Asymptomatic TB Transmission in Indonesia and South Africa (ATTIS Study)

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ATTIS
Asymptomatic TB Transmission in Indonesia and South Africa

AHRI
AFRICA HEALTH RESEARCH INSTITUTE

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LIST OF ACRONYMS AND DEFINITION OF TERMS

ACRONYMS

AHRI	Africa Health Research Institute
ART	Anti-retroviral therapy
aTB	Asymptomatic TB
aTB-C	Asymptomatic TB - Confirmed
aTB -U	Asymptomatic TB - Unconfirmed
dCXR/CAD	Digital Chest X-Ray with Computer-Aided Detection
DNA	Deoxyribonucleic Acid
EBC	Exhaled Breath Condensate
HH	Household
IGRA	Interferon-Gamma Release Assays
KZN	KwaZulu-Natal
LAM	Lipoarabinomannan
MGIT	Mycobacteria Growth Indicator Tube
mRNA	Messenger Ribonucleic Acid
Mtb	Mycobacterium tuberculosis
NHLS	National Health Laboratory Service
NHREC	National Department of Health Ethics in Health Research
NAAT	Nucleic Acid Amplification Test
PCR	Polymerase Chain Reaction
RC3ID	Research Center for Care and Control of Infectious Disease
sTB	Symptomatic TB
sTB-C	Symptomatic TB - Confirmed
sTB-U	Symptomatic TB - Unconfirmed
QGIT	QuantiFERON Gold In-Tube
TB	Tuberculosis
TPT	TB Preventative Therapy
TST	Tuberculin Skin Tests
W4SS	WHO 4 Symptom Screen
WHO	World Health Organisation
Xpert	Xpert MTB/RIF Ultra

Term	Definition
W4SS (WHO-recommended Four Symptom Screen)	A symptom-based screening tool recommended by the World Health Organization for tuberculosis (TB) screening. It involves asking about (i) cough of any duration, (ii)fever, (iii) night sweats, or (iv)unexplained weight loss. In **children** the W4SS is adapted to include (i) cough of any duration (ii) fever (iii) Poor weight gain or weight loss (iv) close contact with a person with TB disease.
W4SS Screen-positive	An individual who reports one or more symptoms on the W4SS
W4SS Screen-negative	An individual who reports all four W4SS symptoms are absent (regardless of whether they have other TB symptoms)
Household contact	A person who shared the same enclosed living space for one or more nights or for frequent or extended periods during the day with the index patient during the 3 months preceding TB screening
Suggestive of TB (radiological)	Chest X-ray findings interpreted by digital CXR with computer-aided detection (dCXR/CAD) and confirmed by a radiologist as consistent with TB.

Screening Definitions

Abbreviation	WHO- aligned terminology	W4SS	Molecular evidence of Mtb	dCXR/CAD
aTB-C	bacteriologically-confirmed asymptomatic TB	negative	Sputum Xpert positive, any grade OR Tongue swab# NAAT positive	any
sTB-C	bacteriologically-confirmed symptomatic TB	positive	Sputum Xpert positive, any grade OR Tongue swab# NAAT positive	any
aTB-U	bacteriologically-unconfirmed asymptomatic TB	negative	Sputum Xpert negative OR Tongue swab# NAAT negative	suggestive of TB*
sTB-U	bacteriologically-unconfirmed symptomatic TB	positive	Sputum Xpert negative OR Tongue swab# NAAT negative	suggestive of TB*
noTB	no evidence of TB	negative	negative	normal

* As interpreted by dCXR/CAD and confirmed by a radiologist

Tongue swabs will only be collected if approved by WHO and/or national authorities (South Africa or Indonesia) and in participants unable or refuse to produce sputum

Case Definitions

Abbreviation	WHO- aligned terminology	W4SS	Results of 3 sputum tests	dCXR/CAD	Other Clinical Data
aTB-C	bacteriologically-confirmed asymptomatic TB	Negative	1) Xpert >trace (1-3/3 sputum) **OR** 2) MGIT Mtb positive (1-3/3 sputum)	any	
sTB-C	bacteriologically-confirmed symptomatic TB	Positive	1) Xpert >trace (1-3/3 sputum) **OR** 2) MGIT Mtb positive (1-3/3 sputum)	any	
aTB-U	bacteriologically-unconfirmed asymptomatic TB	negative	1) 3/3 sputa Xpert Ultra negative AND 3/3 Mtb MGIT negative **OR** 2) 1-3/3 sputum Xpert Ultra trace AND 3/3 Mtb MGIT negative[#]	suggestive of TB *	clinical picture not explained by non-TB aetiology
sTB-U	bacteriologically-unconfirmed symptomatic TB	positive	1) 3/3 sputa Xpert Ultra negative AND 3/3 Mtb MGIT negative **OR** 2) 1-3/3 sputum Xpert Ultra trace AND 3/3 Mtb MGIT negative[#]	suggestive of TB *	clinical picture not explained by non-TB aetiology
noTB	no evidence of TB	negative	3/3 sputa negative by Xpert Ultra **AND** 3/3 MGIT Mtb negative[#]	normal*	

* As interpreted by dCXR/CAD and confirmed by a radiologist

[#] If one of the three collected MGIT cultures is contaminated rather than negative, this will be considered acceptable, provided that the remaining two MGIT cultures are negative and all three sputum Xpert results are also negative

PROTOCOL SUMMARY

Title	**Asymptomatic Tuberculosis Transmission in Indonesia and South Africa**
Short Title	ATTIS Study
Protocol number	A-ATTIS-001
Settings	Contiguous geographical areas (study areas) in King Cetshwayo and uMkhanyakude Districts, KwaZulu-Natal, South Africa and Bandung City, Indonesia
Study Duration	30 months
Summary of Approach	This is a **cross-sectional study comparing the Mtb infection status in child household contacts of people with asymptomatic TB, people with symptomatic TB and community controls** *(core study)*. These households will be identified through **community-based TB screening.** **A case-control phenotyping study** *(sub-study 1)* will be conducted on participants classified as aTB-C, sTB-C and noTB and will include additional diagnostic tests, clinical phenotyping and biobanking. **A TB-Unconfirmed cohort** *(sub-study 2)* study will be conducted on participants with bacteriologically-unconfirmed TB (TB-U) that does not meet criteria for TB treatment and will include serial clinical evaluation, diagnostic testing and biobanking. **A mathematical modelling study** *(sub-study 3)* will utilise empiric data from the study to estimate the contribution of asymptomatic TB to local and global TB transmission and incidence and the impact of potential interventions.
Co-primary Objectives	Compare Mtb infection, as defined by interferon release assay (IGRA) positivity, in child household contacts (2 to 14 years) of adults with bacteriologically-confirmed asymptomatic TB (aTB-C), with: (1) children in households with no adult with TB (2) children who are household contacts of adults with bacteriologically-confirmed symptomatic TB (sTB-C).
Secondary Objectives	(1) Evaluate the diagnostic performance, feasibility and acceptability of current diagnostic tools to detect aTB, including digital chest x-ray with computer aided diagnosis (dCXR/CAD), sputum Xpert, and sputum Mycobacteria Growth Indicator Tube (MGIT). (2) Evaluate the performance of novel diagnostic and screening tools to detect aTB including tongue swab NAAT, bioaerosol NAAT, exhaled breath

	condensate for lipoarabinomannan, cell free Mtb DNA and Mtb-specific T cell activation. (3) Characterize the features and longitudinal outcomes of sub-groups of people with aTB, including those with dCXR/CAD findings suggestive of TB but who are sputum Xpert & culture negative. (4) Establish a biobank and conduct studies of immune responses to understand the biology of aTB and define correlates of progression and resolution. (5) Model the contributions of aTB to global TB transmission and incidence and the potential impact of interventions to detect and treat this disease phenotype. (6) Evaluate the proportion of child household contacts who convert from baseline negative to positive IGRA at 10-week visit or have a positive blood biomarker (including cell free DNA assay or positive Mtb-specific T cell activation assay). (7) Define the proportion of participants with TB screening results consistent with TB-U who have a subsequent positive sputum Xpert, sputum MGIT, tongue swab NAAT or bioaerosol collection facemask NAAT. (8) Identification of transmission clusters using whole genome sequencing of Mtb strains obtained from participants with aTB-C, sTB-C and their household contacts.
Sample size	**Community-based TB screening: 90,000 (60,000 in Bandung and 30,000 in KwaZulu Natal)** **IGRA assessments in children: 2,400 (900 in Bandung and 1500 in KwaZulu Natal)** **Case-control phenotyping study: 1,200 (600 in each setting)** **Cohort study: 1,000 (500 in each setting)** We estimate that we will need to screen 60,000 adolescents/adults in Bandung and 30,000 adolescents/adults in KwaZulu-Natal to identify 150 cases with aTB-C in each setting. Mtb infection status in child HHC will be measured in 600 households in each setting (150 aTB-C cases, 150 sTB-C cases, 300 controls). The estimated average number of children aged 2-14 years per household is 1.5 in Bandung and 2.5 in KwaZulu-Natal. Therefore, we will enrol approximately 900 children in Bandung and 1,500 children in KwaZulu-Natal for IGRA assessments. Once 20% of the community has been screened in each setting, an interim analysis will be conducted to assess assumptions and inform final sample size calculations. In the **cross-sectional study of household Mtb infection** *(core study)***,** 150 index cases with aTB-C and 300 community controls (1:2 ratio) will provide ≥80% power to detect a difference between the IGRA positivity of child household contacts of adults with aTB-C and community controls if the true risk difference is 10-15%. Assuming that the true risk ratio comparing child

	household contact IGRA positivity between aTB-C and sTB-C index cases is 0.90, 150 index cases of each TB phenotype in each setting will give us ≥80% power to demonstrate that the transmission risk with aTB-C is not less than 50% of the transmission risk of sTB-C. The index adults from the 600 households in each setting will be enrolled in the **case-control phenotyping study**. Assuming that the true sensitivity and specificity of the diagnostic tools are between 70-95%, with 150 aTB-C and 150 sTB-C cases, we will be able to estimate the sensitivity with a precision of ±7.6% to ±4%. With 300 controls, we will be able to estimate the specificity with a precision of ±5.3% to ±2.6%. In each setting, after community screening, we expect to identify 500 participants with aTB-U who are eligible for the TB-U **cohort**. Based on the assumed annual progression rate, this sample size is expected to yield 31-66 individuals with progressive disease. With 50 individuals who progress and 100 who do not, we will have ≥80% power to detect a difference of 0.50 standard deviations in mean biomarker concentrations between the two groups. If the prevalence of biomarkers in individuals who do not progress is 10-20%, we would have ≥80% power to detect an increase to 30-40%.
Study Population	**Cross-sectional study of household Mtb infection** *(core study)* • *Community screening*: Adolescents and adults (≥15 years) who reside in households with children 2-14 years and located within the study areas in each setting. • *Child contacts*: Children (2-14 years) who are household contacts of participants with aTB-C, sTB-C, or of randomly selected neighbourhood households with adults without TB **Case-control phenotyping study** *(sub-study 1)* Adolescents and adults (≥15 years) who were identified through community TB screening to have aTB-C, sTB-C and no TB **Cohort** *(sub-study 2)* Adolescents and adults (≥15 years) who were identified through community TB screening with bacteriologically-unconfirmed TB (TB-U)
Outcomes/ Analyses	**Cross-sectional study of household Mtb infection** *(core study)* Primary endpoint: Cumulative prevalence of child household contact IGRA positivity and/or confirmed TB (TB-C) at 10 weeks after diagnosis of the index case. Additional outcomes/ analyses: (1) Proportion of children that convert from negative IGRA status at baseline to a positive IGRA status at 10-week visit.

	(2) Proportion of children with a positive plasma cell free Mtb DNA assay and positive Mtb-specific T cell activation assay, stratified by household group. (3) Whole genome sequencing of Mtb isolates from people with TB-C and household contacts. **Case-control phenotyping study** *(sub-study 1)* (4) Sensitivity, specificity, positive predictive value, negative predictive value, yield, feasibility, acceptability of digital chest x-ray with computer aided diagnosis (dCXR/CAD), sputum Nucleic Acid Amplification Test (NAAT), Mycobacteria Growth Indicator Tube (MGIT), tongue swab NAAT, and bioaerosol collection facemask mask NAAT to detect aTB-C. (5) Symptoms, health outcomes, quality of life, comorbidities, nutritional status, and TB history, relative to group and W4SS status at screening. (6) Proportion and characteristics of participants with detectable exhaled breath condensate (EBC) lipoarabinomannan (LAM), positive plasma cell free Mtb DNA assay, positive Mtb-specific T cell activation assay and additional EBC and blood-based TB biomarkers, stratified by group. **Cohort** *(sub-study 2)* (7) Time to event and factors associated with microbiological progression in participants with initially untreated aTB-U. (8) Plasma biomarkers (including cell free Mtb DNA, Mtb-specific T cells, transcriptional signatures) that correlate with progression from aTB-U in the 12 months after TB screening. (9) Proportion and characteristics of participants with TB screening results suggestive of (TB-U) who have a subsequent positive sputum Xpert, sputum MGIT, tongue swab NAAT or bioaerosol collection facemask NAAT.

1. BACKGROUND

TB EPIDEMIOLOGY

Tuberculosis (TB) continues to be a major cause of morbidity and mortality, with 10.8 million people falling ill from TB and 1.25 million deaths in 2023[1]. Following two years of COVID-19 related disruptions a substantial global recovery in the TB response was reported in 2022[2]. However, many countries are still off track in achieving the sustainable development goals (SD3) and the 2012 UN member states commitment to eliminate TB by 2030. For example, the TB global incidence rate reduced by 8.7% from 2015 to 2022, which is far from the END TB strategy milestone of a 50% reduction by 2025[2]. It is estimated that if the current trends continue, there could be 31 million deaths from TB by 2050 and a TB disease-associated economic cost of US$17.5 trillion[3]. Universal implementation of the current tools and creation of new innovative tools and approaches is critical to change the trajectory and reduce TB deaths by 90% by 2030[4].

ASYMPTOMATIC TB

The natural history of TB is still not completely understood and definitions and literature on the various states of TB disease is evolving. More data has emerged that indicate that not all people with TB disease with bacteriological confirmation report symptoms such as the classical four symptoms of cough, fever, weight loss and night sweats. Based on the consultation convened by the World Health Organization (WHO) in October 2024, the global tuberculosis report 2024 outlines new definition of asymptomatic TB (aTB) for TB programmes and research[1]. The main distinguishing criterion is symptoms suggestive of TB during screening. aTB is defined as a person with bacteriologically confirmed or unconfirmed TB disease who did not report symptoms suggestive of TB during screening[1].

A systematic review of prevalence surveys (23 national and 5 sub-national) from 23 African and Asian countries found approximately half (50.4%; IQR: 39.8%–62.3%) of the people with TB disease did not have symptoms of TB[5]. In the South African national TB prevalence survey conducted in 2018, 58% of the participants with bacteriologically confirmed TB had radiological features suggestive of disease while not having symptoms[6]. The latter could still be an underestimate of aTB prevalence as most prevalence surveys only request a sputum specimen in people with symptoms or radiological features suggesting TB, consequently excluding those with aTB and no or very subtle radiological changes. In a study conducted in Cape Town, South Africa, where TB contacts were screened with sputum culture, irrespective of chest X-ray findings, only 47% of those with aTB had abnormal chest X-ray findings suggestive of TB[7]. Consequently, aTB may represent a large proportion of prevalent TB disease and this group of people would be missed or not diagnosed by most current TB screening programs, which are largely based on symptom screening.

This new WHO definition of aTB is comparable to what was described as subclinical TB in some of the literature. Subclinical TB refers to *"Individuals who are bacteriologically positive for Mycobacterium tuberculosis (Mtb) but are without, not aware of, or do not report any symptoms during a symptom screen or medical history, and have no physical signs that would be recognised as indicative of TB upon clinical examination"* [8]. However, the absence of physical signs suggestive of TB on clinical examination has not been incorporated into the new WHO definition of asymptomatic TB.

This definition of subclinical TB was part of the four states of early Mtb disease that have been described following a Delphi exercise by experts in 2023. Based on combination of the presence of three (macroscopic pathology, infectiousness and symptoms) the four dimensions, are infectious/non-infectious subclinical TB and infectious/non-infectious clinical TB[9-13.] The presence of macroscopic pathology is the minimum prerequisite for a TB disease state[8]. Macroscopic pathology is described as the cellular infiltration as a result of failure to contain Mtb that can be observed on biopsy, autopsy, clinical examination or by imaging[8].

Another factor that complicates prevalence estimates of aTB is that various screening and diagnostic algorithms are currently in use. Most prevalence surveys use either the presence of symptoms or lung field abnormality on chest x-ray to trigger collection of sputum, which is then subjected to NAAT. Because of its cost and requirement for laboratory infrastructure, sputum MGIT is rarely performed at scale, but when it is, it identifies additional cases of bacteriologically-confirmed TB[14].

The efficiency of chest x-ray surveys to detect asymptomatic TB has been greatly enhanced by the development of portable hardware for the capture of high-quality digital chest x-ray images and the development of computer-assisted diagnostic algorithms that can identify lung field abnormalities suggestive of TB with comparable accuracy to human radiologists[15, 16]. Despite their efficiency, surveys in which sputum collection is triggered by chest x-ray, fail to detect the subpopulation of people with asymptomatic TB who have normal chest x-rays (recently shown by Mendelsohn et al. to comprise up to 43% of asymptomatic bacteriologically-confirmed TB among household contacts of a symptomatic index case)[17]. Chest x-ray screening surveys also identify people with chest x-rays suggestive of TB whose screening sputum tests are negative. This group presents a diagnostic and management challenge to health systems because the chest x-ray abnormalities of people in this group may be attributable to early progressive TB, inactive previously treated TB, non-TB infectious or non-infectious aetiologies. The stochasticity of sputum sampling and the limited sensitivity of sputum Xpert and MGIT for paucibacillary TB, means that collection of additional sputum samples for microbiologic evaluation would confirm TB-C in a proportion of these individuals. This knowledge and information about the proportion of the remaining TB-U group that progressed to bacteriologically-confirmed TB in the year after screening is important unknown information to guide future screening efforts. Addressing key knowledge gaps about the comparative performance of existing diagnostic tests to identify aTB and the characteristics and outcomes of sub-groups identified by different combinations of these tests can be addressed through a large-scale screening study that collects sputum for Xpert and MGIT and dCXR on all participants.

Infectiousness is defined as the ability to generate viable Mtb from the respiratory tract that can cause new Mtb infections in others[8]. Although infectiousness has classically been viewed as predominantly associated with cough, viable Mtb droplets can be produced in the absence of symptoms, through talking, singing and breathing[18-21]. The potential infectiousness of people with aTB is of major public health concern as they could substantially contribute to Mtb transmission at population level.

What remains unclear is how infectious is aTB compared to sTB, and as a consequence, what the contribution of aTB is to global Mtb transmission. Mathematical modelling from prevalence surveys in African and Asian countries suggests that 68% of TB transmission could be attributable to aTB[22]. If individuals with aTB are found to contribute significantly to Mtb transmission, there will be a need for wide-ranging changes in global TB policy, including case finding strategies[23]. To inform such policy

decisions and the associated redirection or commitment of resources, it is key that the relative infectiousness of aTB is more accurately established than current data allow[22]. Generating high quality empiric evidence to close this evidence gap has been identified as the highest priority research question by the WHO during the 2024 Technical Consultation on aTB. The aim of this study is to collect empiric data on household aTB transmission in Africa and Asia.

There are multiple factors that drive the transmission of, and new infections with, Mtb, such as prevalence and infectiousness of people with TB disease, the number of susceptible people they come in contact with and the duration and frequency of that contact. Environmental factors such as overcrowding, high humidity and inadequate ventilation may increase transmission[24]. Measuring Mtb transmission is extremely difficult as not all people that are infected progress to have TB disease and there is also variability in how long it takes to progress to disease.

Genotyping Mtb isolates has been utilized to study TB transmission. In a study that evaluated people with aTB and their index TB case, 7/10 people with culture-positive aTB had drug resistance similar to that of the index person and three of these were confirmed with genome sequencing[7]. The high costs of genotyping, mutation rate of Mtb and within-host variation are some of the limitations for such an approach. Although some of the Mtb transmission and new infection occurs outside the household or not from a known contact, especially in high burden settings, presence of TB infection in known contacts is still the most feasible recommended approach to estimate Mtb infection at community level. The relative ease of measuring Mtb transmission and new TB infection within a household, household TB infection prevalence and incidence, and case notification rates in children have been used to quantify population level impact[24].

WHO FOUR SYMPTOM SCREENING (W4SS)

The WHO-recommended four-symptom screen (W4SS) is a standardised symptom-based screening tool for TB disease. It was first recommended by WHO in 2011 to facilitate detection of TB in PLHIV and allow the safe initiation of TB preventative therapy in high-burden, low resource settings[25]. The tool involves assessment for the presence of four key symptoms: cough, fever, night sweats and weight loss.

If any one of these symptoms is present, the individual is considered *screen-positive* and requires further TB investigation. The absence of all four symptoms is considered *screen-negative* and has been shown to have a high-negative predictive value in high-burden settings [25]. The W4SS was originally developed as a simple rule-out tool to be used by healthcare workers for PLHIV being evaluated for isoniazid preventative therapy (IPT), now referred to as TB Preventative Therapy (TPT).

Subsequent systematic reviews have demonstrated an overall sensitivity of 83%, and a specificity of 38% for the detection of TB in PLHIV. Among outpatients not yet on antiretroviral therapy(ART), sensitivity is slightly higher (84%) with a similar specificity (37%), whereas among PLHIV already on ART, sensitivity drops to approximately 53%, with specificity increasing to 70% [63]

Despite its value, limitations of the W4SS have been recognised. Performance is suboptimal in specific subpopulations (e.g. children and pregnant women) who may present atypically or with minimal symptoms [26, 27] In addition, variability in how the questions are asked can introduce interviewer bias, emphasising the need to standardise implementation and training [28].

The W4SS remains firmly embedded in TB/HIV programme delivery and national policy across high-burden settings. Since its recommendation by WHO, it has become the standard screening tool within national TB guidelines. Despite its recognised limitations, the W4SS continues to serve as a standard for TB symptom screening in both programmatic and research contexts.

GEOGRAPHICAL SETTING

This study will be conducted in Bandung City, West of Java Province in Indonesia and northern KwaZulu-Natal (KZN) in South Africa. Both countries are classified as middle-income countries with some recovery to their economies following the COVID-19 pandemic[29]. These two countries have been chosen to provide the required evidence for a possible policy change by WHO. WHO follows a structured evidence-based process before recommending or endorsing a policy change on TB screening that includes a demonstration that the policy is implementable in low resource settings where TB is often most prevalent based on evidence from at least two WHO regions. Globally undernutrition, alcohol use disorder, smoking and HIV infection are the leading risk factors for TB **(Appendix A),** these factors are prominent in Indonesia (smoking and undernutrition) and South Africa (HIV infection and alcohol use disorder). Therefore, results from these two low middle income countries with different TB epidemics will provide useful data from settings with high TB prevalence but differing epidemiology, particularly with respect to HIV.

Both countries have a high burden of TB (with a TB incidence of 387/100 000 in Indonesia and 427/100 000 in South Africa **(Appendix B)**[1]. The major difference between the two countries is that the proportion of people co-infected with HIV and TB is very low (3.5%) in Indonesia, compared to South Africa (54%)[1]. In both countries there are efforts to reduce the burden of TB [30] by increasing screening for TB contact tracing and access to TPT. However, the high HIV prevalence in South Africa, smoking, undernutrition and poverty, continue to make people in these countries susceptible to TB.

Based on data from a health and demographic surveillance system in northern KZN, children aged 2-14 years old are 32.0% of the resident population, and 39.8% of households have at least one resident child in this age group. In the households with children in this age group, the median number of children per household is 2 (IQR 1-3) and mean is 2.58 (SD=1.63). A study done across two provinces (Limpopo and Free State) showed tuberculin skin test positivity of 17%.[30] The recently completed (not published) household (HH) contact tracing conducted in the Hlabisa sub-district in northern KZN found 18.4% (95% CI:15.-21.5%) IGRA positivity in child contacts of symptomatic clinic diagnosed people with TB. The IGRA positivity was 16.4% (95% CI: 7.8%-28.8%) amongst child contacts of people with aTB diagnosed at the clinic and 7% (95% CI: 3.5% to 10.5%) in community controls with no person with TB in the household.

In Bandung city, 59.0% of households have at least one child aged 2-14 years, with a mean of 1.5 (SD=0.7). In a study comparing IGRA and TST in Bandung City in 196 households with a person with TB, 299 household-exposed children aged 6 months to 9 years old and 72 age and sex matched neighbourhood-exposed children were recruited and tested. Of the household contacts, 156 (52%) tested positive using IGRA and 144 (48%) using TST[31]. Of the neighbours 15 of 72 tested (21%) were IGRA positive and 7 (21%) were TST positive. Of 38 children with a follow-up IGRA test at 3 months, 8 of 23 (35%) initially IGRA negative individuals had IGRA conversion and 3 of 15 (20%) initially IGRA positive individuals had IGRA reversion. Another study recruited 1,347 TB household contacts of 462 known TB

cases[32]. Of the contacts, 170 were children aged 5 to 9 years; 96 of 168 (57%) tested were IGRA positive among the children, and a further 15 amongst 67 (22%) tested after 14 weeks became IGRA positive amongst the 72 who were initially IGRA negative.

INTERFERON-GAMMA RELEASE ASSAY

In addition to assessment of genotypic links, other approaches that are recommended for estimating Mtb infection resulting from transmission are TB notification rates, tuberculin skin tests (TST) and or Interferon-gamma release assays (IGRA)[24]. IGRA such as the QuantiFERON Gold In-Tube (QGIT) test, detect immunoreactivity to highly specific Mtb antigens from a blood sample. The drawback of the QGIT test is that it requires laboratory processing; however, many laboratories in South Africa and other high TB prevalence setting already have sufficient expertise and experience with these tests. The QGIT test involves a simple blood draw and participants are not required to return for another 'in person' visit for interpretation. Results also do not rely on the subjectivity of TST readers, for example, and QGIT is more sensitive and specific than TST in people living with HIV (PLHIV), including those with previous BCG vaccination[33, 34]. In addition to test conversion, IGRA tests are subject to significant early test reversion in case contacts, which has not been fully explained beyond that which occurs close to the cut-off value[35-37]. Therefore in this study we will measure Mtb transmission and new infection from people with aTB by using the IGRA/QGIT status in child household contacts as the endpoint to quantify infectiousness and compare the rates with those with community controls.

NOVEL TB DIAGNOSTIC TOOLS

The identification of novel screening and diagnostic tools capable of detecting aTB is a research priority for WHO. Existing modalities, demonstrate limited sensitivity for paucibacillary disease and are often constrained by cost, infrastructure requirements, and operational complexity, restricting their applicability in field settings. Advances in biomarkers, molecular diagnostics, and non-sputum-based sampling strategies have yielded a pipeline of promising tools at various stages of development. However, evaluation of their feasibility and performance to detect asymptomatic disease is a critical knowledge gap that needs to be addressed in order to inform their potential role in scalable aTB detection strategies.

BIOAEROSOL SAMPLING

Mycobacterium tuberculosis (Mtb) is an obligate respiratory pathogen reliant on extracorporeal survival when exhaled from an infected individual and passing to a new host. In the 1950s, the infectious nature of aerosols was confirmed experimentally in a minority of sputum-positive individuals in a guinea pig infection model[38] . However, identification of Mtb in exhaled aerosols has been technically very challenging owing to the paucibacillary nature of bioaerosol samples. Recent interest in non-sputum diagnostics and measures of Mtb infectivity have refocused attention on this critical stage in the Mtb infection cycle. In response, new modalities have been investigated including mask sampling, tongue swabbing, exhaled breath condensate (EBC), and cyclone aerosol collection[39] .

Understanding the dynamics of Mtb transmission, including transmission from individuals with asymptomatic TB, is crucial for developing effective strategies to reduce TB incidence and interrupt community spread. For over a decade, Wood and colleagues have used innovative technologies such as the respiratory aerosol sampling chamber (RASC) to study bioaerosol generation from volunteers[40] . By

combining the RASC with DMN-trehalose-based detection to visualize Mtb bacilli, they reported that nearly 90% of clinic attendees with suspected TB were positive for Mtb bioaerosols, regardless of whether they were ultimately diagnosed with TB or initiated treatment[41] . In addition, Mtb bioaerosol levels declined similarly in both groups during a six- month follow-up, with around 20% of participants, including those who were treated and those without a TB diagnosis, continuing to test bioaerosol-positive despite clinical resolution. Follow-up work in a randomized community sampling study demonstrated high (~80%) Mtb bioaerosol point prevalence in the same setting, with >90% of participants associated with Mtb bioaerosol positivity over three samplings conducted at baseline, two week, and two-month timepoints [42]. In combination, these findings suggest that Mtb bioaerosol release can persist independently of active disease status, highlighting the potential for ongoing transmission even after symptom resolution[22, 44]

Building on research using the RASC, the team recently developed a portable, tabletop EBC collection device that uses linear airflow and cooling of the airstream within a copper block maintained at 0°C ± 3°C, causing the condensation of exhaled breath, which is collected in sterile tubes. Preliminary comparative studies with the RASC have shown at least equal sensitivity of Mtb detection, with successful identification of Mtb-specific RD9 DNA sequences and positive Xpert results following EBC MGIT culture (Patterson, Wood, et al. unpublished).

Facemask have emerged as a promising novel tool to capture bioaerosolized Mtb and have been shown by Williamson et al, to identify 8 individuals who were sputum Xpert-negative, 50% of whom subsequently developed Xpert-positive sputum[19]. In addition to the facemasks used in this study, there are additional designs that have shown reasonable sensitivity and specificity for detection of Mtb[45]. Larger studies are needed to evaluate the sensitivity of facemasks for the detection of aTB and to assess the proportion of individuals in whom exhalation of detectable aerosolized Mtb precedes sputum-positivity.

While providing key insights, studies utilizing new sampling tools have highlighted the impact of clinical phenotypes, collection times, aerosol collection efficiency, and MTB detection sensitivity on the results obtained, making comparative analyses challenging. In high-burdened settings, Mtb transmission from known sputum-positive cases explains <33% of transmission chains, suggesting an important contribution from other community sources. This study will quantify transmission from people with aTB-C. In addition, based on previous observations suggesting the potential for individuals to cycle into and out of an infective state without overt TB disease, we propose to evaluate longitudinally the release of Mtb bioaerosols among the TB-U cohort participants with aTB-U, a group of potential TB importance to TB transmission.

TONGUE SWABS

Given the operational challenges of sputum collection, tongue swabs offer the possibility of an easier-to-collect specimen enabling rapid scaling up of TB testing. Tongue swabs can be readily collected and tested using existing courier and laboratory logistics and molecular tests; collection does not pose as great an aerosol generation risk as sputum collection. They also offer the possibility of obtaining a specimen from individuals unable to expectorate sputum on demand, common in children, and severely ill patients. Although preliminary studies suggest that tongue swabs subjected to Xpert have reduced sensitivity (range 78-88%) relative to sputum[46, 47]. It is posited that the ease of sample

collection may result in an increased test coverage in the target population. Current published data on tongue swab accuracy and diagnostic yield is limited; and WHO has not yet published recommendations on the use of tongue swabs for the diagnosis of TB.

Research to optimize tongue swab collection devices, their transport and processing and diagnostic assays is ongoing. Different approaches to oral cavity sampling have been evaluated including swabbing the dorsum of the tongue, and combinations of buccal, palate and gum line swabbing.[48, 49] Preliminary data suggests that swabbing of the dorsum of the tongue has the highest TB yield.[49, 50] Additionally, different types of swabs and materials have also been evaluated, with Copan flocked swabs (FLOQSwabs™) swabs having higher yield compared to other swab types.[51]Non-commercial assays that target qPCR for *IS*6110 or bacterial ribosomal rRNA have been evaluated ('manual PCR').[49] Manual PCR methods are readily adapted to process swabs instead of sputum and studies have demonstrated higher yield for TB detection than commercial PCR assays like Xpert.[52, 53] This may be due to pre-analytic pathogen enrichment steps and the ability to set detection thresholds (Ct value cut-offs). However manual PCR methods can also have higher rates of false positives due to contamination than closed systems such as automated Xpert.[54] Xpert requires less technical expertise than manual PCR and can be done closer to the point of care, but additional work is still needed to adapt oral tongue swabs for use on other automated PCR platforms designed for processing and testing sputum specimens.[50]

Tongue swab sensitivity and specificity: Tongue swab assay performance varies greatly depending on the studied population (e.g. high background prevalence, hospitalized, HIV), PCR method (manual vs. commercial PCR), location of oral sampling, and type of swab used. The sensitivity of tongue swabs ranges from 36.3% to 92,8%.[50, 55] In a meta-analysis done by Wang et al., results from 23 studies found a pooled sensitivity of 50% (95%CI, 37-63%).[56] Tongue swab sensitivity in paediatric studies was lower (22%-43%) compared to adults. [54, 57, 58] However, in PLHIV, tongue swab sensitivity of 81,3% compared to sputum Xpert Ultra and culture. Across studies, the specificity of oral swabs is very high with specificities of 100% in multiple studies.[51, 58, 59] However, there is no data describing tongue swab yield, compared to yield in sputum in patients who either have early/minimal TB disease or aTB.

Acceptability of tongue swabs: Data from interviews with South African healthcare workers with extensive experience in sputum and swab collection indicated that they will prefer a tongue swab method above sputum collection, provided there is increased education to the public on this method to ensure cooperation from patients for sample collection.[60] However, the preferences of patients for either sputum or tongue swab or other specimen or diagnostic process has not been widely canvassed, especially when these are used to diagnose TB in those with either early/minimal TB disease or asymptomatic/subclinical TB. The effort, discomfort, and possible loss of privacy or dignity from collecting a specimen may impact on acceptability. Moreover, the value core believability that patients place on a laboratory result from a tongue swab may differ from that of a sputum specimen and may impact their willingness to start TB treatment if the test actually results as *M tuberculosis* complex detected.

T-CELL ANTIGEN-SPECIFIC ACTIVATION (TB-TASA)

Tuberculosis diagnosis remains challenging and is still widely dependent on sputum-based microbiological methods that miss or delayed many cases, especially asymptomatic, paucibacillary, paediatric, and extrapulmonary TB. T-cell antigen-specific activations assays are emerging as promising

non-sputum diagnostic tools[61]. These assays rely on stimulating whole blood or PBMC with *M tuberculosis* antigens and measuring upregulation or loss of surface markers such as CD38, HLA-DR and CD27 on Mtb-specific T cells. Studies have shown that the presence of the MTB-specific activated T-cells indicates infection (latent or active, depending on clinical context).

However, larger evaluations are still needed to validate their performance, particularly in aTB.

MTB CELL FREE DNA

There is growing interest in blood-based (non-sputum) approaches that can detect fragments of circulating *Mycobacterium tuberculosis* as complementary or alternative diagnostic biomarkers. CRISPR-mediated detection platforms have emerged as promising tools due to their ability to amplify and detect short, low-abundance Mtb cfDNA fragments with high analytical sensitivity, potentially enabling non-sputum diagnosis and earlier detection[62].

Several studies have demonstrated that Mtb cell free DNA (cfDNA) can be recovered from plasma and detected by CRISPR-based assays, where Cas12a complexes generate enzymatic signal amplification to improve detection of low concentrated target sequences. Huang et al, reported high diagnostic accuracy of Mtb cfDNA, with sensitivity of 96% (80-100) and specificity of 94% (71-100) in microbiologically and clinically confirmed HIV-negative TB cases, and sensitivity of 83% (36-100) and specificity of 95% (77-100) in paediatric and extrapulmonary cases. These findings support the potential Mtb cfDNA CRIPR-mediated assay as a rule-in test or as an adjunct to existing workflows.

Critical questions remain regarding their performance in detecting bacteriologically-confirmed aTB and whether they may be able to improve specificity and provide bacteriologic confirmation in people who would otherwise meet the definition of aTB-U.

2. OBJECTIVES

The **co-primary objectives** of the study are to compare Mtb infection, as defined by interferon release assay (IGRA) positivity, in child (2-14 years) household contacts of adults with bacteriologically-confirmed asymptomatic TB (aTB-C), with:

1. children in households with no adult with TB
2. children who are household contacts of adults with bacteriologically-confirmed symptomatic TB (sTB-C)

The **secondary objectives** are to:

1. Evaluate the diagnostic performance, feasibility, and acceptability of current tools for detecting aTB, including digital chest X-ray with computer-aided diagnosis (dCXR/CAD) and sputum Xpert, and sputum Mycobacteria Growth Indicator Tube (MGIT)
2. Evaluate the performance of novel diagnostic and screening tools to detect aTB including tongue swab NAAT, bioaerosol NAAT, exhaled breath condensate for lipoarabinomannan, cell free Mtb DNA and Mtb-specific T cell activation.
3. Characterize the features and longitudinal outcomes of sub-groups of people with aTB, including those with dCXR/CAD findings suggestive of TB but who are sputum Xpert & culture negative.

4. Establish a biobank and conduct studies of immune responses to understand the biology of aTB and define correlates of progression and resolution.
5. Model the contributions of aTB to global TB transmission and incidence and the potential impact of interventions to detect and treat this disease phenotype
6. Evaluate the proportion of child household contacts who convert from baseline negative to positive IGRA at 10-week visit or have a positive blood biomarker (including cell free DNA assay or positive Mtb-specific T cell activation assay).
7. Define the proportion of participants with TB screening results consistent with sTB-U who have a subsequent positive sputum Xpert, sputum MGIT, tongue swab NAAT or facemask NAAT.
8. Identification of transmission clusters using whole genome sequencing of Mtb strains obtained from participants with aTB-C, sTB-C and their household contacts.

3. METHODOLOGY

3.1 STUDY DESIGN

This is a multi-country, cross-sectional study designed to assess household Mtb infection and transmission among asymptomatic (aTB) and symptomatic TB (sTB) cases and their household contacts. Community-based screening will be implemented across contiguous geographic areas within each participating country. It is estimated that approximately 60,000 adults in Indonesia and 30,000 adults in South Africa will undergo screening. Eligible participants identified through community screening will be enrolled into the core study and related sub-studies for further diagnostic evaluation, clinical phenotyping, and biobanking. The core study is a **cross-sectional study comparing the Mtb infection status in child household contacts of people with aTB-C, sTB-C and community controls (randomly selected noTB).** These households will be identified through **community-based TB screening.**

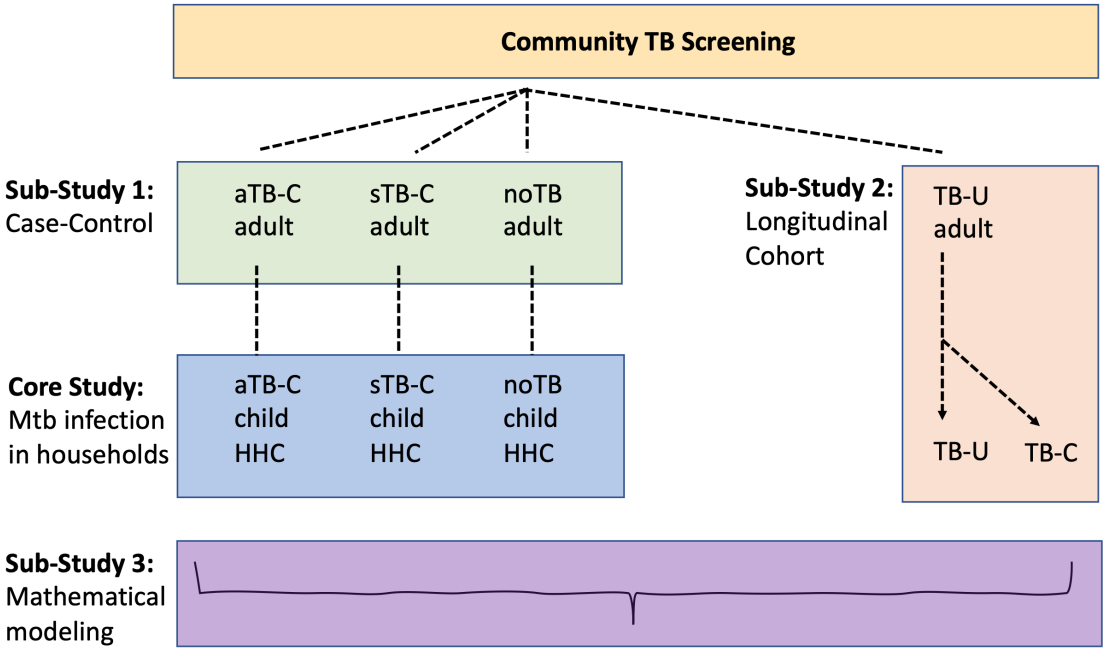
The **case-control phenotyping study** *(sub-study 1*) will be conducted in people with asymptomatic TB symptomatic TB and identified controls in the core study and will comprise additional diagnostic tests, clinical phenotyping and biobanking.

A TB-U cohort study *(sub-study 2)* will be conducted in individuals identified during the community screening with bacteriologically unconfirmed TB that do not meet criteria for TB treatment, and will comprise serial clinical evaluation, diagnostic testing and biobanking.

A mathematical modelling study *(sub-study 3)* will utilize empiric data from the study to estimate the contribution of asymptomatic TB to local and global TB transmission and incidence and the impact of potential interventions.

The study flow chart provides an overview of the visit schedule and depicts how the core and sub-studies are interrelated **(Figure 1).**

Figure 1. Schematic representation of the study procedures and interrelations of the studies



The study is expected to be completed within 36 months following the grant funding award, with an anticipated 30-month implementation period. Implementation will commence in South Africa, followed approximately two months later by Indonesia, once site preparations are complete. A stage-gate review will be conducted when each country has screened around 20% of the target sample (approximately 5,000 participants in South Africa and 12,000 in Indonesia). At this point, estimated for August 2026 (South Africa) and October 2026 (Indonesia), key parameters informing sample size, including the number of aTB cases identified, number of child household contacts and child IGRA positivity across control, aTB, and sTB households, will be reviewed.

3.2 SETTINGS

This study will be conducted in contiguous geographical areas within:

1. King Cetshwayo and uMkhanyakude Districts, KwaZulu-Natal (KZN) Province, South Africa. The study will start in the uMfolozi subdistrict (King Cetshwayo) and subsequently move to uMhlathuze (King Cetshwayo) and the Big Five Hlabisa subdistrict (uMkhanyakude), depending on enrolment progress and the stage-gate review.
2. Bandung City, West of Java Province, Indonesia

3.3 STUDY POPULATION

The study population for the **cross-sectional study of household Mtb infection (*core study)*** will be adolescents and adults (≥15 years) who undergo community screening. In addition, the core study will also enrol child household contacts (2–14 years) of community-screened adolescents and adults who meet the inclusion criteria for the core study (aTB-C, sTB-C, or noTB)

In the **case-controlled phenotyping study *(sub-study 1)*** the study population are adolescents and adults with aTB-C, sTB-C or no TB controls who participated in the cross-sectional study (core study)

In the **TB-U cohort study *(sub-study 2)*** the study population are adolescents and adults (≥15 years) who were identified through the community TB screening as having TB-U.

The **study population and eligibility criteria** for each of the studies are defined below **(Table 1)**

Table 1: Study Populations and Eligibility Criteria for the individual studies

Study	Study Population	Eligibility Criteria
Cross-sectional study of Household Mtb infection (core study)	**Community screening:** Adolescents and adults (≥15 years) who reside in households located within the study areas in each setting **Child contacts:** Children (2-14 years) who are household contacts of people with sTB-C, people with aTB-C or of randomly selected adults in households without TB	**1. Adolescent/Adult participants for community screening** Inclusion criteria: 1. Age ≥15 years 2. Residence in the designated study area 3. Residence in a household with children (2-14 years) Exclusion criteria: 1. Unable or unwilling to consent (>18 years) 2. Unable to unwilling to assent (15-17 years) 3. Parent or guardian unwilling to consent (15-17 years) 4. Residence in a household with a participant in a TB vaccine trial (currently or previous 2 years) **2. Child household contacts** Inclusion criteria: 1. Age 2-14 years 2. Household residence with an adolescent/adult participant with: a. aTB-C, **or** b. sTB-C, **or** c. an adolescent/adult participant who has been selected as a healthy control (no TB) Exclusion criteria: 1. Unable or unwilling to assent (≥7 years) 2. Parent/guardian unable or unwilling to consent 3. Residence in household in which any resident has been diagnosed with TB within the past 2 years 4. Residence in a household with more than one adolescent/adult with current co-prevalent TB-C
Case-control phenotyping study *(sub-study 1)*	Adolescents and adults (≥15 years) who participated in cross-sectional study of household Mtb infection (core study)	**1. Participants with aTB-C** Inclusion criteria: 1. Adolescent/adult participant in TB screening 2. Screening W4SS negative 3. Screening sputum Xpert positive (any grade) OR Mtb MGIT positive* OR tongue swab NAAT positive **2. Participants with sTB-C** Inclusion criteria: 1. Adolescent/adult participant in TB screening 2. Screening W4SS positive 3. Screening sputum Xpert positive (any grade) OR Mtb MGIT positive* OR tongue swab NAAT positive

Study	Study Population	Eligibility Criteria
		3. **Participants selected as healthy controls** Inclusion criteria 1. Adolescent/adult participant in TB screening 2. Screening W4SS negative 3. Screening sputum Xpert negative; AND Mtb MGIT negative* **4.** Selected as a healthy control in the Cross-sectional study of Mtb infection in households (see above) **ALL 3 groups above** Exclusion criteria 1. Unable or unwilling to consent ** MGIT results returning after enrolment may result in study group re-categorisation*
Cohort study *(sub-study 2)*	Adolescents and adults (≥15 years) who participated in TB screening.	**Participants who meet the study case definition for bacteriologically-unconfirmed TB (TB-U)** Inclusion criteria: 1. Adolescent/adult participant in TB screening 2. All three sputa Xpert are negative/ 'trace' AND all three sputum are Mtb MGIT negative 3. Screening dCXR/CAD interpretation is "suggestive of TB" 4. Clinical assessment at phenotyping visit does not suggest a non-TB aetiology for dCXR/CAD findings and is consistent with TB-U 5. Participant did not commence TB treatment after phenotyping visit Exclusion criteria 1. Unable or unwilling to consent to longitudinal follow-up

3.4 STUDY SCHEDULE

A summary of study visits is provided in **Table 2**, and the flowchart of Schedule of Procedures is presented in **Appendix C**.

Table 2: Schedule of study procedures for participants enrolled in the asymptomatic TB transmission in Indonesia and South Africa study

Cross-sectional study of Household Mtb	**Visit 0: Household identification and consent** • Identify geospatial areas for enumeration • Informed consent by household head • Minimal Household enumeration to determine eligibility • Dwelling/House description data

infection *(core study)*	**Visit 1: Screening and enrolment** • Consent and enrolment of all adolescents and adults HH members • Demographics • Basic Health Questionnaire • Symptom screening (including W4SS) • Minimal physical examination (weight, height) • Sputum collection for Xpert and MGIT culture • Tongue swab NAAT collection for participants unable or refuse to produce sputum [#] • Digital chest x-ray with computer aided detection (dCXR/CAD)
	Visit 2.1 and 2.2: Community or clinic IGRA measurements (weeks 0-2 and or 8-10) • Individual assent and parent/ guardian consent for child household contacts • Structured questionnaire on contact patterns with index person and detailed dwelling/House description data • Symptom screening (W4SS modified for children) • If TB symptoms, sputum collection • Weight, height, mid upper arm circumference (MUAC) • Venous blood for IGRA testing (all children at visit 2.1; at week 8-10 (visit 2.2), only repeat IGRA if visit 2.1 IGRA was negative) • Venous blood samples -for HIV[$], cell-free Mtb DNA, Mtb-specific T cells and biobanking • If any symptoms or signs suggestive of TB are identified, refer to the appropriate health facility and provide support for TB investigation and treatment in accordance with national guidelines • Referral for TB preventative therapy (TPT) for all HH contacts of people with TB (visit 2.2)
	Visit 2.3: Community, clinic or telephonic review and referral (weeks 2-14), Provide IGRA status • Issue any outstanding results for adults • Follow-up on TB treatment or TPT initiation • End of study visit for cross-sectional study

Case-control phenotyping study *(sub-study 1)* *AND* Cohort study *(sub-study 2)*	**Visit 1.1 Facility based clinical assessment (weeks 1-8)**
	• Individual consent/ assent
	• Referral to research clinic for phenotyping
	  ○ Blinded structured questionnaire, include symptoms, quality of life, health outcomes
	  ○ Physical examination
	  ○ Collection of 2 sputum samples (Xpert, MGIT culture)*
	  ○ Collection of samples for novel diagnostic tests
	  ▪ Tongue swabs
	  ▪ Facemask
	  ▪ Exhaled breath condensate
	  ▪ Venous blood – HIV; VL; HbA1C; FBC/smear; Mtb cell free DNA; Mtb-specific T cell activation, biobanking specimens
	  ○ Physician review of participant's TB screening results
	• Clinical referral for TB and non-TB care
	• Telephonic review of linkage to care and well-being
	• Electronic / physical record review to confirm TB treatment initiation and completion

TB-U Cohort study *(sub-study 2)*	**Visit 1.2, to 1.5: Longitudinal follow-up (Months 3, 6, 9 & 12)** • Clinical assessment including symptoms, quality of life, health outcomes • Physical examination • Sputum samples x 2 (Xpert, MGIT culture) • Venous blood – Mtb cell free DNA; Mtb-specific T cell activation, biobanking specimens • Digital chest x-ray • Facemask and Exhaled Breath Condensate, in a subset of participants • Physician review • Clinical referral for TB or non-TB care

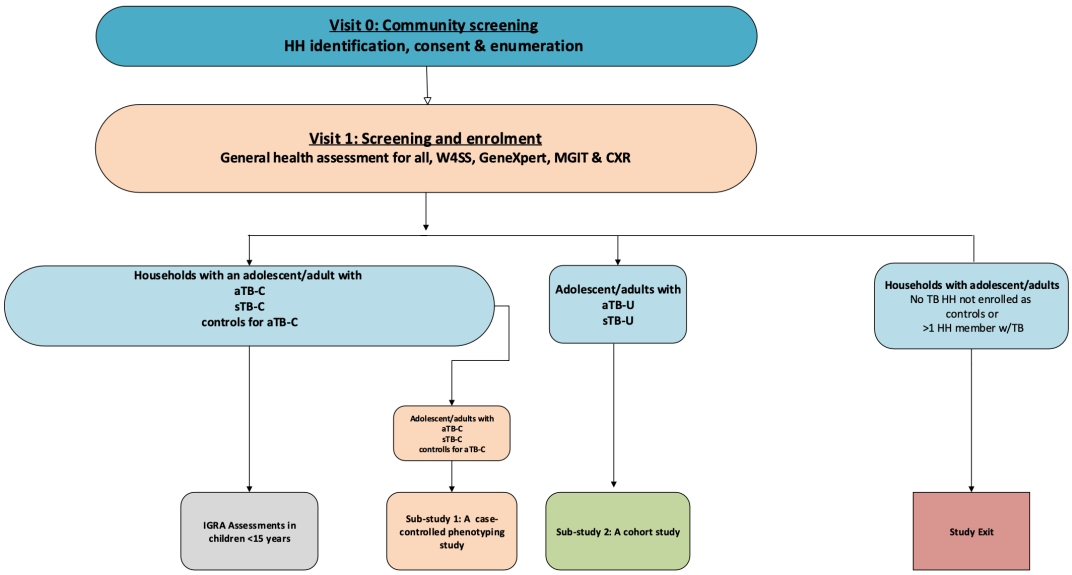
 # Tongue swabs will only be collected if approved by WHO and/or national authorities (South Africa or Indonesia) and in participants unable or refuse to produce sputum
** If eligibility is based on a positive tongue swab collected due to unsuccessful sputum collection at visit 1.0, three sputum samples will be collected at visit 1.1*
$ HIV testing in children will only be performed in South Africa

3.5 STUDY PROCEDURES

Large scale community screening will be conducted among adolescents and adults aged 15 years and older who reside in the study areas and in households (HH) with children aged 2–14 years. To better understand Mtb transmission, households will be enrolled from contiguous geographic areas, allowing for the assessment of transmission while minimising geographic variability and potential confounding introduced by non-adjacent areas.

Prior to entering each selected area, the study team will obtain permission from the local area leader or community authority. Study areas will be mapped using Quantum Geographic Information System (QGIS) software or other equivalent geographic information system tools to define boundaries and identify households for enumeration. All households within these contiguous areas will be visited to enumerate inhabitants **(Figure 2).** Household enumeration will be part of visit 0 and may be done on the same date as visit 1.0. Informed consent will be obtained from the household head before any study activities are conducted. Following consent, household enumeration will be performed to determine eligibility for participation, and minimal dwelling characteristics will be recorded. The GPS coordinates of participating households will also be documented as part of the household-level assessment.

Figure 2. Schematic overview of participant flow across study components



At the **screening and enrolment (visit 1.0),** all adolescent and adult household members will be approached for informed consent/ assent. Following enrolment, demographic information and responses to a basic health questionnaire will be collected. Participants will undergo symptom

screening using W4SS. They will also answer a question about the presence and nature of any additional symptoms they are experiencing, and whether any of their symptoms have prompted them to seek health care. A minimal physical examination, including measurement of weight and height, will be performed. Following coaching, each participant will provide a minimum of 3ml sputum/respiratory tract sample for Xpert and MGIT culture testing

If following coaching, the participant is still unable or refuses to produce a 3ml sputum sample, a tongue swab will be collected for NAAT. Tongue swabs will only be collected if their programmatic use has been approved by WHO and/or national authorities (South Africa or Indonesia), and in participants unable or refuse to produce sputum.

In addition, a digital chest X-ray with computer-aided detection (dCXR/CAD) will be conducted as part of the TB screening assessment. All dCXR/CAD X-Rays which are reported as suggestive of TB by the CAD software will be reviewed by a radiologist.

Within 2 weeks of the screening visit (visit 1.0), the **adolescent/adults** screened will be categorised into one of 5 screening groups **(Figure 2).**

(1) **aTB-C Participant**: W4SS screening - negative; Xpert -positive (any grade) OR tongue swab-NAAT – positive; dCXR/CAD – any result
(2) **sTB-C Participant**: W4SS screening - positive; Xpert -positive (any grade) OR tongue swab-NAAT – positive ; dCXR/CAD - any result
(3) **aTB-U Participant**: W4SS screening - negative; Xpert -negative-OR tongue-swab NAAT – negative; dCXR/CAD – suggestive of TB
(4) **sTB-U Participant:** W4SS screening -positive; Xpert-negative OR tongue-swab NAAT – negative; dCXR/CAD – suggestive of TB
(5) **noTB Participant:** W4SS screening -negative; Xpert – negative OR tongue swab NAAT – negative ; dXR/CAD – normal

Adult and adolescent participants (≥15 years) will be identified **(Figure 2)** for potential enrolment into either the case–control phenotyping-study (Sub-study 1; aTB-C, sTB-C) or the TB-U cohort study (Sub-study 2; TB-U). For each aTB-C participant, a control participant (1:2 ratio) will be randomly selected among noTB participants within the neighbourhood (250–700 m radius depending on the population density, using GIS coordinates) of the aTB-C participant's household.
The **child household contacts (aged 2–14 years)** of those with aTB-C and their controls, and sTB-C will be eligible for inclusion in the cross-sectional study (IGRA assessments) of household Mtb infection (core study).

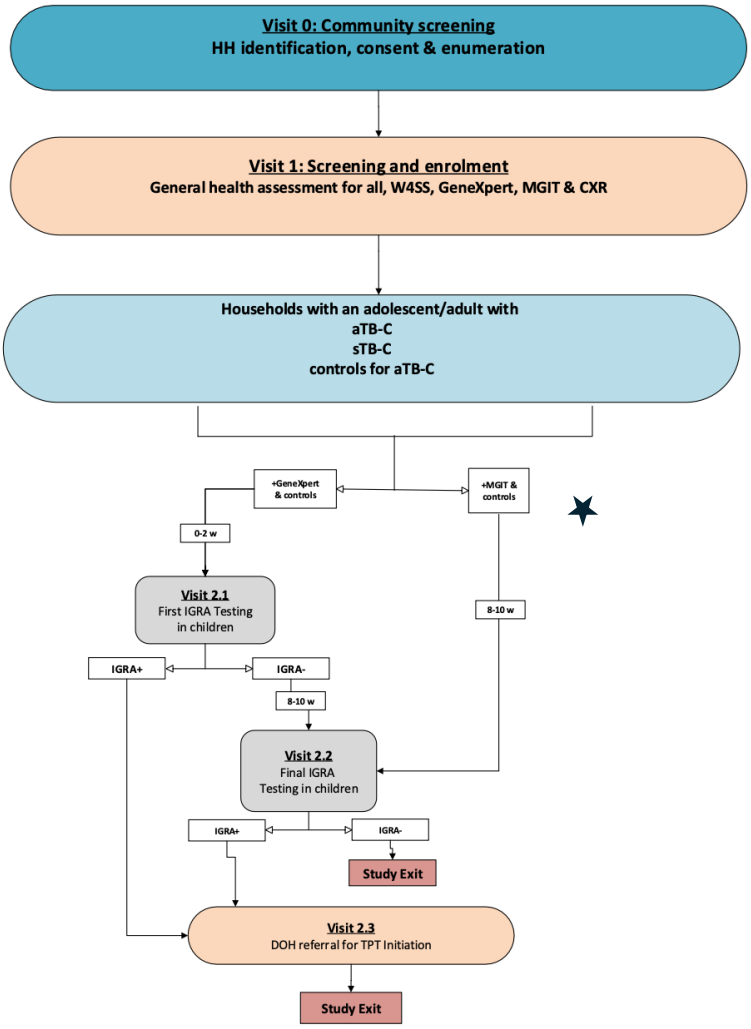
Most participants will be categorized according to their screening results which include Xpert or tongue swab NAAT and dCXR/CAD. These results are expected to be available within approximately two weeks of sample collection. The **MGIT culture results** may require up to 6 weeks to become available.-Those participants that are newly diagnosed with TB (as aTB-C, sTB-C) based on the MGIT culture results will be considered for the sub-study 1 and 2; while their child household contacts will be invited to undergo IGRA assessments. If the adolescent or adult MGIT result is positive within 2 weeks of collection, the child HHC will be invited to participate in visits 2.1 and 2.2. If the MGIT result becomes positive later than 2 weeks, only visit 2.2 will be conducted, due to study visit scheduling constraints.

All adolescent/adult participants with positive screening results (Xpert; tongue swab, MGIT) will be referred to Visit 1.1 within 2 weeks of their positive result and will receive appropriate referrals at the end of the study visit. Participants who are not eligible for any additional study visits will have their screening results returned, appropriate referrals will be made as needed, and participants will be thanked for their participation. When all results are normal and no referrals are required, participants may be informed of their results by telephone or SMS.

Households of **aTB-C participants (aTB-C household) and sTB-C participants (sTB-C household)**, as well as selected **noTB participants (Control Household)** serving as controls, will undergo a follow-up visit (Visit 2.1).

At the **community or clinic visit (visit 2.1; Figure 3), if** the child or children meet the eligibility criteria, initially parental/individual assent and/or guardian consent will be obtained for each child during this visit.

Figure 3. Core study participant flow, including IGRA testing among child contacts.



★ ***If the adolescent/adult MGIT is positive within 2 weeks, the child household contact will be eligible for both visit 2.1 and 2.2***

A **structured questionnaire** will be administered to an adult household member to collect information on household characteristics, living arrangements, and factors influencing Mtb transmission. Data will include dwelling type, ventilation, contact patterns, hygiene practices, and key individual factors such as socioeconomic status, health history, vaccination, and exposure risks. Social mixing patterns and time spent at home versus in the community will also be captured through the interviewer-administered questionnaire.

TB symptom screening will be conducted using a version of the W4SS adapted for children. Symptomatic children will have **sputum collection** attempted for testing with Xpert and MGIT. All children will have the height (or length if appropriate), weight and mid-upper arm circumference (MUAC) measured, where applicable.

Between 4 to 10 mL of **venous blood** (no more than 1ml/kg) will be collected from all children for interferon-gamma release assay (IGRA) testing, analysis of cell-free Mtb DNA and Mtb-specific T cells, and for biobanking.

In **South Africa**, **HIV testing** will be performed for children where parental or guardian consent and child assent have been obtained. Testing will be conducted with age- and maturity-appropriate pre- and post-test counselling in accordance with national guidelines

Children with a **negative baseline IGRA** result will be revisited 8–10 weeks later **(Visit 2.2)** for repeat TB symptom screening and collection of a second blood sample for IGRA, cell-free Mtb DNA, Mtb-specific T cells, and biobanking. If the child is symptomatic at that visit, sputum collection for Xpert and MGIT will again be attempted. . At both study visits, children identified with TB symptoms will be referred to the appropriate health facility for further TB assessment. The study team will provide support to strengthen local healthcare facilities by building capacity in the investigation and management of children with TB symptoms in line with national guidelines.

A community, or clinic-based follow-up visit or telephonic contact will be conducted between weeks 2 and 14 (**Visit 2.3)** for participants with positive results. This visit will be used to provide IGRA and sputum results (where sputum was collected) and to confirm that appropriate referral to the local health clinic for TPT initiation or TB treatment, as indicated, has occurred. The study team will provide support to strengthen local health care facilities by building capacity in the provision of TPT for household contacts of participants diagnosed with TB, in accordance with national guidelines.

In South Africa, HIV results will be provided with post-test counselling using language appropriate to the child's age and maturity. Children aged 12–14 years may receive their results without a parent or guardian present but will be encouraged to involve their parent or guardian in the discussion and ongoing support.

Participants with negative results will be contacted via telephone or SMS. This visit will mark the End-of-Study visit for household contacts.

SUB-STUDIES 1 and 2

Within two weeks of TB screening, eligible participants will be invited to attend the research clinic for **Visit 1.1**. Participants will remain blinded to their screening results at the time of the visit. At Visit 1.1,

individual consent or assent will be obtained for participation in the sub-studies. This visit will include all participants being enrolled in either Sub-study 1 (aTB-C, sTB-C, and control participants) or Sub-study 2 (TB-U participants).

At the clinic, **a blinded structured questionnaire** will be administered to collect detailed information on symptoms, quality of life, functional status, and health outcomes. This will be followed by a comprehensive physical examination, including assessment of vital signs, height, weight, lymphadenopathy, and breath sounds. Two samples, either an early-morning and spot specimen or two spot specimens, as per site procedures will be collected for Xpert and MGIT culture testing. Participants whose screening molecular test was a positive tongue swab NAAT, will have 3 sputum specimens collected.

Additional samples will be obtained for novel diagnostic assays, including tongue swabs, bioaerosol collection using a facemask, and exhaled breath condensate. Exhaled breath condensate (EBC)samples will be collected by trained study staff using a hand-held or desktop EBC device. Participants will breathe tidally, with or without coughing, into a straw connected to the device for 10 minutes, repeated twice. Venous blood (40mL) will be collected for HIV, viral load testing, HbA1c, full blood count, Mtb cell-free DNA, Mtb-specific T cell activation and biobanking.

A study physician will review each participant's TB screening results and advise the participant of their results at the end of the visit. They will ensure appropriate clinical referral for TB and non-TB care in line with national guidelines. The study physician will conduct scheduled remote reviews of all sputum results and the clinically reportable blood results (HBA1c, HIV, viral load, full blood count). If a positive result is identified at any time, the physician will contact the participant to ensure prompt referral to a health facility, accompanied by all relevant test results.

Ongoing contact will be maintained with all TB-C participants to encourage initiation of TB treatment and continue to assess participant well-being until linkage to care and commencement of TB treatment is documented (verification by chart review or electronic record linkage). TB treatment outcomes will be ascertained in all those that start TB treatment. Participants who decline TB treatment despite referral will be offered continued follow-up and monitoring within the TB-U cohort study. When all results are normal and no referrals are required, participants may be informed of their results by telephone or SMS.

Once all MGIT results from Visit 1.1 have final results, the study team will confirm study case definitions and communicate with participants regarding their next steps in the study. Participants in Sub-study 1 will complete their clinic-based visits after Visit 1.1 (other follow-ups telephonic or as the research team conducts IGRA assessments in the community). Participants enrolled in Sub-study 2 will continue follow-up in accordance with the established schedule, attending Visits 1.2 through 1.5 as outlined in schedule of events **(Table 3; Appendix C)**.

TB-UNCONFIRMED (TB-U) COHORT STUDY (SUB-STUDY 2)

Eligible participants for this TB-U cohort study will be evaluated in the research clinic every three months (month 3,6,9 &12) for one year **(Visits 1.2-1.5)** to monitor progression.

At each follow-up visit, participants will undergo a structured clinical assessment, including an interim health history, review of recent antibiotic or TB treatment use, detailed symptom screening, evaluation of quality of life, functional status, and health-seeking behaviour.

A comprehensive physical examination will be performed, including assessment of vital signs, height, weight, lymphadenopathy, and breath sounds.

Sputum specimens will be collected for TB phenotyping, consisting of two samples, either an early-morning and spot specimen or two spot specimens, as per site procedures. Venous blood will be drawn for phenotyping assays, including Mtb-specific T cell activation, Mtb cell-free DNA, and samples for biobanking. A dCXR/CAD will be performed at each visit. Facemask and Exhaled breath condensate will be collected from a subset of participants

A study physician will regularly review each participant's clinical, radiological, and microbiological results, including sputum findings, to assess disease progression or resolution. If a positive sputum result is identified at any time, the participant will be promptly contacted and referred for TB evaluation and treatment initiation in line with national guidelines. Referrals will be made for non-TB care as required. Ongoing contact will be maintained with all participants referred for TB treatment to encourage initiation of TB treatment and to continue to assess participant well-being until linkage to care and commencement of TB treatment is documented (verification by chart review or electronic record linkage). Individuals who commence TB treatment during follow-up will be considered to have reached the progression endpoint and will not continue with further TB-U cohort study visits.

Visit 1.5 will serve as the End-of-Study visit for participants in Sub-study 2. At this visit, clinically relevant results will be returned, participants will be counselled to seek clinical care if any symptoms or physical conditions worsen, and they will be thanked for their participation

3.6 LABORATORY PROCEDURES

SPECIMEN COLLECTION, PREPARATION, HANDLING AND SHIPPING

Maximal infection control precautions will be implemented by research staff during all study visits and specimen collection procedures. These precautions include the use of gloves, safe venepuncture equipment, and a fit-tested N95 respirator during sputum collection. Sputum will be collected in well-ventilated areas, at an adequate distance from research staff and bystanders, to minimize infection risk.

All specimens will be collected in designated containers and labelled with printed, bar-coded labels containing the participant identification number, study visit, specimen type, intended assays, and specimen destination. Specimens designated for processing at the central laboratory will be stored at the appropriate temperature and transported under controlled conditions. At the central laboratory, samples will be aliquoted for testing or storage. All specimen preparation and handling will be conducted in BSL-2 or BSL-3 facilities (as approved by the AHRI/RC3ID Biosafety Committee), which are equipped for the safe handling of specimens potentially containing Mtb or HIV.

A specimen tracking log will be maintained to document all transfers between sites, including confirmation of appropriate temperature maintenance during transport. In instances where specialized laboratory assays required for the scientific aims of the project are not available in the central study

laboratory, samples may be exported to collaborating laboratories within South Africa or internationally. These transfers will be reviewed and approved by AHRI and the UKZN Biomedical Research Ethics Committee (BREC). Appropriate Material Transfer Agreements (MTAs) will be executed between collaborating institutions, and all necessary export permits will be obtained prior to shipment.

Non-human bacterial specimens may be stored indefinitely for future analyses, while human biological material may be stored for up to 20 years. Participants will be asked to provide broad informed consent for storage and future use of their biological samples including potential genetic testing, with all relevant information regarding genetic testing, storage duration, and governance included in the consent process.

LABORATORY EVALUATIONS/ ASSAYS

All laboratory procedures will be performed in accordance with approved biosafety and quality control standards and the relevant Standard Operating Procedures (SOPs). Samples will be processed at accredited study or reference laboratories using validated methods.

(1) **Sputum**. Sputum samples (minimum 3mL) will be processed for Xpert **(GeneXpert Ultra)** or other similar assay, and for MGIT culture. Processing includes sample decontamination, aliquoting of specimens, testing of positive MGIT cultures to confirm Mtb status, and genetic sequencing of Mtb strains. Residual aliquots of sputum, MGIT culture, Mtb strains or extracted Mtb DNA may be stored.

(2) **Venous blood.** 4-10mL of venous blood will be collected from child household contacts and 40mL of venous blood will be collected from participants in the case-control phenotyping and longitudinal TB-U sub-studies.

a. Whole blood will be instilled in **QuantiFERON Gold** assay tubes, incubated according to manufacturer instructions, and plasma analysed for interferon-gamma (IFN-γ) concentration. Supernatants may be stored.
b. Whole blood will be incubated with ESAT-6/CFP-10 peptide pools and appropriate controls. Following incubation, cells will be fixed, permeabilised, stored, and later assessed by flow cytometry using the **TB-TASA assay or other similar T-cell activation assay**.
c. Whole blood will also be processed for extraction and quantification of **cell-free Mtb DNA** using validated methods, or other similar assays for the detection of Mtb products.
d. **Clinical testing** will be performed at an accredited clinical lab and will include HIV testing and reflex viral load, HbA1c and full blood count.
e. Whole blood will be processed for biobanking and will include peripheral blood mononuclear cells (PBMCs), plasma, PaxGene tubes for RNA preservation, and buffy coats. DNA, RNA or other components may be extracted and stored. To address study objectives, stored biosamples may be used for assays to characterize cellular components and function, proteomics, lipidomics, transcriptomics, metabolomics, genomics, T cell receptor analysis, serologies and pharmacologic analyses. These

samples will be stored for future studies of TB disease and genetic studies under approved biobanking procedures.

(3) Tongue swabs will be processed for detection of Mtb DNA by **PCR (e.g., PlusLife or other similar platform)**. A subset of swabs or eluted DNA may be frozen and stored for biobanking.

(4) Facemask bioaerosol samples will be analysed for Mtb DNA using validated extraction and **PCR protocols** or other similar assay.

(5) Exhaled breath condensate (EBC)samples will be collected in sterile tubes and condensed in an ice-filled cooling chamber mounted around the sterile tube within the device and shipped to the Desmond Tutu Health Foundation (DTHF) or other approved laboratory for analysis of **LAM or other biomarkers**.

3.7 POTENTIAL RISKS AND BENEFITS OF STUDY PROCEDURES

POTENTIAL RISKS

(1) Risk of spontaneous sputum collection.
Sputum collection is very safe for the research participant but does pose a risk to the study staff and/or bystanders or aerosolized transmission of a respiratory pathogen. Sputum collection will be explained to the participant by a qualified research nurse who will ensure the participant has access to a well-ventilated area, preferably outdoors, that is isolated from other people. The research nurse will wear a fit-tested N95 respirator at all times.

(2) Risk of chest x-ray.
Chest x-ray is a very safe procedure that exposes adults to a very low dose of radiation. All chest x-rays will be performed by licensed radiographers according to their safety protocols. It has been shown that even for pregnant women the benefit of dCXR for TB screening outweighs the risk of exposure of the foetus to radiation[63]. To minimize risk of exposure, a lead shield apron will be worn around the waist of all participants.

(3) Risks of venepuncture.
Venepuncture for blood sampling carries a minimal risk of pain, bruising bleeding and rarely infection. These reactions usually last only a short time (1-3 days). The risk of infection will be minimized by the use of sterile, single-use needles. Fainting or light-headedness may also occur, and usually lasts only a few minutes. Experienced, skilled staff will obtain blood samples.

(4) Stress response to previously unknown HIV or TB diagnoses.
Learning of a new TB or HIV diagnosis may cause emotional distress. Results will be communicated sensitively by trained nurses or physicians, who will provide post-test counselling and assess the need for additional support. Participants experiencing severe distress or suicidal ideation will receive immediate psychological or medical referral as appropriate.

(5) Risk of breach of confidentiality.
Strict confidentiality will be maintained. All study data and records will be de-identified, coded, and stored in secure, access-controlled databases. Disclosure of results will occur only where required by local regulations (e.g., notification of TB or HIV results to health authorities) and in accordance with institutional agreements and participant consent.

POTENTIAL BENEFITS

(1) All participants will benefit from being screening for TB. Any positive cases will be referred for treatment.
(2) All participants who are tested for HIV will receive pre- and post-test HIV counselling and learn their HIV status. Those adolescent and adult participants who are HIV-positive will learn their HIV viral load. The results and counselling will improve their knowledge of their personal health and empower them to make positive health choices.
(3) All participants who have results that require clinical action will be promptly referred to address abnormalities revealed. Standard of local care will be strengthened using study resources to support appropriate TB investigation, management, and implementation of TPT use in line with national guidelines
(4) Participants may derive satisfaction from knowing that the study results could contribute to a better understanding of asymptomatic TB and its impact on the global TB epidemic.

3.8 STUDY OUTCOMES/ ANALYSES

The study is designed to assess both the transmission of Mtb within households and the diagnostic performance of current and novel tools for detecting asymptomatic TB. The outcomes are grouped into primary and additional outcomes categories as outlined below.

CORE STUDY

Primary endpoint: Cumulative prevalence of child household contact IGRA positivity or confirmed TB (TB-C) at 10 weeks after diagnosis of the index case.

Additional outcomes/ analyses:

(1) Proportion of children that convert from negative IGRA status at baseline to a positive IGRA status at 10-week visit.
(2) Proportion of children with a positive plasma cell free Mtb DNA assay and positive Mtb-specific T cell activation assay, stratified by household group.
(3) Whole genome sequencing of Mtb isolates from people with TB-C and household contacts.

CASE-CONTROL PHENOTYPING STUDY (SUB-STUDY 1)

(4) Sensitivity, specificity, positive predictive value, negative predictive value, yield, feasibility, acceptability of digital chest x-ray with computer aided diagnosis (dCXR/CAD), sputum Nucleic Acid Amplification Test (NAAT), Mycobacteria Growth Indicator Tube (MGIT), tongue swab NAAT, and bioaerosol collection facemask mask NAAT to detect aTB-C.
(5) Symptoms, health outcomes quality of life, comorbidities, nutritional status, and TB history, relative to group and W4SS status at screening.

(6) Proportion and characteristics of participants with detectable exhaled breath condensate lipoarabinomannan (LAM), positive plasma cell free Mtb DNA assay, positive Mtb-specific T cell activation assay and additional blood-based TB biomarkers, stratified by group.

TB UNCONFIRMED (TB-U) COHORT (SUB-STUDY 2)

(7) Time to event and factors associated with microbiological or clinical progression in participants with initially untreated aTB-U.

(8) Plasma biomarkers (including cell free Mtb DNA, Mtb-specific T cells, transcriptional signatures) that correlate with progression from aTB-U in the 12 months after TB screening

(9) Proportion and characteristics of participants with TB screening results suggestive of (TB-U) who have a subsequent positive sputum GeneXpert Ultra, sputum MGIT, tongue swab NAAT or Bioaerosol collection facemask mask NAAT.

4. DATA MANAGEMENT

4.1 DATA COLLECTION

All study information will be collected electronically using password-protected tablets, with direct entry into the Research Electronic Data Capture (**REDCap**) database. REDCap is a validated software system, compliant with ICH-GCP and regulatory requirements and can support online and offline data capture.

4.2 DATA MANAGEMENT AND STORAGE

The management of study records will prioritize confidentiality, integrity, and security of data. Access to the REDCap database will be restricted to authorized study team members, using individual password-protected accounts with role-based permissions. Audit trails will automatically document data entry, modifications, and access.

To protect participant confidentiality:

- All case report forms (CRFs) will be anonymized using the study identification code.
- Laboratory samples will be labelled with unique barcodes linked to the participant's study identification code but not to personal identifiers.
- Datasets will be de-identified prior to analysis, ensuring that no directly identifying information is used in reports or publications.
- Personally identifiable information (including names, identify numbers, address, geolocation data, telephone numbers) will be stored in a separate table in the database with restricted access and identified by a separate identifier. This information will not be included on the data repository. If access to this data is required for research purposes, including for linkage to health system data (NHLS or Tier.net) it will be made available on a secured data enclave that restricts download or copying of data.

Data will be stored on secure, access-controlled servers with routine back-up procedures in place. Hard-copy study records, where required, will be kept in locked cabinets in restricted-access areas. Data management procedures will comply with institutional policies, Good Clinical Practice (GCP) standards, and relevant national data protection regulations.

4.3 PUBLICATION & DATA SHARING POLICY

Study findings will first be shared with local stakeholders and the Department of Health. Results will subsequently be disseminated through peer-reviewed publications and presentations at scientific meetings in the form of posters and oral communications. All proposed publications and presentations, including manuscripts, conference abstracts, oral presentations and posters, that include ATTIS data will be submitted to the ATTIS Scientific and Management Steering Committee for review in the form of a written proposal. Each proposal will outline the objectives, data required, proposed analyses and plans for publication or presentation. Once initial approval is granted, we will share the draft manuscript or presentation, including the proposed author list, citations of ATTIS datasets and acknowledgments with the Steering Committee for final review and approval prior to external submission.

Final peer-reviewed manuscripts will be submitted to open-access journals indexed on PubMed to ensure wide accessibility of the study outputs. In line with data-sharing principles, de-identified datasets and associated metadata will be made available to qualified researchers upon reasonable request, subject to approval by the ATTIS Scientific and Management Steering Committee and in compliance with data protection regulations.

5. STATISTICAL CONSIDERATIONS AND MODELLING

5.1 SAMPLE SIZE CALCULATIONS

CORE STUDY

The core study has two co-primary objectives: (1) to compare IGRA positivity in child household contacts of participants with aTB-C with that of community controls; (2) to compare IGRA positivity in child household contacts of participants with aTB-C cases with that of participants with sTB-c. We are using child IGRA positivity as a proxy measure of TB transmission.

Based on our existing data on the prevalence of aTB-C in each setting, we expect to screen 60,000 individuals in Bandung and 30,000 in KwaZulu Natal to identify 150 cases of aTB **(Table 3)**. We expect ~20% of children to be IGRA positive in control households in Bandung and 7% to be positive in KwaZulu Natal, and an average of 1.5 (SD=0.7) and 2.5 (SD=1.6) children per household, respectively, in each setting. We have assumed an intra-cluster correlation coefficient (ICC) of 0.20-0.30 in each setting, based on our data in KwaZulu Natal[64].

Table 3: Assumptions used to determine overall scale of screening and power for the asymptomatic TB transmission in Indonesia and South Africa study

Parameter	Indonesia	South Africa
Number screened	60,000	30,000
Asymptomatic TB prevalence (Clinical -, CXR positive, micro +/-)	0.25%	0.50%
Number of asymptomatic TB cases detected	150	150
Number of control HH	300	300

IGRA positivity in children aged 2-14 years	STB index HH: 40% Control HH: 20%	STB index HH: 18% Control HH: 7%
Household size (children aged 2-14 years)	Mean = 1.5 SD = 0.7	Mean = 2.5 SD = 1.6
Intra cluster correlation (ICC)	0.20 to 0.30	0.20 to 0.30

For our first co-primary objective, we have used the absolute risk difference in child IGRA positivity (rather than the risk ratio) as our effect estimate of interest, because of the difference in duration of exposure (lifetime exposure to community transmission for children in control households, vs a relatively short duration of exposure to the index case for children in aTB-C households).

If the true risk difference between IGRA positivity in aTB-C households compared to control households is 10-15%, inclusion of 150 aTB-C households and 300 control households will provide ≥90% power to detect a significant difference in household IGRA positivity between these groups. We will have 80% power to detect a minimum difference of 12% in Bandung and 7% in KwaZulu Natal **(Table 4).**

Table 4: Minimum risk difference that can be detected in each site with 80% power, comparing aTB and control households

Number aTB cases	Number controls[1]	Mean children per HH	ICC	% IGRA positive in control HH	% IGRA positive in aTB HH	Risk difference[2]	Power
Indonesia							
150	300	1.5	0.30	20%	32%	12%	80%
South Africa							
150	300	2.5	0.30	7%	14%	7%	80%

[1]Number of control households. [2]Absolute difference = proportion of children IGRA+ in aTB households minus proportion of children IGRA+ in control.

For our second co-primary objective, we have used the risk ratio (relative risk) as the effect estimate of interest, because the duration of exposure for children in aTB-C and sTB-C households is expected to be similar. For policy decisions, it would be useful to know that the transmission risk of aTB-C is not substantially lower than that of sTB-C; therefore, we have used a non-inferiority approach for our power calculations. We have assumed that the prevalence of IGRA positivity in children in sTB-C households is 40% in Bandung and 18% in KwaZulu Natal (our best estimates, based on available data). With 150 sTB-C and 150 aTB-C index cases, we will have at least 80% power in each setting to demonstrate that the transmission risk with aTB-C is not less than 50% compared with that of sTB-C, assuming that the true risk ratio (RR) is at least 0.90 **(Table 5).**

Table 5: Relative reduction that can be excluded with 80% power, comparing aTB and sTB households[1]

Number sTB cases	Number aTB cases	% IGRA positive in sTB HH	% IGRA positive in aTB HH	Risk ratio (aTB/sTB)	Relative reduction that can be excluded[2]
Indonesia - aTB vs sTB					
150	150	40%	28%	0.70	0.46
150	150	40%	32%	0.80	0.55
150	150	40%	36%	0.90	0.63
South Africa - aTB vs sTB					
150	150	18%	12.6%	0.70	0.43
150	150	18%	14.4%	0.80	0.49
150	150	18%	16.2%	0.90	0.55

[1]Assuming ICC=0.30.[2]Lower limit of 95% confidence interval for risk ratio

CASE-CONTROL STUDY (SUB-STUDY 1)

The case-control study will enrol 150 aTB-C and 150 sTB-C cases, and 300 community controls with no TB. Assuming that the true sensitivity and specificity of the Plus Life tongue swab and bioaerosol collection facemask diagnostic tools are between 70-95%, with 150 aTB-C and 150 sTB-C cases, we will be able to estimate the sensitivity with a precision of ±7.6% to ±4.0%. With 300 controls, we will be able to estimate the specificity with a precision of ±5.3% to ±2.6%.

TB-UNCONFIRMED (TB-U) COHORT STUDY (SUB-STUDY 2)

A total of 1000 individuals (500 from Bandung and 500 from KwaZulu Natal) identified through the community-based screening who have chest x-rays suggestive of TB and negative baseline sputum will be followed prospectively. Based on published data that found that individuals with radiographic evidence of TB progressed to microbiologically-positive disease at an annualized rate of 10% (95% CI 6.2-13.3)[65], we anticipate 31-66 progressors to microbiologically-confirmed TB from each site.

With 50 individuals who progress and 100 who do not, we will have ≥80% power to detect a difference of 0.50 standard deviations in mean biomarker concentrations between the two groups. If the prevalence of biomarkers in individuals who do not progress is 10-20%, we would have ≥80% power to detect an increase to 30-40%.

STAGE GATE

We have used our best estimates of the prevalence of TB-C, prevalence of IGRA positivity, number of children per household, and value of the ICC to inform our sample size calculations, based on the available data in each setting. However, given the limited data available, there is uncertainty in these assumptions and a small change in any one of these (e.g. in household size, ICC, or the prevalence of

IGRA positivity) can have a large impact on our power. Therefore, after 20% of the population has been screened at each site (12,000 in Bandung and 6000 in KwaZulu Natal), we will assess the accuracy of the key assumptions used in our sample size calculations. Specifically, we will analyse the available data to determine the population prevalence of aTB-C, the proportion of children aged 2-14 years who are IGRA positive, average household size, and the estimated ICC. We will then use these revised estimates to further refine our power calculations for the co-primary endpoints. The results will be discussed with the funders and, if necessary, we may adjust the project sample size, budget and timelines to ensure sufficient power to address both co-primary endpoints. We will also evaluate any data available for the TB-U cohort (proportion of participants meeting the screening definition of TB-U who are confirmed aTB-C by 2 additional sputum specimens, proportion of TB-U participants who progress to TB-C) and we may adjust the TB-U cohort size to address its powering endpoint.

5.2 ANALYTICAL APPROACH

Descriptive statistics will be used to summarise participant characteristics separately for each study setting, including household-level characteristics (e.g. socioeconomic status, household construction, number of household members), index-case level characteristics (e.g. age, sex) and child-level characteristics. The analyses for each endpoint will be conducted separately for each setting.

CORE STUDY

Comparison of aTB and control households: For the primary analysis, we will use random-effects logistic regression with a fixed effect for exposure group (aTB vs control) and a random intercept for household. Since control houses will be selected from the same neighbourhood as the aTB index cases, we will also include a random effect for matched set. From the fitted model, we will estimate the marginal risk difference in the proportion IGRA positive between the two groups.

Matching on neighbourhood will provide some control of confounding by neighbourhood-level factors (e.g. socioeconomic status, background exposure in the community). However, we will also conduct multivariable analyses, explicitly controlling for confounding using child-level (e.g. age, sex and degree of contact with index, such as sleeping in same room etc) and household-level covariates (e.g. household crowding). Covariate adjustment will be guided by theoretical causal diagrams, defined a priori.

Comparison of aTB and sTB households: For the primary analysis, we will use random-effects logistic regression with a fixed effect for TB exposure group (aTB vs sTB) and a random intercept for household. The marginal risk ratio will be estimated from the fitted model.

We will also conduct multivariable analyses, controlling for confounding using child-level, index case-level (age, sex, HIV status) and household-level covariates, as described above, with covariate adjustment guided by theoretical causal diagrams, defined a priori. If there is evidence of a difference in the infectiousness of aTB compared with sTB we will explore whether this is mediated through characteristics of index case disease severity (bacillary burden, cavitation etc) or other mechanisms.

Sensitivity analyses: In a high TB incidence setting, IGRA positivity in household members may be result of contact with infected individuals outside the household, rather than with the index case. This would lead to outcome misclassification since the assumption underlying our analysis is that IGRA-positivity is

a result of exposure to the index case. This effect should be non-differential so would bias estimates towards the null. We will conduct sensitivity analyses restricted to younger children (e.g. aged <10 years), with the assumption that younger children are more likely to have been infected by the household index case than through community or non-household contact. Additionally, quantitative bias analyses will be performed using alternate definitions of 'asymptomatic' and 'symptomatic' TB-C index cases to account for 'misclassification' between these groups based on discrepancies between W4SS status at screening and the additional blinded symptom data collected at Visit 1.1.

CASE CONTROL STUDY (SUB-STUDY 1)

We will assess the performance of the standard and novel tools for diagnosing aTB-C and sTB-C by calculating the sensitivity, specificity, PPV, NPV and yield, and 95% confidence intervals, of each tool. We will use ROC curves and Chi-squared tests to explore the association of tool performance with child household contract IGRA positivity, to assess the ability of these tools to identify individuals with aTB who contribute to Mtb transmission.

We will compare bioaerosol status between aTB and sTB index cases and controls. Bioaerosol measurements will also be compared with tongue swab NAAT results. We will also explore their association with child IGRA positivity, to assess their performance as a surrogate for Mtb household transmission.

TB UNCONFIRMED (TB-U)COHORT STUDY (SUB-STUDY 2)

We will use survival analysis methods to estimate the time to progression. The primary progression endpoint will be defined as a positive sputum test. An alternative progression endpoint will include worsening of symptoms or clinical status, radiological progression and/or starting TB treatment. Person-time will be calculated from date of entry to the TB-U cohort until the earliest of date of the progression endpoint, the last visit seen, or death. Participants who have not met the progression endpoint by the end of follow-up will be censored administratively at the date of the 12-month visit. Since we expect <15% to progress (based on published data), we will use restricted mean survival time (RMST) to estimate the average survival over follow-up. We will use linear regression to compare log-transformed plasma biomarkers between progressors and non-progressors, assuming a normal distribution, or Wilcoxon rank sum tests if data are non-normally distributed. Biomarkers that are measured as binary variables will be compared between groups using Chi-squared tests. Factors associated with time to progression will be explored using Cox regression.

6. MODELLING

The mathematical modelling in the ATTIS study will quantify the contribution of aTB to transmission and assess the potential impact of screening interventions in two high-burden settings - Indonesia and South Africa. These countries were selected because they represent diverse epidemiological contexts: South Africa has a high burden of TB and HIV co-infection, while Indonesia has a high TB burden with low HIV prevalence, driven by structural determinants like undernutrition and smoking. Modelling in these contrasting settings will generate insights that are relevant for other high-incidence countries and requires different models.

MODEL STRUCTURE AND ADAPTATION

We will build on two recently developed, calibrated transmission-dynamic models of TB:

> The South Africa model has been developed over the past two years to evaluate the population-level impact of the Gates Foundation TB Research & Development portfolio. It is calibrated to country-level epidemiological, programmatic, and prevalence survey data using a Bayesian parameter estimation process.
>
> The Indonesia model will build on the core structure used in an existing Wellcome Trust project, including both bacteriologically-confirmed and unconfirmed asymptomatic TB, calibrated to prevalence survey, demographic and other relevant data.

Both models will be adapted to include assumptions about the natural history of aTB, including infectiousness, duration, rate of progression to sTB, and the probability of self-cure. Models will be aligned through collaborative discussions with country teams and partner modelling groups to ensure consistency in structure and assumptions.

Estimate the short and long-term impact of aTB directed screening approaches in each country

(1) Review empirical evidence and consult with partners to revise model structures and define parameter ranges.
(2) Incorporate key epidemiological determinants for each country/region and the interaction with new model structure (e.g. HIV/ART and bacteriologically unconfirmed TB in South Africa, Smoking and Undernutrition with aTB in Indonesia).
(3) Implement Bayesian calibration approaches to ensure model parameters reflect uncertainty and are consistent with observed data (TB prevalence, notifications, etc.).
(4) Quantify the proportion of disease and Mtb transmission attributable to unaddressed aTB in the absence of targeted interventions.
(5) Simulate aTB sensitive community-based screening scenarios using current and novel diagnostic tools, under multiple implementation and scale-up strategies.
(6) Compare universal screening (including asymptomatic individuals) with symptom-based screening strategies.
(7) Conduct scenario analyses to assess how variations in key natural history parameters of aTB influence model projections (e.g., infectiousness, duration, self-cure, progression).
(8) Evaluate external validity by comparing findings across the two countries, which span different TB epidemic contexts.
(9) This work will be led by each modelling team with expertise of local epidemiology and relevant determinants.

Estimate the cost-effectiveness of community-based screening approaches

We will link epidemiological models to cost data from the literature, expert opinion and project expenditure to estimate cost-effectiveness of interventions from health system perspective. We will leverage cost estimates from ongoing modelling studies including the Wellcome Trust funded work in Indonesia, SA TB investment case, Gates Foundation TB portfolio analysis in SA, and the BMGF-funded tongue swab yield (TSwaY) study. These exercises will be led by the country modelling teams using local/regional data where possible.

7. ETHICAL CONSIDERATIONS

The research outlined in this protocol will be conducted in accordance with the Declaration of Helsinki; the principles of Good Clinical Practice as laid down in the ICH Harmonised Tripartite Guideline for Good Clinical Practice; and the ethics guidelines of the Department of Health of the Republic of South Africa.

The research team is aware of the need to consider the additional ethical concerns arising from working with potentially vulnerable individuals. Vulnerability may be defined due to age, potential marginalisation, disability or due to disadvantageous power relationships in personal settings. We are aware that participants may not fall into a conventionally vulnerable group but because they are in a dependent relationship, they could feel coerced or pressured into taking part.

The protocol will be submitted for review by the **Biomedical Research Committee (BREC) of the University of KwaZulu-Natal (UKZN).** In addition, the study will be reviewed and approved by the AHRI community advisory board, the uMkhanyakude and King Cetshwayo health districts and the KwaZulu Natal Provincial Department of Health research committees. In Indonesia, the study will be submitted to **Health Research Ethics Committee Universitas, Univesritas Padjadjaran (UNPAD);** Health Research Ethics Committee (HREC), Ministry of health, Kementerian and National Institute of Health Research and Development (Balitbangkes)

7.1 COMMUNITY ENGAGEMENT

Both AHRI and RC3ID have robust and well-developed relationships with the local community as well as regional and national TB, health and health research governing bodies. In both settings, preliminary discussions of this study have been conducted with local, regional and national community members, laying the groundwork for more extensive and specific engagements throughout the study lifecycle. As part of preparations for this study community representatives or Community Advisory Board (CAB) members will be engaged to review the planned study protocol, informed consents and any other study related material. The community leaders will be informed of the study for administrative approvals. In addition, community wide engagement and sensitization through dialogues and media will be conducted in the study selected communities. Throughout the study, feedback will be provided to community representatives or CABs and leadership at each critical milestone or at minimum twice a year. This will ensure that the study team addresses any challenges or community concerns regarding the study. The study team will also engage civil society groups in the relevant communities, and representatives will be invited to be part of the study advisory group.

7.2 INFORMED CONSENT/ ASSENT PROCESS

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the participants and their families. In South Africa, the Informed Consent process will be conducted by an isiZulu- and English-speaking research nurse who will be able to converse in whichever language the participant prefers.

Consent forms describing in detail the study procedures and risks are given to the participant and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB approved and the participant will be asked to read and review the document. Consent forms will be available in English and isiZulu according to patient preference. Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise.

The participants will sign the informed consent document prior to being enrolled in the study. The participants will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participants may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

At the time the study nurse will seek informed consent, the study nurse will ask the eligible participant (or the participant's representative) if the participant is literate. If the eligible participant reports he or she is not literate, then the study nurse will request that a witness be present while the study nurse reads and explains the study and what participation will entail. If the eligible participant accepts to participate, he or she will make a mark on the signature line of the consent form. The witness will also sign and date the form, if the witness is confident that the participant has understood the explanation and is participating willingly. In addition, the witness will complete the date line for the participant.

Participants aged 2-17 years
In this study adolescents aged 15 to 17 years will be screened in the community-based TB screening and will be eligible to participate in the nested case-control phenotyping study (sub-study 1) and TB-U cohort study (sub-study 2). Children aged 2- 14 years will be eligible to participate in the cross-sectional study of household Mtb infection (core study).

The parent or guardian will first be approached to provide permission for the child to be invited to consider participation in the research, prior to any contact being made with the child. The study will then be explained to the child in age-appropriate language, and the child will be asked simple questions to confirm that they understand the study well enough to decide whether they want to take part.

Where a child demonstrates adequate understanding and wishes to participate, their assent to take part will be obtained in addition to written parental consent. Where a child is too young (under 7 years) or is unable to demonstrate sufficient understanding to make this decision, the parent or guardian will be asked to decide on the child's behalf, in the child's best interests.

Pregnant women:
Pregnant women will be included in the study and will receive appropriate counselling on all study procedures. The procedures are non-invasive and carry negligible risk to both the mother and the foetus. These include questionnaires, symptom screening, digital chest X-ray performed with appropriate abdominal shielding in accordance with WHO recommendations, and collection of biological samples. Participation will be entirely voluntary, and all information regarding potential risks and benefits will be explained in clear and understandable language.

Prisoners:

Since there are no prisons within the study area, prisoners will not be included in this study.

Adults lacking mental capacity

"Mental capacity" refers to an individual's ability to understand, retain, and use information to make an informed decision. Given the complexity of the biosampling and testing involved in this study, it is unlikely that individuals with limited mental capacity will be able to fully comprehend or provide informed consent for participation in research procedures, including genetic testing or future-use investigations.

However, to ensure equitable access to potential health benefits, such individuals may be offered participation in routine TB screening procedures only. No research-specific samples or data will be collected from participants who lack capacity, and their inclusion will not extend beyond activities required for clinical screening or routine referral.

Participants in Dependent Relationships

Other participants in dependent or unequal relationships, who do not fall within the specific vulnerable groups described above, may also experience forms of vulnerability. Fieldworkers will be trained and sensitised to recognise potential coercion or undue influence and will take all reasonable steps to ensure that consent is freely given and fully informed. Vulnerability will be assessed on a case-by-case basis, acknowledging that individuals or groups not typically considered vulnerable may become so through circumstances related to study participation, which could increase or exacerbate their vulnerability.

7.3 PARTICIPANT CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators and study staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating participants with the exception of test results for which there is a data sharing agreement between AHRI and the Department of Health. These include sputum Mtb GeneXpert and Mtb culture results and HIV test results which in accordance with a memorandum of understanding with the District Department of Health (DOH) must be notified to local health services.

In South Africa, permission will be sought from the Department of Health (DoH) to access the National Health Laboratory Service (NHLS) online platforms and Tier.Net, the DoH electronic patient management system, in order to ensure that participants are appropriately linked to care and to follow long term TB outcomes of study participants.

The study protocol, documentation, data and all other information generated will be held in strict confidence.

Demographic, clinical and laboratory data will be collected. Each participant will be assigned a unique study number. The electronic database will only contain participants' study numbers – no other personal identification will be captured. Data will be entered into an access-protected databases and backed-up on an external server. Participant identification data, including contact details and telephone numbers, will be kept separately in a database to which only the Research Data Management team will have access.

7.4 CHILD TB MANAGEMENT AND REFERRAL SUPPORT

The diagnosis and management of TB in children within existing national TB programmes remains challenging, particularly in terms of systematic screening, access to diagnostic tools, and clinical capacity for paediatric TB management. Through this study, it is anticipated that additional children with TB symptoms or TB infection may be identified and referred to local healthcare facilities for further investigation and treatment, thereby increasing the number of children requiring clinical care.

The study team undertakes to strengthen and capacitate these facilities to manage children with TB, at the expense of the study, through training and linkage support. While this may place temporary additional demand on local health services, the study will have the broader benefit of facilitating earlier identification and appropriate management of children with TB, thereby contributing to improved health outcomes and strengthening of the existing healthcare system. All activities will be implemented in close collaboration with the Department of Health to ensure full alignment with national policies, service delivery structures, and referral pathways.

7.5 FUTURE OF STORED SPECIMENS

Broad consent will be sought for storage of biospecimens for future use by researchers since establishing a biobank to advance understanding of TB is an objective of this study. Biosamples collected in Indonesia will be governed by Indonesian laws and the local IRB (**the Health Research Ethics Committee Universitas, Univesritas Padjadjaran (UNPAD**)) while those in South Africa will be governed by South African laws and the local IRB (**Biomedical Research Committee (BREC) of the University of KwaZulu-Natal (UKZN**). During the informed consent process, participants will decide whether they agree to the future use of his/her specimens as approved by the IRB. Any requests for access to and use of the stored specimens from the study biobank will need to be reviewed and approved by the study Principal Investigators and the ATTIS Scientific and Management Steering Committee. Ethics approval from the IRB will be required for use of stored specimens for purposes outside those described in the protocol. Specimens from South African participants will be maintained in the AHRI biorepository and specimens from Indonesian participants will be maintained in the UNPAD biorepository. In each biorepository they will be catalogued by study identification number without any name or identifying information. The IRB will review future studies prior to their being undertaken. Any future studies with the stored specimens will have the following protections of confidentiality: specimens will be coded, bar-coded and de-linked. Genetic testing may be performed in participants who have provided specific informed consent for it. Unless specifically indicated by the IRB, participants will not be re-contacted to consent use of their specimens for this research. Participants will be able to withdraw their consent to storage of their specimens at any time.

7.6 REIMBURSEMENT

As recommended in the NHREC guidelines and in accordance with the community engagement principles of AHRI, we will calculate our reimbursement schedule based on three components: Time, Inconvenience and Expenses. For study visits involving children, reimbursement will be provided to the parent or legal guardian. Reimbursement will not be provided directly to the child. Age-appropriate refreshments will be offered to child participants as a gesture of appreciation.

Reimbursement for each ATTIS study visit (except visits 0 and visits 2.3): R150

The ATTIS study visits will require no more than 2-3 hours of the potential participant's time.

Component 1: TIME (R50)

Time off work is remunerated at the rate of unskilled labour which is approximately R20 per hour. The expected time for the visit is no more than 2.5 hours, therefore reimbursement for the time component will be R20 x 2.5 hours = R50.00.

Component 2: INCONVENIENCE (R50)

Participants will be reimbursed for inconvenience for procedures that are greater than that which the participant encounters in daily life. The ATTIS study procedures may include a dCXR/CAD, sputum collection, blood draw and bioaerosol sample collection. For the inconvenience of these procedures, which are somewhat more than that which the participant encounters in daily life, the participants will be reimbursed at a rate of R50

Component 3: EXPENSE (R50)

The participant will be reimbursed with R50 to cover missed meals during the time spent at the study visit. Transportation to and from the study visit will be provided by the study team, so will not be reimbursed to the participant.

8. STUDY GOVERNANCE

8.1 THE ATTIS SCIENTIFIC AND MANAGEMENT STEERING COMMITTEE

The **ATTIS Scientific and Management Steering Committee** will provide overall oversight of the study, including governance of data and sample use, approval of protocol amendments, and review of associated grants. The Committee will guide the evaluation of biomarkers, authorize the use of biosamples, and oversee decisions regarding publications and authorship.

Any decision to introduce an additional diagnostic test or emerging digital tool will be based (i) the value of the scientific knowledge to be gained, (ii) the operational implications of the additional test and the capacity of the field team to execute these activities, and (iii) the availability of funds to support the additional staff requirements, equipment, consumables, or laboratory costs.

8.2 NOVEL TOOLS SELECTION COMMITTEE

A **Novel Diagnostics External Advisory Committee** will provide up to date guidance on novel diagnostics that should be tested in the study based on evolving evidence. This Committee will convene at regular intervals and on an ad hoc basis as required, maintaining close coordination with the ATTIS Scientific and Management Steering Committee.

8.3 CLINICAL SAFETY COMMITTEE

The **Clinical Safety Committee** will be responsible for ensuring participant safety and adherence to national and international standards of care throughout the study. Clinical safety oversight will include

local clinical experts, including infectious disease specialists and paediatricians, who are independent of the study to ensure objective assessment and guidance. A Clinical Safety Committee will be established in each participating country, with regular cross-country communication to ensure alignment and consistency across study sites.

Its responsibilities will include:

(1) **Guideline Review**
Regularly reviewing national and local guidelines for TB diagnosis, management, and treatment to ensure that study referral procedures remain consistent with current standards of care.

(2) **Oversight of SOPs**
Reviewing and providing input on standard operating procedures (SOPs) that govern treatment referral following phenotyping or longitudinal visits.

(3) **Case Review**
Review individual participant cases that fall outside of established SOPs following phenotyping or TB-U cohort visits, including those with difficult categorization or results interpretation, and will advise on

This committee will convene at regular intervals and on an ad hoc basis as required, maintaining close coordination with the ATTIS Scientific and Management Steering Committee to ensure that safety oversight is integrated with overall study governance.

8.4 STUDY ADVISORY COMMITTEE

An independent advisory group, that consists of two researchers, a statistician and a department of health and community representatives from each country will provide oversight to the study. We will also approach the World Health Organization for a recommendation of a representative to join the study advisory group. This group will meet every six months to review progress, adherence to protocol, and participants' safety, providing advice on all aspects of the study. Major protocol amendments will also be shared with the group. Observers such as all the investigators/work plan leads, and the funders' representatives will be invited to join the advisory group meetings.

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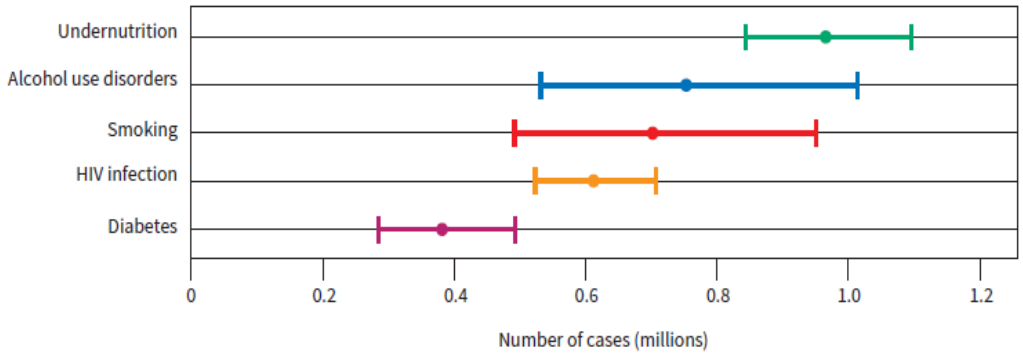
APPENDICES

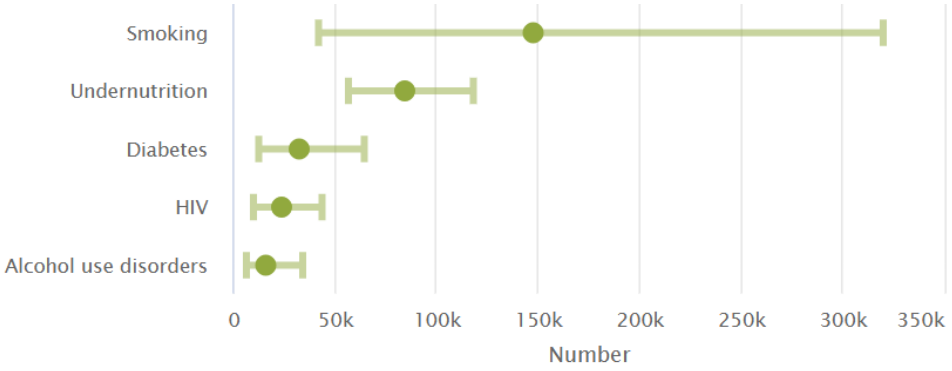
APPENDIX A: TB RISK FACTORS IN INDONESIA AND SOUTH AFRICA

Globally, undernutrition, alcohol use disorder, smoking, and HIV infection are the main risk factors for TB, with smoking and undernutrition most prominent in Indonesia, and HIV infection and alcohol use disorder in South Africa (A = global; B = Indonesia; C = South Africa).

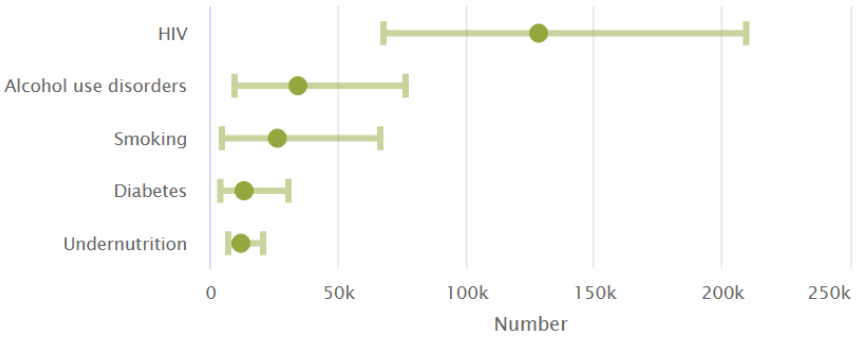
A) Cases attributable to the five TB risk factors globally



B) Cases attributable to the five TB risk factors Indonesia



C) Cases attributable to the five TB risk factors South Africa



APPENDIX B: TB NOTIFICATIONS AND BURDEN IN INDONESIA AND SOUTH AFRICA

Table 6: TB notifications and estimated TB Burden in Indonesia and South Africa in 2023[1]

	Indonesia	**South Africa**
Total TB incidence (n) **Total TB incidence rate per 100 000**	1.1M (995K-1.2M) * 387 (354-432)	270K (168K-395K) * 427 (265-626)
HIV negative TB mortality	125K (108K-143K) *	25K (23K-26K) *
HIV positive TB mortality	6.2K (5.3K – 7.1K) *	31K (9K-66K) *
TB Notifications		
New and Relapse Cases (n) **New and Relapse Cases rate per 100 000**	804.8K * 286/ 100 000	211.8K* 335/100 000
Percentage of new and relapse tested with rapid diagnostics at diagnosis	57%	83%
Total cases notified (n)	821.2K*	222K*
Percentage new and relapse with known HIV status	61%	84%
People with TB with known HIV-positive status	3.5% (16.2K*)	54% (95.8K*)
Estimated percentage of new TB cases with MDR/RR-TB	2.1% (2-2.1)	3.1% (3.1-3.2)

*Note: *Number: K= 000 or M= 000 000; ^Rate: (no. per 100 000 population per year*

APPENDIX C : SCHEDULE OF STUDY PROCEDURES

Table 7: Schedule of Study Procedures

Study	Cross- Sectional study (core study)					Phenotypic (Sub-study 1)	Cohort (Sub-study 2)			
Study population	Adolescents & Adults (> 15 yrs.)		Children (2-14 years)			Adolescents & Adults				
Visit	Visit 0	Visit 1	Visit 2.1	Visit 2.2	Visit 2.3	Visit 1.1	Visit 1.2	Visit 1.3	Visit 1.4	Visit 1.5
Time point		screening	Week 0-2*	Week 8-10	Week 2-14	Week 1-8	Month 3	Month 6	Month 9	Month 12
Household census and consent	x									
Informed consent / assent		x	x	x [b]		x				
Demographics		x								
Medical History		x				x				
Structured questionnaire			x	x [b]		x				
TB Symptom Screening (W4SS)		x	x	x						
TB Symptom Assessment (full)						x	x	x	x	x
Clinical Assessment incl. quality of life, functional status							x	x	x	x
Physical Examination -limited		x	x	x [b]						
Physical Examination - full						x	x	x	x	x
Sputum sampling – NAAT & MGIT		x	x[a]	x[a]		x	x	x	x	x
Blood sample- HIV Counselling and testing $			x	x [b]		x				
Blood sample- IGRA			x	x[c]						
Blood sample – HbA1c, full blood count						x				
Blood sample – Mtb-specific T cell activation; Mtb cell free DNA; biobanking			x	x		x	x	x	x	x
Digital X-Ray with CAD		x					x	x	x	x
Novel tests – tongue swab NAAT; facemask; exhaled breath condensate						x	x[d]	x[d]	x[d]	x[d]
Physician review						x	x	x	x	x
Referral to DOH – TB assessment, as required			x	x	x	x	x	x	x	x
Referral to DOH – TPT, as required			x	x	x					

x[a]: if child is symptomatic; **x [b]**: if adult diagnosed on MGIT> 2 weeks after collection, child consent/assent, structured questionnaire and HIV will be taken at visit 2.2; **x [c =]** : IGRA repeated at visit 2.2 if negative at visit 2.1 **x[d]**: facemask and exhaled breath condensate to be collected at all study visits in a subset of TB-U cohort participants (first n=100); ***** If adolescent/ adult MGIT positive within 2 weeks the child HHC will be eligible for visit 2.1 & 2.2; if MGIT positive> 2weeks, the child HHC will only attend visit 2.2; **$:** HIV testing of children will only occur in South Africa