent explicitly express the will that the child's data be removed from the database and the laboratory samples be destroyed, the study team will carry out such will. When parent(s)/guardian(s) 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.

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

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

7.12.2. Loss to follow-up

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

A child who has not withdrawn consent or transferred out, and who does not show up at the M6 visit is considered definitely lost to follow-up in the trial, unless he/she is known to be deceased. The date of lost to follow-up will be the date of his/her last contact with the trial team (either at the hospital, via telephone, or at home).

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

7.13. Post-study care conditions

At the end of the study, children will continue to receive care and treatment according to the conditions defined by their country authorities. Children with ongoing TB treatment at M6 will be followed-up and treated under the responsibility of NTPs. Until the ongoing TB episode is cured, the study investigators, in close collaboration with NTPs, will actively contribute to facilitate their access to the best available TB disease management.

7.14. Safety measures in the context of the Covid pandemic

- Specific SOPs for IPC measures were developed for each study activities based on risk assessment. CTUs were required to provide training on IPC to the study staff, and the TB-Speed project provided the following Personal Protective Equipment (PPE) to all sites per WHO recommendations
- For study staff, children and their parents: surgical masks, hand sanitizers

- For study staff (nurses, clinicians, laboratory technicians) only, depending on the procedure:
  - Droplets and contact precautions (clinical examination, blood, stool, and DBS collection): long sleeve gowns, gloves, googles, surgical masks
  - Aerosol-generating procedures (NPA and GA collection, samples processing at the laboratory): aprons or isolation gowns, long gloves, goggles and high filtration masks
- For specific activities:
  - Safety boxes to dispose contaminated sharps and waste appropriately
  - Triple packaging boxes to ensure biosafety during sample transportation

8. LABORATORY AND RADIOLOGIC EVALUATIONS

8.1. Biological specimen collection

NPAs, stool and blood samples are collected at inclusion (see Table 5) for laboratory tests performed on-site or at the country reference laboratory, as well as for biobanking (see chapter 8.3).

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

- **Nasopharyngeal aspirates**

The collection of the contents of the oropharynx is done by mechanical suction through a graduated suction tube inserted into the nostril while the child is seated (See appendix 4). NPAs will be performed under SpO2 monitoring.

- **Stool sample**

Stools cannot be tested with the GeneXpert device without prior processing to avoid indeterminate or falsely negative results due to the presence of PCR inhibitors. In this study, stool processing will be performed using the flotation method based on Sheather's sucrose solution previously used in the PAANTHER 01 study (Appendix 5). Stool processing optimization will be largely developed as a separate work-package of the TB-Speed project (Output 4). It will assess centrifugation-free methods for stool processing hence generating evidence on the diagnostic value of stools as alternative specimen for TB diagnosis in resource-limited settings.

- **Gastric aspirates**

The collection of gastric contents will be done in the morning through a nasogastric tube in a child fasting since midnight the previous day, in supine position, before any rise in case of hospitalization and after 1 hour of supine for children seen in consultation. If it is impossible to collect at least 5 ml of gastric fluid, 10 to 20 ml of sterile water will be injected through the catheter and aspirated.

- **Expectorated sputum**

The sputum collection will be attempted in children aged 5 years and more, able to self-expectorate. A first sputum collection will be performed on the spot after NPA collection on the day of inclusion. If successful a second sample will be collected the following day, early morning after wake up. If an expectorated sputum cannot be obtained, it will be replaced by a GA.

- **Urine samples**

A fresh urine sample (20 mL approximately) will be collected at inclusion in a sterile urine container.

- **Blood samples**

Table 6: summary of blood samples collected according to age and weight

	Tube	**Volume**	**Inclusion D0**	**Month 2** *(+/-2 weeks)*	**Month 6** *(+/-2 weeks)*
Complete blood count	EDTA	2 mL	X	X	X
Transaminases + CRP	Heparin	2 mL	X	X	X
CD4 count	EDTA	2 mL	X		X
Biobank:					
- Whole blood	PAXgene	2,5 mL	X		
- Plasma	EDTA	2 mL	(X)*		
Number of tubes collected			5 (4)*	2	3
Maximal volume of blood collected			10,5 mL (8,5 mL)*	4 mL	6 mL

** Plasma sample for biobank not collected in children weighing <5 kg or those presenting with signs suggestive of severe anaemia (conjunctival or palmar pallor or haemoglobin level below 6.5 g/dL). Volume of blood draw must not exceed 3 mL/kg/visit and 7 mL/kg/6 weeks.*

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

8.2. Laboratory assessment

8.2.1. TB bacteriological tests

The Ultra assay will be performed within 3 days of inclusion (D0-D2) on the following samples:

- one untreated NPA
- one stool sample with prior processing
- two GAs (or expectorated sputum)

The Ultra assay will be done at the hospital laboratory on standard G4 platforms.

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

Results will be interpreted as follows:

- In case of positive result for the presence of MTB (including "trace call" positive result) on any sample, the global result will be given as "detected".
- In case of negativity of Ultra performed on all samples, the global result will be given as "not detected".
- In case of an invalid or error result for MTB detection, the test will be repeated if sample volumes allow for it.

  If volumes are insufficient to repeat testing, no additional sample will be collected unless the child has symptoms or signs suggestive of TB (persistent cough or fever for more than 2 weeks, and failure of antibiotics treatment). The child will be evaluated for TB using routine sample collection (gastric aspirate or other sample according to country practices) for Ultra testing, as recommended by WHO.
- In case of rifampicin resistance detected on one or more sample, the result will be given as "MTB detected, rifampicin resistance detected". Otherwise the result will be given

as "MTB detected, rifampicin resistance not detected (or indeterminate)". Children with rifampicin resistance detected by Ultra will have culture and phenotypic DST performed on leftovers from NPAs and stool (and additional samples if needed), and will be started on empirical MDR-TB treatment.

Ultra results will be communicated by the laboratory to the nurse or clinician as soon as the result is available.

Leftover of NPAs, stool samples, and GA or sputum will be kept at -80°C for future ancillary/sub-studies.

8.2.2. Liquid culture and detection of TB drug resistance

Mycobacterial culture will be performed on two GAs or sputum samples.

Culture and DST will be done at the country Central Laboratory level.

Mycobacterial culture will be done in liquid medium (MGIT) by an automated method (BACTEC 960), and on solid media (Lowenstein Jensen), if available.

Identification of mycobacteria will be done by the molecular method Gen-Probe®, or Niacin test, or MPT64 antigen test depending on availability at the laboratory level. Detection of TB drug resistance will be performed using first-line DST on liquid media (MGIT) or solid media or line probe assays (LPA).

8.2.3. Blood tests

Blood analyses including leucocyte differential counts, red blood cells values, CRP, and transaminases measurements will be performed using standard procedures usually implemented on sites and local reference ranges.

MLR will be calculated secondarily as the quotient of absolute monocyte and lymphocyte counts.

8.2.4. Laboratory quality control

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

Internal quality control (QC) should be routinely performed for Ultra testing, including calibration tests and procedures provided by the manufacturer. Internal QC results and logs should be available for monitoring.Frozen samples

Leftovers from NPA, stool, GAs or sputum, as well as whole blood, plasma and urine samples will be frozen and stored at the Central Laboratory level.

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

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

8.2.5. Type of samples and purpose

- **NPA, stool, GA, sputum**

Bacteriological analyses such as culture and mycobacterial antigens assays could be performed retrospectively on NPA, stool, GAs or sputum leftovers.

- **Urine**

Urine samples could be used for evaluation of biomarkers such as LAM, including the novel Fujifilm SILVAMP TB LAM assay.

- **Whole blood**

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

Whole blood could be used for potential genomics or transcriptomics analyses to discriminate between TB and other diseases, including mRNA transcripts and micro RNAs, based on state-of-the-art subsequent to the study.

- **Plasma**

A 1.5 mL plasma aliquot will be collected from ETDA tubes used for whole blood collection at inclusion.

The plasma sample for biobank will not be collected in children weighing <5 kg or presenting with signs suggestive of severe anaemia (conjunctival or palmar pallor or haemoglobin level below 6.5 g/dL).

Plasma samples (and serum leftover) could be used to further characterize the proteomic, metabolic and immunologic profile of HIV-infected children with presumptive TB, as well as pharmacokinetics/pharmacodynamics studies of TB drugs in this population.

8.2.6. Storage

During the study, biological samples will be stored at -80°C at the country reference laboratory. 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.

8.3. Radiological assessment

CXR is performed systematically at the inclusion, M2 and M6 final study visit (see Table 5), as well as any time during follow-up in children presenting with clinical signs suggestive of TB.

A 2-view chest radiography (anteroposterior and lateral for children aged less than 5 years and posteroanterior only for older children) will be performed using standard analogue X-ray machines with digital plates, or digitalized radiography machines where possible.

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

For the purpose of TB reference diagnosis classification, CXRs will be reviewed independently by two readers blinded to clinical and biological data to identify CXR lesions consistent with TB, as proposed in the Clinical Case Definition for Classification of Intrathoracic Tuberculosis in Children [59]. Discordant opinions will be resolved by a third reader.

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.

Example: a SAE which could be associated with the study procedures and which could modify the conduct of the trial 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 [67].

- **Causality**

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

9.2. Expected adverse events related to the study intervention

Expected AEs occurring from NPA collection procedure include, by decreasing order of frequency: cough (this induced cough reflex is expected as it is the mechanism by which sample is obtained), nausea, local trauma/nose bleeding, sneezing, vomiting, and in rare cases dyspnea/low O2 saturation and heart rate deceleration <60/mm [32].

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 [68].

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

9.3. Reporting of adverse events

In order to document safety and morbidity, all AEs will be reported in the CRF. This includes AEs occurring as a consequence of NPA collection, drug-induced toxicity for ART or TB treatment, opportunistic infections, and IRIS.

9.4. Notification of serious adverse events

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

- Death
- Life-threatening AEs, defined as grade 4 clinical AEs, excluding asymptomatic biological AEs of grade 4
- SAE related to NPA collection.

9.5. Responsibilities of the investigators

The investigators are responsible for:

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

The assessment on expectedness will be done by the sponsor.

SAEs, as defined above, should be reported as soon as they are known to the country CTU according to the last updated SOPs. 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

9.6. Responsibilities of the sponsor

9.6.1. SAE Recording and assessment

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

The sponsor is responsible for the assessment of the causality of the SAE in 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 (SAR).

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

9.6.2. New fact reporting

When a new event is likely to affect the safety of participants, the sponsor and the investigator 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 collected

10.1.1. Individual patient data

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

- Individual identifiers: month and year of birth, sex
- Anthropometric and clinical data: weight, height, vital signs, symptoms, treatments and adherence, AEs
- Data on the tolerability of NPA collection (qualitative and quantitative)
- Radiological and ultrasonography data: digital images and interpretation
- Laboratory data: mycobacterial culture and Ultra assay, blood tests
- Samples collected for biobanking
- TB treatment, if initiated
- Comorbidity management: antiretroviral treatment, treatment of other opportunistic infections, other
- Outcome data: end of study status or early study termination (death, lost to follow-up, withdrawal)

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

10.1.2. Cost data

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

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

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

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

10.2. Definition of source data

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

The following information should be collected from source medical records filled by site physicians:

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

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

In addition to the source medical records, radiological data will be collected as digital imaging and communications files (.dicom files). CXRs interpretation will be directly reported in the patient's CRF using standardized forms developed as part of the capacity building component of the TB-Speed Output 1.

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

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

10.3. Electronic data entry

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

10.3.1. eCRF

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

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

Design and conception of the eCRF will be done by the international trial manager in close collaboration with the international data manager.

10.3.2. Data hosting

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

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

10.3.3. Data security

The eCRF will be accessible 24/24h 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).

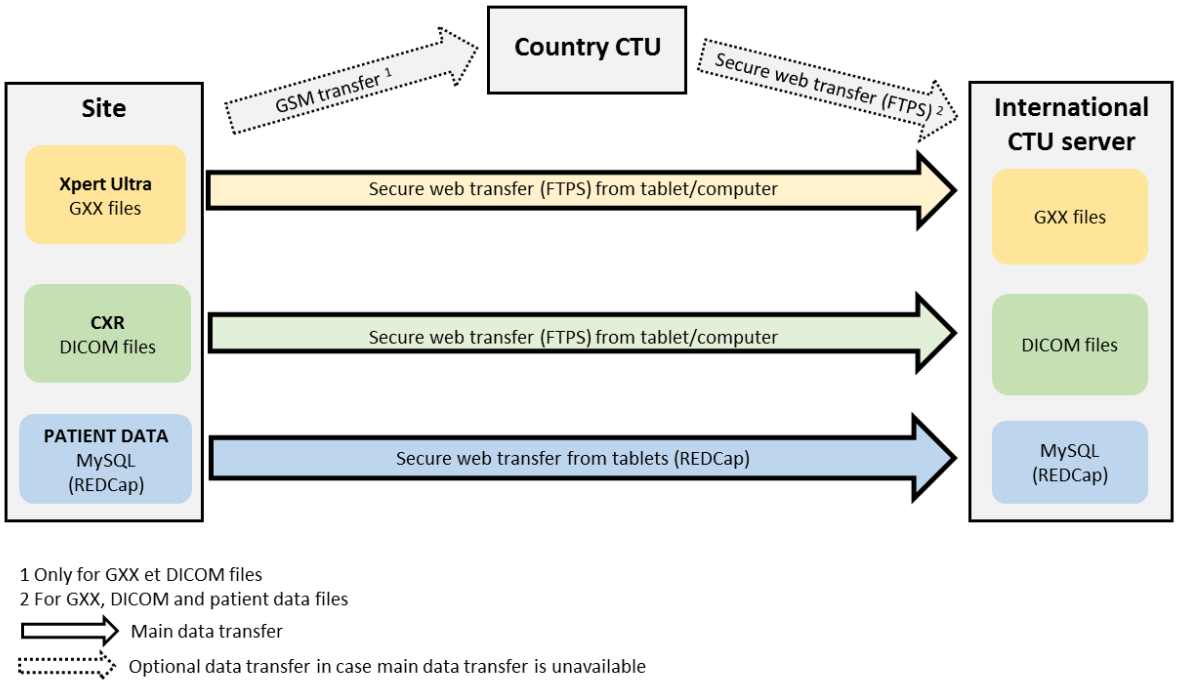
The server hosted at UBx will be saved every weekend on a hard drive, and send to bands (rotation frequency of the bands will be every five weeks). The database will be saved incrementally on a hard drive every working days.

10.3.4. Data entry

Field-based users will be able to access REDCap either through a classical Internet-connected tablet or computer, or through the REDCap mobile App application. The mobile App also enables offline data entry through a tablet or an Android mobile phone. In such a case, the tablet or mobile phone will be brought by the Clinical Research Assistant (CRA) to the country CTU and further synchronized with the central database once connected to the Internet (Figure 3).

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

Figure 3: Secure data flow



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 QC. The investigator is responsible for ensuring that all sections in the eCRF are completed correctly and that entries can be verified against source data. If the investigator authorizes other persons in their staff to make entries on the eCRF, the names, positions, and signatures must be documented in writing. eCRF must be completed during/after each study visit. Any person entering data in the eCRF must be trained beforehand and appointed to do this task.

10.3.5. Data coding

The Anatomical Therapeutic Chemical (ATC) system will be used for drug classification and coding. As part of safety monitoring, AEs will be coded using the Medical Dictionary for Drug

Regulatory Affairs (MedDRA, version 17.1). Coding will be performed by country CTUs based at TB-Speed consortium members institutions.

10.3.6. Data transfer

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

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

In the context of the Covid pandemic, due to travel restrictions, remote site monitoring visits might be conducted by the international CTU. Where needed, pseudonymized source documents will be forwarded by the country CRA through a ftps for monitoring purpose only; source documents will be destroyed afterwards.

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

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

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

The investigator, co-investigators, head of laboratory must allow access to relevant hospital, laboratory or clinical records, to confirm their consistency with the CRF entries. All research staff working in the study, including study nurses, national CTU team (PMs, CRAs), PIs, international coordination team (CRA, Trial Manager, Coordinating Investigators) will sign a confidentiality agreement with regards to access to individual patient data and medical records.

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

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

10.5. Lenght 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 French regulations 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. Essential study documents will be retained at the coordinating centre for fifteen years after study completion.

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

10.6. Study documents archiving conditions and management

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

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

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

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

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

11. STATISTICAL DATA ANALYSIS

11.1. Statistical analysis manager

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

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

11.2. Description of the statistical analysis plan

11.2.1. Analysis of the primary endpoint and algorithm validation

We will evaluate the proportion of missed TB cases in HIV-infected children with presumptive TB not initiated on treatment as per the PAANTHER TB treatment decision algorithm (i.e. false negative cases). We will then estimate the NPV (NPV=1-proportion of false negative) of the PAANTHER TB treatment decision algorithm and its 95% confidence interval. The NPV will be compared to the 75% minimal acceptable lower confidence interval limit.

The algorithm will be considered validated if its NPV remains significantly higher than 75% if the prevalence is at 50%, corresponding to a sensitivity significantly higher than 80%. If a lower diagnostic accuracy is found, we will update the existing prediction model and adjust or recalibrate it to the validation sample.

11.2.2. Intermediate analyses

Intermediate analyses will be performed every 4 to 6 months and submitted confidentially to the IDMC (see chapter 13.2). These will include the proportion of missed TB cases in the group of children not initiated on TB treatment as per the PAANTHER TB treatment decision

algorithm, as well as global morbidity and mortality data in this group. The first intermediate analysis will be performed after 4 months of enrolment. The timing of the following intermediate analyses will be decided with the IDMC.

11.2.3. Analysis of secondary endpoints

The median time to TB treatment decision and interquartile range, and the proportion of children with presumptive TB having completed the PAANTHER TB treatment decision algorithm will be estimated.

We will assess the proportion of HIV-infected children with unlikely TB among those initiated on treatment as per the PAANTHER TB treatment decision algorithm (false positive cases) identified by the international Expert Committee. We will estimate the positive predictive value (PPV) and its 95% confidence intervall. Once PPV and NPV are assessed, we will estimate sensitivity and specificity of the algorithm/scoring system and TB prevalence.

Discrimination and calibration, which are key measures of a model's predictive performance, will be quantified for the original model and the model incorporating new predictors. To avoid adding optimism, the original model will not be redeveloped. Discrimination of the model, measured by the C statistic, or area under the receiver operating characteristic (AUROC) curve, will be estimated for the original model and parameters with and without potential new predictors such as MLR and CRP. AUCs of the models will be compared using bootstrapping technique. Calibration examines the agreement between predicted and observed risk of disease and can be quantified by a calibration slope and the expected/observed (E/O) statistic (ratio of the total expected to have disease to the total observed with disease). TB diagnosis as assessed through the Expert Committee review of false negative and false positive cases will be used as reference standard.

Sensitivity analyses will be conducted to assess impact of age, ART, CD4, and nutritional status on the performance of the score. Furthermore, the score will be assessed country-wise to identify potential heterogeneity in score performances between countries.

If a lower predictive accuracy is found, we will update the existing prediction model and adjust or recalibrate it to the validation sample. As a result, the updated model combining the information from the original PAANTHER model with new information from the TB-Speed study, will probably have improved transportability to other populations.

Sensitivity, specificity, and predictive values of Ultra in NPA and stool will be measured for each specimen independently and for the combination of the 2 specimens against mycobacterial culture.

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

11.2.4. Cost-effectiveness analyses

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

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

Sensitivity analyses will be conducted to assess uncertainties around the estimates and the robustness of our findings. Variation of parameters such as TB prevalence, TB incidence and

costs will help to simulate different scenarios of implementing the TB-Speed approach, adapted to the countries' specific contexts. The latter will be important for the generalization of the results and to inform more general TB guidelines.

12. COMMUNICATION AND PUBLICATION POLICY

12.1. Findings publication procedure

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

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

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

" / Ethics statement / * / This study is part of **** 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 trials registry (**** CT XXXX **)./ Funded by the Unitaid and 5% initiative/. All study participants gave their informed written consent to participation, in line with ethical guidelines.*

12.2. Procedure for writing up the final report

The international CTU will establish the final report of the study as well as summary report within a year after the end date of the study, i.e. the last visit of the last patient. The report and its summary are established according to ICH recommendations (International Conference for Harmonisation – ICH Topic E3 – Structure and Content of Clinical Study Reports CPMP/ICH/137/95. Accessible at: https://database.ich.org/sites/default/files/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 authorities of each country involved 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 site investigator or, where appropriate, the qualified person who represents it.

During the study, any clinically significant abnormality detected in the examination or test results will be communicated 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 (OSC) is the operational team that will undertake the day-to-day decisions related to study implementation in each country, based on the model applied in all clinical trials currently managed by the IDLIC team at UBx.

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

The OSC will be in charge of the reporting and formulation of proposals for the Executive Committee regarding work-plan and budget reallocation and execution of the decisions taken by the project coordination committee (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 SAB is an expert consultative committee providing scientific advice to the project management teams. It gives input on the relevance and scientific validity of the project design and implementation, monitors progress and ensure scientific and ethical integrity of the project.

- **Role**

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

- The relevance of the project objectives within the context of the paediatric TB research landscape;
- The appropriateness of designs and methods of the proposed studies (outputs) to the research questions;
- The scientific strength, safety and feasibility to meet the stated objectives of the project;
- The complementarity of the project with other ongoing or planned external trials/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), or based on the reports from the IDMC;
- The use of data and biological samples, and their utilisation for analyses not listed in the protocol;
- Any publication ahead of submission to international peer-reviewed scientific journals;
- Confidential scientific reports transferred to WHO for consideration and inclusion of outcomes into development and update of WHO normative guidance.

➢ **Composition and appointment**

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

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

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

13.3. Independent Data Monitoring Committee

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

It will review regular 4 to 6-monthly reports on the primary endpoint, i.e. incident TB cases in children not initiated on TB treatment as per the PAANTHER TB treatment decision algorithm. These reports will include a brief clinical description as well as individual outcomes for each child. The report will include global morbidity and mortality data in this group. They will oversee global safety and provide advice on the continuation of the study or adaptation of the protocol in case of safety concerns in this group of children.

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

IDMC members will be selected in collaboration between the coordinating investigators and the sponsor before to the start of the study. All IDMC members must be free from any direct involvement in the study. Any competing interests, both real and potential, must be declared. The list of members will be provided to relevant Ethics Committees when the IDMC is constituted, prior to launching the study. The IDMC will meet before the first inclusion, and every 4 to 6 months until the end of the study. The sponsor, the SAB or the IDMC may request to increase the frequency of these meetings.

13.4. Endpoint Review Committee

In each country, an endpoint review committee will consist of: (i) the country PI; (ii) one or several other adequately trained physicians, selected by the country PI; (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 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 PIs for additional information whenever needed.

13.5. Expert Committee

At the international level, the expert committee will consist of 3 to 4 independent experts not involved in the management of the study, nor members of the SAB. The expert committee will clinically validate final TB diagnosis. They will have access to CXR external reviews.

13.6. Country Project Committee

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

13.7. 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 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, Epicentre (Technical Partner) in Uganda, University of Zambia in Zambia, and Instituto Nacional de Saude in Mozambique. 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 trial manager, who will work in close collaboration with the international trial (output) manager, and monitored by a country CRA who will work in close collaboration with the international CRA.

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

14. CONFIDENTIALITY

14.1. Procedure for respecting the confidentiality of participants

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

The study ID assignment log (only in paper form) will be kept shut-away on site under the responsibility of the investigator. Direct personal identifiers (including names, dates, demographic and contact information) will only be made available to those whose job within the operational activities of the trial makes having such information absolutely essential, subject to signature of a confidential agreement. This includes routine hospital staff involved in the child clinical management, as well as research study staff when they may be visiting the

study site for monitoring, coordination, or event validation purposes. They will not have access to direct personal identifiers outside of the trial site premises.

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

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

15.1.1. Risks

HIV infected children with presumptive TB have a high risk of death independent from the study assessment. Risks due to para-clinical investigations are well known and will be explained to the participants. The potential risks of NPA and blood draw will be limited by insuring they are performed by trained nurses with appropriate supplies and standardized procedures detailed in the study SOP.

- NPA usually causes a reactive cough; epistaxis and discomfort can also occur;
- The NPA does not increase the risk of bronchospasm and should not increase risk of hypoxemia. However sample collection will always be done with access to salbutamol and oxygen..
- 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 antiretroviral treatment programs.

15.1.2. Benefits

This study is providing the following 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 thanks to timely TB treatment initiation as recommended by the national health authorities;

- in case of Rifampicin resistance detected by Ultra, an access to drug susceptibility testing for *Mycobacterium tuberculosis* and the opportunity to receive an adapted treatment according to these results, including access to MDR-TB treatment;
- investigation fees per protocol paid by the study (clinical examinations, laboratory, radiology, hospitalization and transportation fees).

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

15.2. Regulatory provisions

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

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

This 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 Ethics Review Committee, and to the Inserm Ethics Evaluation Committee.

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

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

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

15.4. Additional approvals

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

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

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

15.5. Data protection

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

15.6. Insurance

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

Inserm has taken out a civil liability insurance for the entire duration of the study in accordance with the French legal provisions and regulations on research.

The certificate of insurance relating to this Protocol constitutes Appendix 7.

15.7. Participants amenities

Study investigators will ensure that each subject receives the following benefits throughout the study: reimbursement of transportation fees to the hospital, medical exams, tests, and medications related to the study if not covered by the national health system, as well as hospital stays whenever prescribed or approved by the country trial investigators, within the limits of the available budget.

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 1996) to guarantee the quality of the research and safeguard the health and the rights of the patient. The investigator is above all the guarantor of the quality of the study progress.

The monitoring plan is established by the international coordinating CTU with the sponsor and the coordinating investigators before the start of the study. Key data subject to source data verification are identified in the monitoring plan. Procedures for monitoring will be detailed in 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 data collected in the country. The international coordinating CTU, based at the UBx IDLIC/Mereva team, coordinates and supervises monitoring performed by country CTUs and performs targeted monitoring.

16.2.2. Monitoring by the country CTU

A CRA will regularly visit each implementing site during the all study period, including at set-up, implementation, and at the end of the study. During these visits, the country CTU will be in charge of the following, according to the monitoring plan:

- Check adherence to the protocol, SOPs and Good Clinical Practice, including eligibility criteria, informed consent, patient schedule;
- establish and maintain the investigator's Trial Master Files (TMF) up-to-date;
- check the completeness and the accuracy of patient key data on the CRF (source data verification);
- verify that confidentiality of data is fully respected;
- verify SAEs reporting, documentation and follow-up, and send the forms to the sponsor's pharmacovigilance and to the international coordinating CTU;
- evaluate the progress of patient enrolment;
- check the quality management of samples and biobank;
- ensure that quality controls and quality management for laboratory assessments are implemented;
- monitor CXR interpretation performance;
- follow-up with investigator sites centralized correction requests sent by the international coordinating CTU.

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

Furthermore, the country CRA will also hold regular meetings with the study staff at each sites to discuss any patient file international and country CTUs deemed 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

During the study set-up process, an opening visit will be performed for each site by the international CRA and the project co-investigator on behalf of the sponsor. Only upon completion of equipment, training, ethical and regulatory approvals (including civil liability insurance) will a site be authorized to start enrolling patients.

A member of the international coordinating CTU will visit each study site at least once a year. The purpose of these visits will be to review with the country CTU advances and issues with the local monitoring and data management process, as well as perform a targeted/random monitoring of a limited number of files.

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

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

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

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

A closing visit will be carried out at the end of the study by the international CRA.

In the context of the Covid pandemic, due to travel restrictions, remote site monitoring visits might be conducted by the international CTU. Where needed, pseudonymized source documents will be forwarded by the country CRA through a ftps for monitoring purpose only; source documents will be destroyed afterwards.

16.2.4. Direct access to source data

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

16.3. Audits/inspections

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

17. 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 study 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 during the TB-Speed HIV study. In addition, each country CTU will be provided with a deep freezer to ensure enough space and good condition for the storage of study samples.

Biological samples will be retained for 10 years after study completion, unless objection expressed by parent(s)/guardian(s). Destruction of leftovers or unused samples will be undertaken by country central laboratories in accordance with local regulations relating to the disposal of biological specimens. In the event of samples shipped to external laboratories, those will be disposed of in accordance with applicable regulations in both recipient and supplier countries.

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.

17.2.1. 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.

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

Biobank samples will be collected from children benefitting from a 6-months 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 AMENDEMENTS TO THE PROTOCOL

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

These requirements for approval should in no way prevent any immediate action from being taken by the investigators or by the 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 summary

Clinical trial ID number:

Title: Validation of a tuberculosis treatment decision algorithm in HIV-infected children

Short title: TB-Speed HIV

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

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

Primary Objective:
To evaluate the proportion of missed TB cases in HIV-infected children with presumptive TB not initiated on treatment as per the PAANTHER TB treatment decision algorithm (false negative cases)

Secondary Objectives:
1. To evaluate the feasibility of the PAANTHER TB treatment decision algorithm in HIV-infected children with presumptive TB
2. To assess the proportion of HIV-infected children with unlikely TB among those initiated on treatment as per the PAANTHER TB treatment decision algorithm
3. In HIV-infected children with presumptive TB, including comparison of those initiated and those not initiated on TB treatment as per the PAANTHER TB treatment decision algorithm, to assess:
   a. Incidence of morbidity (drug toxicity, opportunistic infections, IRIS) and mortality during 6 months after enrolment
   b. Time to ART initiation in those who are ART-naïve
   c. Immunologic evolution 6 months after enrolment
4. To assess TB treatment outcomes in HIV-infected children initiated on treatment as per the PAANTHER TB treatment decision algorithm
5. To assess the feasibility of IPT initiation in HIV-infected children not initiated on treatment as per the PAANTHER TB treatment decision algorithm to detect TB
6. To assess the performance of the monocyte-to-lymphocyte ratio (MLR) and the C-reactive protein (CRP) and their potential added value in the PAANTHER score and algorithm
7. To evaluate the diagnostic performance of Ultra performed on one nasopharyngeal aspirate (NPA) and one stool sample against mycobacterial culture performed on standard samples (gastric aspirate in younger children or expectorated sputum in older children) in HIV-infected children
8. To assess the feasibility of stool sample collection, and the feasibility, safety, and tolerability of NPA collection in HIV-infected children
9. To assess the performance of the PAANTHER algorithm for TB diagnosis across various ages, CD4 counts, nutritional statuses and timings of ART initiation
10.To evaluate the cost effectiveness of implementing the PAANTHER TB treatment decision algorithm compared to the estimated effect of the standard of care in children with HIV

Primary endpoint:
Proportion of missed TB cases (false negative cases) in children not initiated on treatment as per the PAANTHER TB treatment decision algorithm

Secondary endpoints:
1.Time to final TB-treatment decision and proportion of children with presumptive TB having completed the PAANTHER TB treatment decision algorithm.
   The algorithm will be considered completed if a decision to initiate TB treatment has been taken at any step of the algorithm or if TB has been excluded after systematic evaluation, and all steps planned in the algorithm have been implemented.
2. Proportion of cases considered as unlikely TB by the Expert Committee in those initiated on treatment as per the PAANTHER TB treatment decision algorithm (false positive)
3. a. Morbidity (drug-induced toxicity – ART and TB treatment-related, opportunistic infections, IRIS) with or without TB treatment, and mortality at 6 months;
   b. Time to ART initiation in ART-naïve children;
   c. CD4 (absolute count and %) gain.
4. Weight gain at 6 months (absolute value and percentage of body weight), TB symptoms resolution and outcomes in children on TB treatment
5. Time to IPT initiation and proportion of children initiated on IPT in those not initiated on treatment as per PAANTHER TB treatment decision algorithm

6. Discrimination (area under the receiver-operating-characteristic curves [AUROC]) and calibration measures of the PAANTHER prediction model including or not MLR and CRP, against the TB composite reference standard as defined by the Expert Committee
7. Proportion of NPAs (or sputum) and stool samples with positive TB detection using Ultra
8. Feasibility defined as proportion of children with NPA and stool samples collected as per study protocol;

Safety defined as proportion of NPA with AEs (vomiting, nose bleeding, low oxygen saturation, respiratory distress) occurring during NPA, and

Tolerability defined as discomfort/pain/distress experienced by the child during NPA as assessed by the child (Wong-Baker face scale), by the parents (visual analog scale), by the nurses (FLACC behavioural scale) (quantitative assessment) in a subset of children
9. Diagnostic accuracy (false negative and false positive rates i.e. NPV and PPV and corresponding sensitivity and positivity) of the PAANTHER algorithm in the different subgroups in terms of age, CD4 counts, nutritional statuses, and timings of ART initiation (on/off ART)
10. Incremental cost-effectiveness ratio (ICER)

Study design:
Prospective, multicentre management study evaluating the safety and feasibility of the recently proposed PAANTHER TB treatment decision algorithm for HIV-infected children with presumptive TB.
Implementing sites: 7 tertiary level hospitals in 4 countries with high and very high TB incidence (Côte d'Ivoire, Uganda, Mozambique, and Zambia) which have not participated in the PAANTHER development study.
Methodology:

- The PAANTHER algorithm will be used for TB treatment decision by site clinicians in all children enrolled in the study.
- Validation of the algorithm will be performed by evaluating the proportion of missed TB cases in children not initiated on treatment as per PAANTHER TB treatment decision algorithm.
- The safety of this strategy will be carefully assessed through review of regular safety reports every 4 to 6 months during study conduct by the Independent Data Monitoring Committee.
- Occurrence of algorithm failures in terms of missed TB cases (i.e. false negatives) and cases with unlikely TB among those initiated on TB treatment as per the algorithm (i.e. false positives) will enable to estimate the negative and positive predictive values of the algorithm.
- A centralized international Expert Committee will clinically review and validate TB diagnosis in children. This will enable to assess the added value of new markers (MLR and CRP) and, if need be, to propose a new version of the score based on an optimised predicted probability.

Follow-up: all children will be followed-up for 6 month upon enrolment, with systematic study visits at day 7, day 15, month 1, 2, 3, and 6.

Sample size: 550 HIV-infected children aged <15 years with clinically suspected (presumptive) TB

Inclusion criteria:
1. Children aged 1 month to 14 years
2. Documented HIV-infection (i.e., confirmed before entry into the study)
3. Presumptive TB based on at least one criteria among the following:
    - Persistent cough for more than 2 weeks
    - Persistent fever for more than 2 weeks
    - Recent failure to thrive (documented clear deviation from a previous growth trajectory in the last 3 months or Z score weight/age < 2)
    - Failure of broad spectrum antibiotics for treatment of pneumonia
    - Suggestive CXR features

   OR
   History of contact with a TB case and any of the symptoms listed under point 3 with a shorter duration (< 2 weeks)

4. Informed consent signed by parent/guardian

Non-inclusion criteria: Ongoing TB treatment or history of intake of anti-TB drugs in the last 3 months (isoniazid alone or rifampin/isoniazid for preventive therapy is not an exclusion criteria)

Diagnostic strategy:
The PAANTHER algorithm and prediction score was designed as a guiding tool for empirical TB treatment decision in HIV-infected children with presumptive TB (see Figure 1 and Appendix 2). The proposed score includes:

- History of close contact with a smear+ TB case

- Suggestive TB symptoms (prolonged fever, unremitting cough, hemoptysis, weight loss in the past 4 weeks)
- Tachycardia
- Chest radiography features (miliary, alveolar opacities, lymph nodes)
- Abdominal ultrasound features (abdominal lymphadenopathy)
- Xpert MTB/RIF assay which will be replaced by Ultra

A score of >100 is highly predictive of TB. In children with a score >100, anti-TB treatment will be initiated immediately. In children with a score of <100, TB treatment will not be initiated except in those with severe/life-threatening conditions for whom TB treatment decision could be made at the discretion of the site clinician. TB could be definitely ruled out after further assessment and decision made as to what treatment the children should receive in accordance with existing national protocols, along with clinical follow-up.

Trial agenda:

- First inclusion: 4th quarter 2019
- Inclusion period: 27 months
- Duration of follow-up for each participant once enrolled: 6 months
- Last visit of the last patient: 2nd quarter 2021
- Overall duration of the study (from the first inclusion to the last visit): 33 months

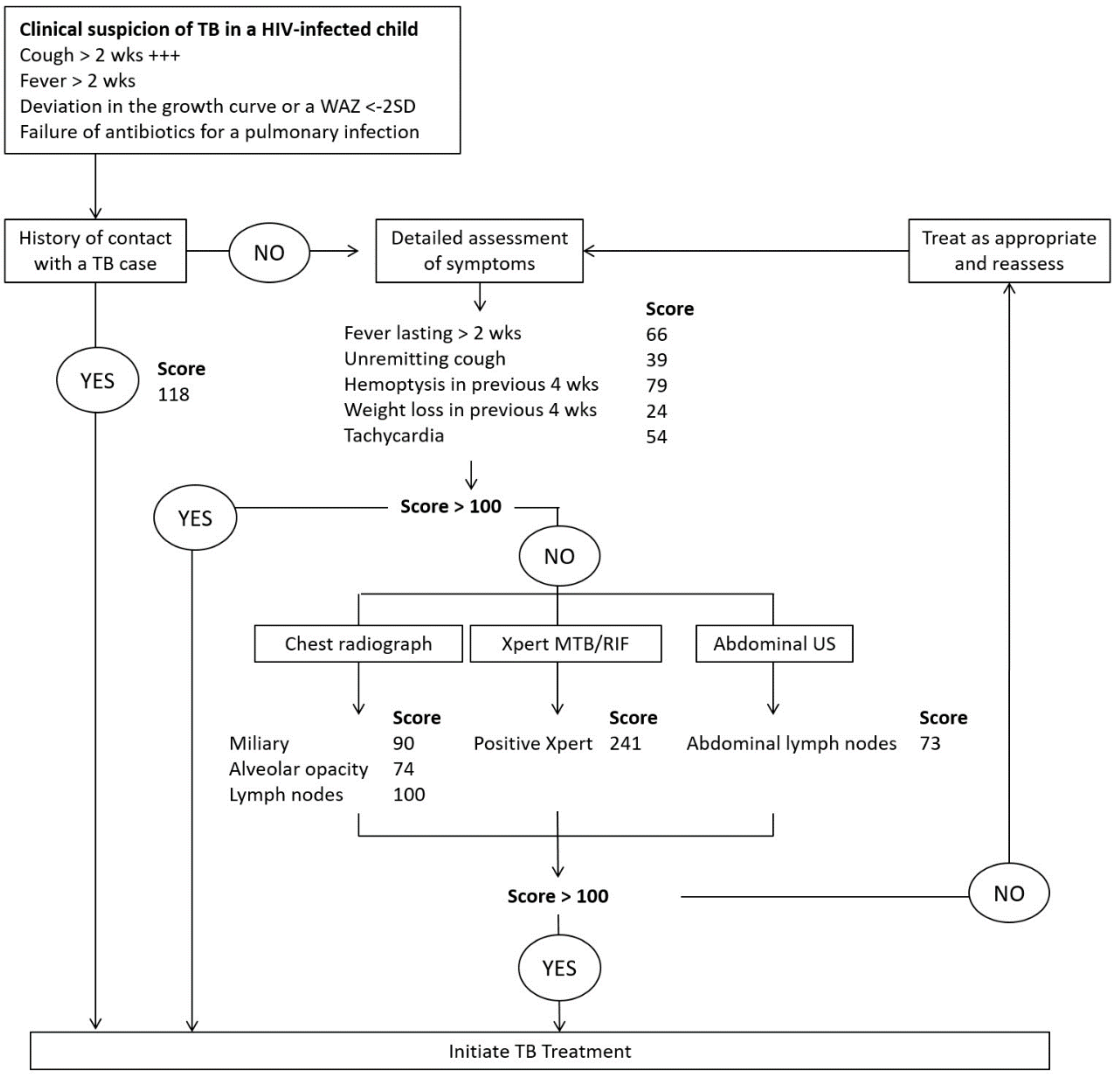
Analysis of the primary endpoint and validation of the algorithm

We will evaluate the proportion of missed TB cases in HIV-infected children with presumptive TB not initiated on treatment as per the PAANTHER TB treatment decision algorithm (i.e. false negative cases). We will then estimate the NPV of the PAANTHER TB treatment decision algorithm and its 95% confidence interval. The NPV will be compared to the 75% minimal acceptable lower confidence interval limit. The algorithm will be considered validated if its NPV remains significantly higher than 75% if the prevalence is at 50%, corresponding to a sensitivity significantly higher than 80%. If a lower diagnostic accuracy is found, we will update the existing prediction model and adjust or recalibrate it to the validation sample.

Expected results:

The main expected benefit of the PAANTHER algorithm is a quick TB treatment decision. Rapid initiation of TB treatment in children with both confirmed and unconfirmed TB will lead to improved health outcomes including reduced risk of death. If validated, the PAANTHER TB treatment decision algorithm could be systematically implemented at the primary or secondary health care level, where screening recommended by the WHO Three I's strategy is implemented and HIV-infected children may present with signs of presumptive TB.

20.2. APPENDIX 2: The PAANTHER algorithm



20.3. APPENDIX 3: Description of study sites

	COTE D'IVOIRE		
SITE		Cocody	Treichville
CITY		Abidjan – capital city	Abidjan – capital city
LEVEL		Teaching Hospital	Teaching Hospital
CATCHMENT AREA (population)		6 000 000 (Abidjan) 447 055 (Cocody area)	6 000 000 (Abidjan) 137 000 (Treichville area)
NB PAEDIATRIC BEDS		47	86
NB CHILDREN ADMITTED		1 394 in 2017	4 520 in 2017
SPECIALIZED WARDS		HIV treatment centre Nutritional Rehabilitation Unit	HIV treatment centre Nutritional Rehabilitation Unit
CLINICAL RESEARCH EXPERIENCE		Yes (HIV, viral hepatitis)	No
HUMAN RESOURCES		Paediatric ward: 1 professor, 14 assistant professors, 10 doctors, 14 nurses	Paediatric ward: 1 professor, 10 assistant professors, 11 doctors, 16 nurses
TECHNICAL RESOURCES		37 oxygen wall outlets, 20 oxygen bubblers, 06 aspirators. CXR. Laboratory: routine blood tests, mycobacteriology.	39 oxygen wall outlets, 19 oxygen bubblers, 09 nasopharyngeal aspirators. CXR. Laboratory: routine blood tests, mycobacteriology, Xpert TB test, PCR.

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

	MOZAMBIQUE	
SITE	José Macamo General Hospital	Maputo Central Hospital
CITY	Maputo - capital city	Maputo - capital city
LEVEL	Secondary level hospital Teaching Hospital	National referral hospital Teaching Hospital
CATCHMENT AREA (population)	451 888	
NB PAEDIATRIC BEDS	65 including 11 beds for lung diseases	335 including 36 beds for lung diseases

NB CHILDREN ADMITTED	2 400 /year	8 000 /year
SPECIALIZED WARDS	TB ward Nutrition ward	Infectious Diseases ward (including TB and HIV) Pulmonology ward Nutrition ward
CLINICAL RESEARCH EXPERIENCE	Yes (HIV)	Yes
HUMAN RESOURCES	Paediatric ward: 6 paediatricians, 4 GPs, 18 nurses	Paediatric ward: 26 paediatricians, 14 GPs, 26 resident physicians, 121 nurses
TECHNICAL RESOURCES	Emergency and wards equipped with oxygen wall outlets. CXR. Laboratory: HIV and routine blood tests, mycobacteriology, Xpert TB test.	Emergency and wards equipped with oxygen wall outlets. CXR. Laboratory: HIV and routine blood tests, mycobacteriology, Xpert TB test.

	ZAMBIA	
SITE	University Teaching Hospital	Arthur Davison Children
CITY	Lusaka - capital city	Ndola, Copperbelt Province
LEVEL	national referral hospital	Provincial referral hospital Only standalone paediatric hospital in Zambia
CATCHMENT AREA (population)	2 000 000 (Lusaka)	2 362 000 (Region)
NB PAEDIATRIC BEDS	352	250
NB CHILDREN ADMITTED	35 000 /year	19 000 /year
SPECIALIZED WARDS	HIV Treatment and Care Centre Nutritional Rehabilitation Unit TB ward Research Clinic	HIV Treatment and Care Centre Nutritional Rehabilitation Centre TB ward
CLINICAL RESEARCH EXPERIENCE	Yes (HIV, TB) GCP training: all staff from the Research Clinic	Yes
HUMAN RESOURCES	Research Clinic: PI, co-PI, and 15 staff (doctors, research nurses, data managers).	Research staff; 2 paediatricians, other research staff to be recruited
TECHNICAL RESOURCES	Laboratory: mycobacteriology, Xpert TB test, microbiology, virology, routine blood tests. CXR. Intensive care unit Oxygen support	Laboratory: mycobacteriology, Xpert TB test, microbiology, routine blood tests. CXR. Intensive care unit Oxygen support

20.4. APPENDIX 4: Summary procedure for nasopharyngeal aspirates collection

Nasopharyngeal aspirates consist in the collection of 2-5 ml of throat contents through a catheter tube connected to a mucus aspirator. Nasopharyngeal aspirate is done for all children, without prior fasting.

The procedure can be performed in a child in 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, when in supine position, young children can be wrapped in a piece of cloth, and an assistant nurse asked to hold the child's head throughout procedure.

After connecting a mucus extractor to the suction pump and catheter, the suction pressure is adjusted. The pressure and catheter size recommended are based on the child's age as follows: in children aged < 1 year, 8 CH catheter with 80-100 mmHg (0.10 bar) suction pressure; in children aged 1 to 10 years, 8 CH catheter with 100-120 mmHg (0.15 bar) suction

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

This procedure should aspirate 2 to 5 ml of secretions. If the volume was not reached by the first aspiration, the procedure is repeated in the other nostril. This procedure is not repeated more than twice. After recapping and cleaning of the specimen container with alcohol/chlorhexidine to prevent cross-infection, and appropriate labeling, the specimens should be transported to the laboratory within 4 hours.

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

20.5. APPENDIX 5: Summary procedure for the preparation of stool samples for Xpert MTB/RIF Ultra

(Subject to further modifications)

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 will be 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 was 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.

20.6. APPENDIX 6: Informed consent forms

20.7. APPENDIX 7: Copy of the insurance policy

20.8. APPENDIX 8: Copy of the Inserm CEEI and WHO ERC approvals

20.9. APPENDIX 9: Copy of the Competent Authority's authorization

20.10. APPENDIX 10: List of required administrative and/or ethical clearance per country

COUNTRY	Ethical clearance	Administrative research clearance	Institutional Review Board
International version			
WHO	Research Ethics Review Committee (WHO ERC)		
STUDY SPONSOR (INSERM)			Institutional Review Board of the French Institute of Medical Research and Health (Inserm IRB)
Corresponding national versions			
CÔTE D'IVOIRE	National Ethics Committee (CNVES)		
MOZAMBIQUE	Comité Nacional de Bioética para Saúde de Moçambique (CNBS)		Comité Institucional de Bioética para Saúde-INS (CIBS-INS)
UGANDA	Research Ethics Committee of the Mbarara University of Science and Technology (MUST-REC)	Uganda National Council of Science and Technology (UNCST)	
ZAMBIA		National Health Research Authority (NHRA)	University of Zambia Biomedical Research Ethics Committee