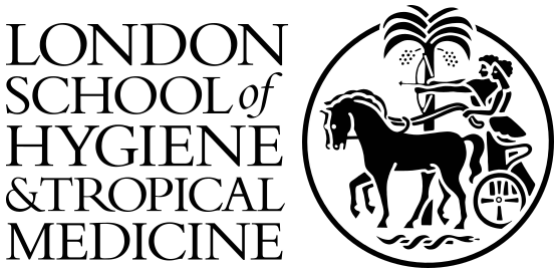

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Prevalence of *Mycobacterium tuberculosis* infection among adolescents in rural KwaZulu-Natal, South Africa

Themba Mzembe

Thesis submitted in accordance with the requirements for the degree of Master of Philosophy of the

University of London

Department of Clinical Research

Faculty of Infectious and Tropical Diseases

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

Funded by the Viiv Healthcare Positive Action Programme

Research group affiliation(s): The TB Centre LSHTM

Declaration

I, Themba Mzembe, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Role of candidate

This thesis reports on the baseline survey from a planned study which aimed to estimate the incidence of *Mycobacterium tuberculosis* infection among adolescents using an interferon-gamma release assay (IGRA). The design was to have survey at baseline and follow up at 12 months. Due to logistical constraints, the follow up will not be conducted within the timeframe of this research degree.

The idea to use adolescents was developed by my supervisor, Alison Grant and the protocol was written by Alison Grant and Richard Lessells. I was appointed after the protocol was already written. I designed the questionnaires and wrote standard operating procedures for the baseline survey with input from my supervisors Alison Grant and Kathy Baisley and from Aaron Karat. I oversaw the implementation of the questionnaires into the REDCap system and checked for errors. I conducted training for the field staff, led the field work and coordinated with the laboratory staff with operational input from Anita Edwards and clinical support from Safiyya Randera-Rees. Throughout the data collection process, I was responsible for the data management. I performed the analyses and wrote the two manuscripts with scientific, statistical and data management input from Alison Grant, Kathy Baisley and Palwasha Khan.

Abstract

Despite effective treatment being available since the 1940s, tuberculosis (TB) remains a leading cause of death by a single infectious agent. Continued transmission is one of the driving factors of the TB epidemic. In high TB and HIV prevalent settings *Mycobacterium tuberculosis* transmission dynamics are still poorly understood. Throughout adolescence, individuals have increasing social contacts with the wider community. Thus, understanding *M. tuberculosis* infection among adolescents has potential for understanding *M. tuberculosis* infection in the population. This thesis reports findings from a cross-sectional study among adolescents in a high TB and HIV prevalence setting.

A sample of adolescents (aged 10-19 years) resident in the southern part of the Africa Health Research Institute(AHRI) demographic surveillance area (DSA) was enrolled from their homes. Blood samples were obtained, and *M. tuberculosis* infection was measured using the QuantiFERON-TB Gold-plus assay.

A total, 1,094 adolescents were enrolled, and the prevalence of *M. tuberculosis* infection was 22.8% (95% confidence interval [CI]: 20.4-25.3%). Older age, history of lifetime household TB contact and living in communities with high HIV prevalence were associated with increased odds of *M. tuberculosis* infection. There was no evidence of association between *M. tuberculosis* infection and Bacillus Calmette-Guérin (BCG) vaccination, household socioeconomic status, and increased cumulative monthly contact hours with either adult males or females. The spatial distribution of *M. tuberculosis* infection showed geographical variation. Communities with increased *M. tuberculosis* infection prevalence were observed on the south eastern part of the study area where population density and HIV prevalence are higher than other parts of the study area.

The findings in this research show that household contacts of individuals with TB disease and individuals from communities with high HIV prevalence remain at risk of infection and should be prioritised for TB prevention and care activities.

Acknowledgements

I would like to express my gratitude to my supervisors Alison Grant and Kathy Baisley for their support and guidance throughout the duration of my research training. I am grateful to my co-supervisor Palwasha Khan for her critical scientific input at the critical time in my studies. I am grateful to Aaron Karat and Richard Lessells for their wonderful input in setting up the study in the early days; Safiyya Randera-Rees for her wonderful clinical input and support in the early months of data collection; Dickman Gareta, Njabulo Dayi and the data management team for their help in setting up and managing the database; Theresa Smit, Siva Danaviah and the lab team for their support in processing the samples and timely delivering results. In addition, I would like to thank my advisors Chris Grundy and Olivier Koole for their input.

I am hugely indebted to Anita Edwards for her wonderful operational and logistical input during fieldwork. I wish to also acknowledge the practical and administrative support from Adrienne Burrough, Elaine Henry and Laure Disson from the school. I am also grateful to Sphephelo Dlamini for his help in coordinating the field team. I am deeply grateful to the field team: Thokozani Bhengu, Sandile Mthembu, Mandlankosi Khumalo, Zinhle Mthembu, Sinothile Ntshangase, Landiwe Ndimande, Phumelele Mthethwa, Zama Mbatha, Nkosingiphile Buthelezi and Gugu Myeni for sacrificing their weekends and working late hours to achieve the recruitment target for a group of individuals who were difficult to find at home.

I would like to thank the participants, their parents and the community for supporting the research work and to the ViiV Healthcare Positive Action Programme for funding and supporting this work.

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List of abbreviations

AHRI	Africa Health Research Institute
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
BCG	Bacillus Calmette–Guérin
BREC	Biomedical research ethics committee of the University of KwaZulu-Natal
CAR	Central Africa Republic
CI	Confidence interval
CFP-10	Culture filtrate protein 10
DOH	Department of health
DR-TB	Drug resistant tuberculosis
DSA	Demographic surveillance area
DSID	Demographic surveillance identifier
ESAT-6	Early secreted antigenic target 6
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
HIV	Human immunodeficiency virus
ID	Identifier
IFN-γ	Interferon-gamma
IGRA	interferon gamma release assay
IQR	Interquartile range
IU	International units
K-RITH	KwaZulu-Natal Research Institute
KZN	KwaZulu-Natal

LiHep	Lithium Heparin
LIMS	Laboratory information management system
LSHTM	London School of Hygiene & Tropical Medicine
ml	Millilitre
M. tuberculosis	*Mycobacterium tuberculosis*
NHLS	National Health Laboratory Service
NS	Not specified
NTM	Non-tuberculous mycobacteria
OR	Odds ratio
PHC	Primary healthcare clinic
PIP	Population intervention platform
PPD	Purified protein derivative
QFT-GIT	QuantiFERON-TB Gold In-Tube test
QFT-Plus	QuantiFERON®-TB Gold Plus
REDCap	Research electronic data capture
SA	South Africa
SSA	Sub-Saharan Africa
SES	Socioeconomic status
SOP	Standard operating procedure
TB	Tuberculosis
TST	Tuberculin skin test
UKZN	University of KwaZulu-Natal
WC	Western Cape
WHO	World Health Organization

Chapter 1 Introduction

1.1. Background

Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* or any of the related members of the *M. tuberculosis* complex family. TB mainly affects the lungs (pulmonary TB) but can also affect other parts of the body (extra-pulmonary TB). TB is transmitted when infectious individuals with pulmonary TB expel the TB bacilli (by for example coughing, sneezing, singing or laughing) into the air and is inhaled by susceptible individuals. Transmission usually occurs indoors in poorly ventilated areas, as the bacilli are quickly destroyed by ultraviolet light in well ventilated areas [1, 2].

Though effective treatment for TB disease has been available since the 1940s, TB remains a major health problem globally. In 2017, an estimated 10.5 million people fell ill of the disease and an estimated 1.5 million TB deaths were reported making TB the leading cause of death by a single infectious agent [1].

The current global approach for TB prevention and care is framed in the “End TB Strategy” which was endorsed by Member States of the World Health Assembly and adopted by the WHO in 2014. The “End TB Strategy” sets out an ambitious goal to end the global TB epidemic with the target of reaching a 90% reduction in the TB incidence rate by 2035 compared to the rate in 2015 [3, 4]. In order to reach this target, there is need to improve our understanding of *M. tuberculosis* transmission dynamics in high prevalence settings.

South Africa has one of the highest annual TB notification rates [5], driven predominantly by the HIV epidemic [5, 6]. Within South Africa, the KwaZulu-Natal province has one of the highest annual TB notification rates. Overall, the annual TB notification rates have been declining in since 2009, but remain above 500 per 100,000 population [7]. Furthermore, the decline is insufficient to reach the global TB targets to reduce incidence by 50% and mortality by 75% in 2025 compared to 2015 rates [3, 5]. In KwaZulu-Natal, the decline has been slower than other provinces [7]. To reach the global targets the current decline needs to be more than doubled by 2020 [3].

1.2. Natural history of *Mycobacterium tuberculosis* infection

The natural history of TB infection is classically divided into two distinct progressive stages; latent TB infection (LTBI) and TB disease. The WHO defines LTBI as:

"…a state of persistent immune response to stimulation by Mycobacterium tuberculosis antigens with no evidence of clinically manifest active TB." [2]

An estimated 5-15% of recently infected individuals progress rapidly (within 2 years) to active TB disease [2]. Approximately 23% of the global population are latently infected [8]. Latently infected individuals do not manifest signs or symptoms of active disease and do not transmit the infection. Infants and individuals with HIV infection or other immunosuppressive conditions are at an elevated risk of progressing from TB infection to TB disease. On the other hand, individuals with active disease manifest signs or symptoms of TB disease (including cough, fever, night sweats and unexplained loss of weight) and can transmit infection [2].

1.3. Measuring *Mycobacterium tuberculosis* infection

There is currently no gold standard diagnostic test for measuring *M*. *tuberculosis* infection. The two main methods widely used are tuberculin skin tests (TSTs) and interferon gamma release assays (IGRAs) [9, 10]. For close to a century, TSTs were the only diagnostic test for *M. tuberculosis* infection. TSTs involve the intradermal injection of standardised purified protein derivative (PPD) on the underside of the arm. This produces a delayed hypersensitivity reaction on the injected site in infected individuals. Diagnosis is made by measuring the size of induration at the injected site within 48-96 hours [10, 11]. The major limitations with TSTs is that they are prone to false positive results due to cross-reactivity with Bacillus Calmette-Guérin (BCG) vaccination, sensitization to other non-tuberculous mycobacteria (NTM) and the 'booster' effect due to repeat testing. False negative results also occur due to severe illness or immune suppression [11-13].

Since the early 2000s, several generations of IGRAs have been developed. IGRAs are whole blood tests for *M. tuberculosis* infection. They measure interferon-gamma (IFN-γ) release by T-cells following stimulation by antigens specific to *M. tuberculosis* infection. There are two main IGRAs available: the QuantiFERON-TB and the T-SPOT.TB (Oxford Immunotec, Abingdon, United Kingdom). The T-SPOT.TB is an enzyme-linked immunospot (ELISPOT) based assay. It measures *M. tuberculosis* infection by quantifying the number of peripheral mononuclear cells producing IFN-γ after stimulation with peptides from early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) antigens. On the other hand, the QuantiFERON-TB is an enzyme-linked immunosorbent assay (ELISA) based assay. It measures *M. tuberculosis* infection by quantifying the amount of IFN-γ produced following stimulation with antigens specific to *M. tuberculosis* infection [12, 14-16].

The latest generation of the QuantiFERON-TB, the QuantiFERON-TB Gold Plus (QFT-Plus) (QIAGEN, Hilden, Germany) uses four tubes: the mitogen tube which acts as a positive control for checking correct blood handling and incubation; the nil tube acts as a negative control i.e. to determine if an individual has a pre-existing immune response and two antigen tubes (TB1 and TB2). Both TB1 and TB2 tubes include peptides from ESAT-6 and CFP-10 antigens. The peptides from the TB1 tube are designed to elicit responses from CD4 T-cells while the peptides from the TB2 tube are designed to elicit additional responses from CD8 T-cells which is expected to improve the test's sensitivity compared to the previous generations. Results are interpreted by comparing IFN-γ values (in international units per millilitre [IU/ml]) obtained from the nil tube to the IFN-γ values obtained from either of the antigen tubes [11, 12, 17-19].

Both TSTs and IGRAs are indirect markers of *M. tuberculosis* exposure and do not discriminate latent *M. tuberculosis* infection from active TB disease. They also do not discriminate individuals who have infection from those who have cleared the infection. Both tests are affected by severe immune suppression [15]. The antigens used in IGRAs are not produced by the strains used in BCG vaccines and most NTMs [11, 19-21]. Thus, IGRAs are

considered to be more specific tests *M. tuberculosis* infection compared to TST. As *ex vivo* tests, IGRAs are not affected by the 'booster' effect due to repeat testing [11-13]. IGRAs have not been extensively used in low income settings due to the high cost of supplies and the requirement for extensive laboratory facilities to perform the test [11]. More details on interpreting results of TST surveys is given in Chapter 2, Section 2.2.2.

1.4. Rationale for measuring *Mycobacterium tuberculosis* infection among adolescents

The WHO defines adolescents as individuals aged 10-19 years [22]. Individuals aged 10-14 years are referred to as young adolescents while 15-19-year olds are referred to as older adolescents. Adolescence is a period of rapid biological, psychological and social transitions [23]. It is a period where individuals learn behaviours that will last their lifetime. Because of their uniqueness adolescents require specific attention in terms of policy and service delivery. However, for a long-time little consideration has been given as adolescents are usually categorised as either children (≤15 years) or adults (≥15 years) [22-24].

Targeting adolescents is critical in order to have a future TB free generation as adolescents form the reservoir for future cases [22] as such adolescents are the target group for prevention of infection (POI) TB vaccines [25-28]. However, little is known about the prevalence and incidence of *M. tuberculosis* infection among adolescents in many settings.

Data on *M. tuberculosis* infection largely comes from TST surveys among young children(<10 years) [29]. *M. tuberculosis* infection in young children acts as a marker of recent transmission from infectious individuals with TB disease [30]. Thus, *M. tuberculosis* infection among young children has been used to make inferences about transmission in the wider population. However, compared to adolescents and adults, young children are more likely to be infected within the household [10, 31, 32]. Though household contacts of individuals with TB disease remain at increased risk of infection due to close contact, both epidemiologic and molecular studies have shown that household transmission accounts for

only between 8-20% of all transmissions [33-36]. This suggests that majority of transmissions are likely to occur in the wider population.

Adolescence is period where individuals undergo rapid changes in their social contact patterns with increased social contacts in the general community. This increases the potential of coming in contact more sources of *M. tuberculosis* infection [37, 38]. This suggests that understanding *M. tuberculosis* infection among adolescents has potential for a better understanding of the dynamics of *M. tuberculosis* transmission in the wider population.

1.5. Aims and objectives

The aim of this thesis is to describe the prevalence of and risk factors for tuberculosis infection among adolescents in a rural South African district with high TB and HIV prevalence. This thesis is based on a study which was originally designed to measure *M. tuberculosis* infection incidence among adolescents, but to date only the baseline survey has been completed, for operational reasons. This thesis reports on findings from the baseline survey.

The specific objectives are:

1. To estimate the prevalence of *M. tuberculosis* infection among adolescents.
2. To describe risk factors for *M. tuberculosis* infection among adolescents
3. To explore geospatial distribution of *M. tuberculosis* infection prevalence among adolescents.

1.6. Thesis outline

In chapter 1, I describe briefly the current state of the global TB epidemic with an overview for the current state in South Africa. I further describe the natural history of TB infection, how M. tuberculosis infection is measured and provide the rationale for understanding infection in adolescents. Finally, I outline the aim and objectives of this thesis.

In chapter 2, I review the published literature on the prevalence, risk factors and spatial distribution of *M. tuberculosis* infection prevalence among adolescents in sub-Saharan Africa.

In chapter 3, I provide a detailed description of the study setting and the methodologies for the design and procedures of the cross-sectional study investigating *M. tuberculosis* infection among adolescents.

The main results from the study pertaining to the characteristics of the study participants, the distribution of the main outcome and the description of the geospatial distribution of *M. tuberculosis* infection are presented in chapter 4. In chapter 5, I present the results for the prevalence and risk factors for *M. tuberculosis* infection.

In chapter 6, I summarize the main findings and discuss the implications on *M. tuberculosis* infection. I discuss the limitations of the study and provide recommendations for policy and future research.

Chapter 2 Literature Review

2.1. Introduction

The aim of this chapter is to provide an overview of the prevalence, risk factors and spatial distribution of *M. tuberculosis* infection among adolescents (10-19 years) in sub-Saharan Africa.

2.2. Methods

2.2.1. Search strategy

Two systematic literature searches were conducted in the MEDLINE (Medical Literature Analysis and Retrieval System Online), EMBASE and Global Health databases. The first search for prevalence and risk factors for *M. tuberculosis* infection among adolescents in sub-Saharan Africa included the following terms: adolescent, latent tuberculosis infection, prevalence and sub-Saharan Africa. The second search for spatial distribution of *M. tuberculosis* infection among adolescents included the terms: sub-Saharan Africa, latent tuberculosis infection and spatial distribution. The terms were adapted for each database. The list of synonyms for each search term and how the searches were performed in the MEDLINE database is shown in Table 2-1. References of the articles included in the review were reviewed for additional articles which might have been missed in the search.

2.2.2. Study selection

Research articles were included in the review if they met the following criteria: published in English, reporting a study conducted in any sub-Saharan Africa country, reported *M. tuberculosis* infection among adolescents (10-19 years) or any part of this age group, population based and conducted after January 2000 as this was the time when antiretroviral therapy (ART) become available in sub-Saharan Africa after the resurgence of TB incidence due to the HIV epidemic in the mid-1990s [39].

Table 2- 1: Literature search terms as performed in the MEDLINE database

Tuberculosis infection (1)	**Adolescent (2)**	**Sub-Saharan Africa (3)**	**Prevalence (4)**	**Geospatial (5)**
(Tuberculosis infection OR TB infection OR Latent tuberculosis infection OR Latent TB infection OR ((Latent*) adj3 (infect*)) OR Mycobacterium adj3 infect* OR ((Mycobacterium tuberculosis) adj3 (infection)) OR ((Latent*) adj3 (mycobacterium tuberculosis)) OR Latent mycobacterium tuberculosis infection) OR exp tuberculin test/ or exp latent tuberculosis/	(Adolescent* OR child OR Children OR Teenage* OR School going children OR school going child OR School going OR Youth* OR Young adult OR Pubescent OR Juvenile OR Underage OR Young person* OR Young people) OR exp adolescent/	(South* Africa* OR Africa OR sub-Sahara* Africa OR sub-Sahara* Africa OR Malawi* OR Botswana OR Namibia OR Swaziland OR Lesotho OR Mozambique OR Zambia OR Zimbabwe OR Tanzania OR Rwanda OR Uganda OR Kenya OR Gambia OR Ethiopia OR Sudan OR Angola OR Congo OR Madagascar OR Chad OR Cameroon OR Guinea) OR exp "Africa south of the Sahara"/	(Prevalen* OR Risk OR Annual risk OR annual risk of infection OR risk factor* OR Predictive factor)	(Geospatial OR Spatial OR Spatial patterns OR Cluster* OR spatial Distribution OR Map OR Mapping OR mapped OR Hotspot)

Search 1 for *M. Tuberculosis* infection prevalence among adolescents in Sub-Saharan Africa: 1 AND 2 AND 3 AND 4
Search 2 for spatial distribution of *M. tuberculosis* infection in Sub-Saharan Africa: 1 AND 3 AND 5

2.2.3. Data extraction

Standard data extracted included: author, year the study was conducted, country (province or city), participants (number included in analysis and selection method), method of testing TB infection, risk factors for and estimates of *M. tuberculosis* infection prevalence. For the geospatial review, the spatial method used, and key results were extracted.

2.3. Results

2.3.1. Study selection

For the *M. tuberculosis* infection prevalence review, a total of 947 records were retrieved. After removing duplicates and titles reporting other diseases or other NTMs, 538 titles and abstracts were screened for reporting *M. tuberculosis* infection among adolescents in sub-Saharan Africa and 17 articles were included in the review (Figure 2-1). 521 articles were excluded for not including individuals aged 10-19 years, reporting TB disease, reporting studies not conducted in sub-Saharan Africa or not population based. The review of references of the 17 articles did not yield additional articles.

For the *M. tuberculosis* infection spatial distribution searches, 194 records were retrieved. Eighteen titles and abstracts were screened for reporting spatial distribution of *M. tuberculosis* infection at population level in sub-Saharan Africa after removing duplicates and titles reporting other diseases, other non-mycobacteria infections or not reporting spatial distribution of the participants (Figure 2-2). Fifteen articles were excluded for either reporting geospatial distribution of TB disease or genomic mapping. Three articles remained and were included in the review. Due to the small number of articles included in the review, relevant articles reporting spatial distribution of TB disease at the population level were selected from the retrieved articles mentioned above to highlight the factors associated with spatial distribution of TB disease and the geospatial methods used.

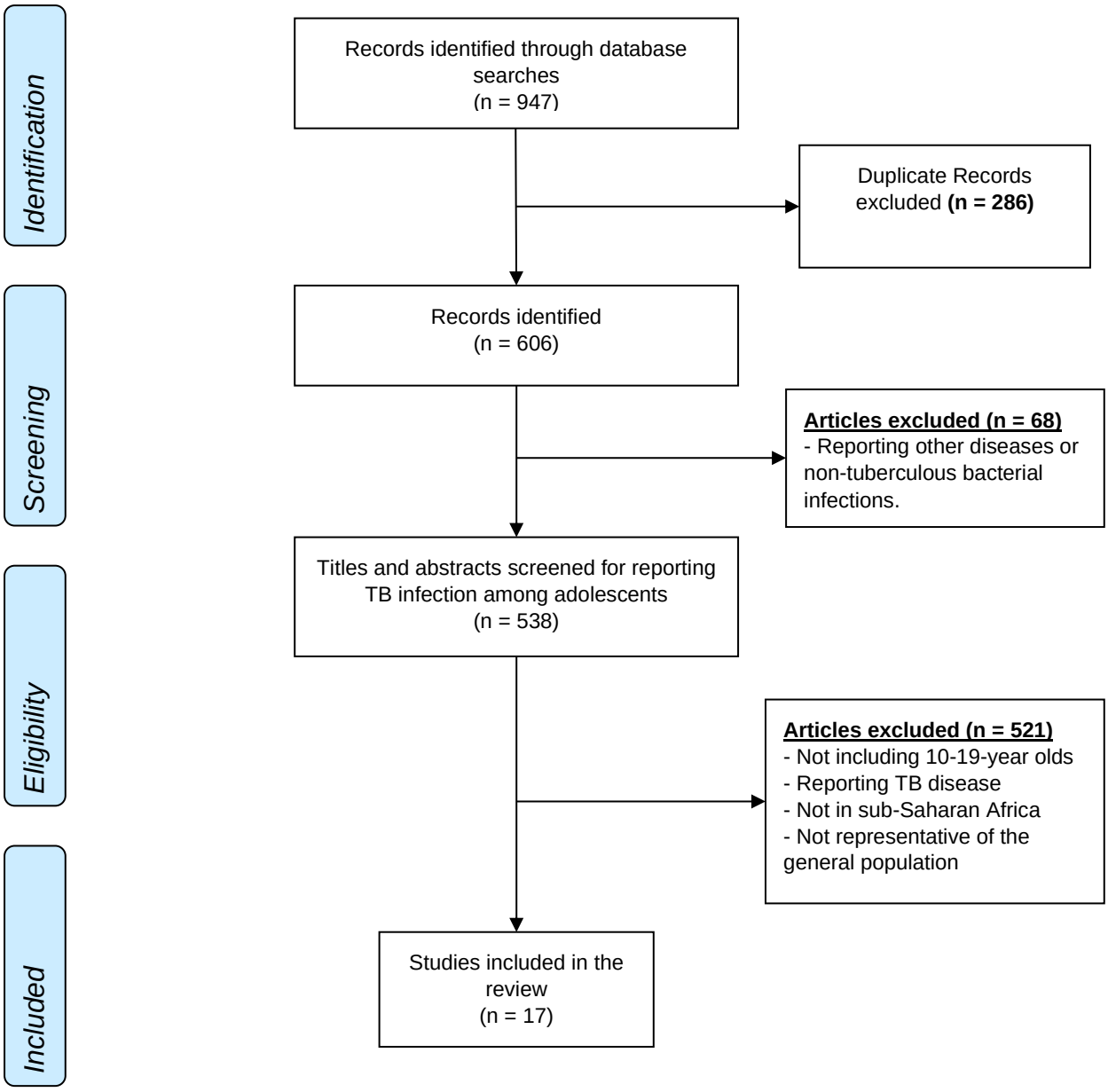

Figure 2- 1: Flow diagram for the screening process of studies reporting the prevalence of *Mycobacterium tuberculosis* infection among adolescents in sub-Saharan Africa

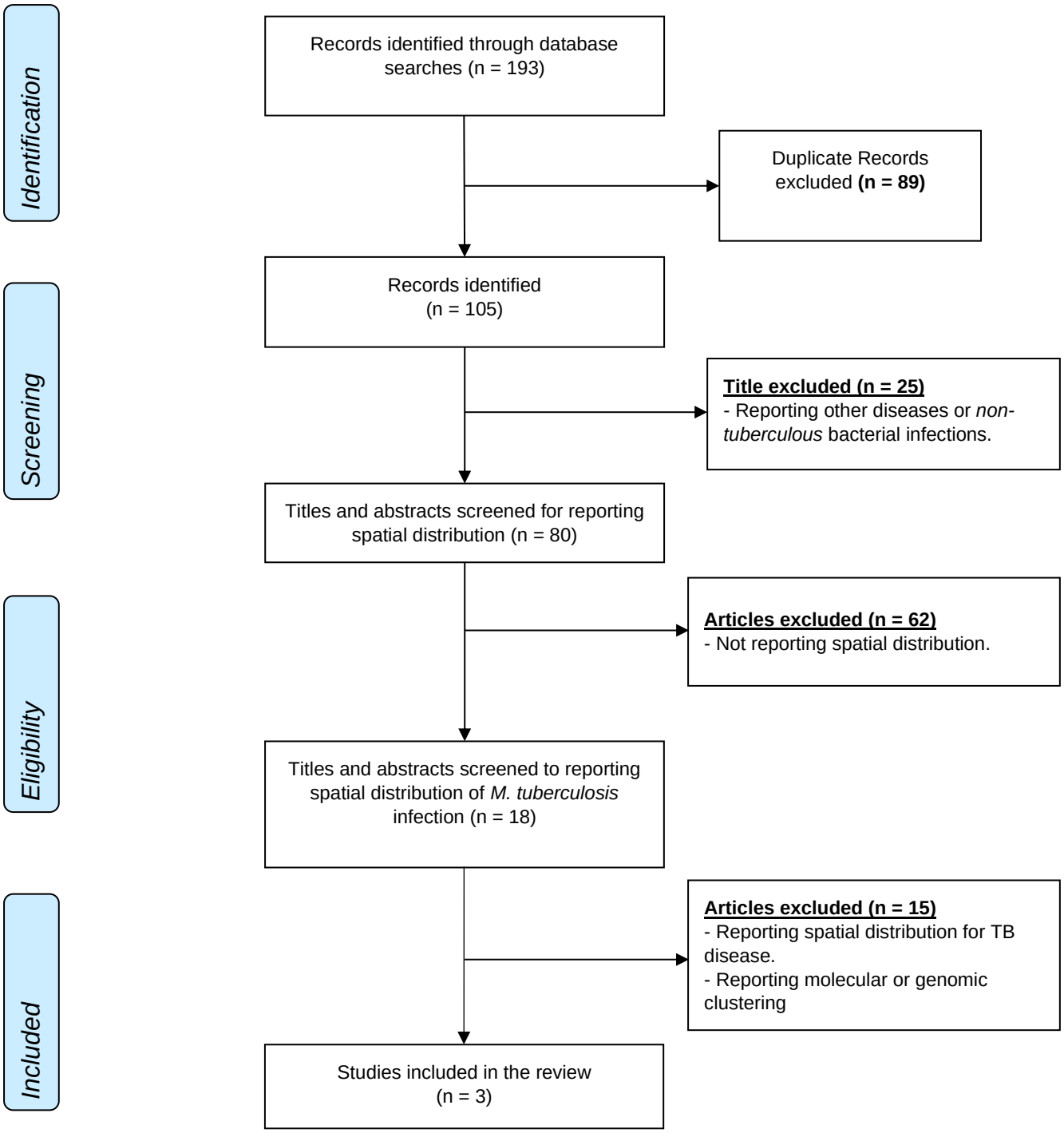

Figure 2- 2: Flow diagram for the screening process of studies reporting the spatial distribution of *Mycobacterium tuberculosis* infection in Sub-Saharan Africa.

2.3.2. Studies reporting the prevalence of *Mycobacterium tuberculosis* infection

Studies reporting the prevalence of and risk factors for *M. tuberculosis* infection among adolescents in sub-Saharan Africa are summarised in Table 2-2. The definitions for *M. tuberculosis* infection (cut-offs) used and the method for determining prevalence are also

presented. The tuberculin skin test (TST) was the most commonly used test. The previous generation of the QuantiFERON-TB, the QuantiFERON TB Gold in tube (QFT-GIT) test was used alongside the TST in three studies; two studies were conducted in Western Cape, South Africa [40, 41] and one study was conducted among individuals aged ≥18 years in South Omo Zone, Ethiopia [42].

Of the 17 studies, nine were conducted in South Africa, and the other studies were conducted among school-going children in The Central African Republic, The Gambia, Ghana, Kenya, Tanzania Uganda and Zambia. Two studies were conducted among adults (≥18 years) in Ethiopia. Among the studies conducted in South Africa, eight were conducted in communities in the Western Cape province and one was conducted in the Gauteng province. For the studies conducted in South Africa, a TST induration ≥10 mm for HIV negative participants and TST induration ≥5 mm for HIV positive participants was used. For the studies in the other sub-Saharan countries other than South Africa, several cut-offs were used including TST induration ≥10 mm, TST ≥15 mm and TST≥17 mm for HIV negative participants and ≥5 mm for HIV positive participants. In addition, mirror and mixture methods were used to determine *M. tuberculosis* infection prevalence. The mirror method assumes that the distribution of indurations among individuals with *M. tuberculosis* infection is symmetrical. The number of participants with *M. tuberculosis* infection is calculated as the number of participants with indurations at the mode plus twice number of participants with indurations bigger than the mode [43, 44]. The fixed mirror method assumes that the indurations due to cross-reactivity with BCG vaccination or NTMs do not reach 17 mm. Therefore, the mode is fixed at 17 mm and the number of participants with *M. tuberculosis* infection is calculated in the same way as the mirror method but fixing the mode at 17 mm regardless of the mode obtained from the fitted data [45, 46]. The mixture method uses advanced statistical modelling to estimate the proportion of participants with indurations due to *M. tuberculosis* infection by fitting separate component models representing participants with indurations due to BCG vaccination, NTMs and *M. tuberculosis* infection.[47-50] The

frequency distributions for non-zero indurations for participants in the studies not conducted in South Africa were bimodal with peaks between 6-8 mm [51, 52] and 12-17 mm [53], thus suggesting cross-reactivity with NTMs as distributions due to *M. tuberculosis* infection constitute a fairly symmetrical distribution [29, 43, 54]. On the other hand, the frequency distributions for non-zero induration for participants in studies conducted in South Africa were relatively symmetrical with peaks between 12-17 mm [40, 52, 55, 56] suggesting little cross-reactivity with NTMs, warranting the use of lower TST cut offs (TST induration ≥10 mm).

2.3.3. Description of studies reporting spatial distribution of *M. tuberculosis* infection

The database search retrieved three studies reporting spatial distribution of *M. tuberculosis* and these are shown in Table 2-3. Two of these studies were conducted in a densely populated township in the Western Cape province, South Africa [57, 58] and one was conducted within a well-defined demographic surveillance area (DSA) in a rural district in northern Malawi [59]. The studies in the Western Cape investigated the effect of residential plot exposure to notified TB disease on *M. tuberculosis* infection among children and adolescents aged 5-17 years. To do this, the authors (Middelkoop *et al*) conducted a TST survey among school-going individuals aged 5–17 years in 2007. Participants were geo-located to their residential plot (about 180 m^2) within the study area. Records of TB patients notified between 1997-2007 in the same area were checked and the TB patients were also geo-located to their residential plots basing on the addresses obtained from the hospital records [57]. Then the authors produced maps showing residential plots of participants with TST positive results and the notified TB patients. The authors repeated the procedure in 2009 using a TST survey among school-going adolescents and children aged 5–22 years and notified TB patients between 1997–2009 in the same study area [58].

In the study in rural northern Malawi, Khan *et al.* estimated the community TB exposure for every child (2–4-year olds) enrolled in a TST survey conducted in a well-defined DSA. The

community TB exposure was estimated as the average annual TB notification for each geographically defined area (consisting of about 450 households) within the DSA. The TB patients (≥15 years) were identified between 2007–2012 (the lifetime of the children enrolled in the TST survey) from a long-term enhanced case finding cohort of all TB patients in Karonga district [60]. The authors also investigated the effect of distance from each child's household to the nearest known smear-positive TB patient during the child's life on the risk of *M. tuberculosis* infection [59].

The limitation with these studies is that classical spatial tools like spatial scanning which help identify areas within the population which have a higher number of cases than expected [61-63] were not used. Classic statistical methods (like logistic regression) were used instead to investigate associations between household and community exposure to TB and *M. tuberculosis* infection prevalence. Furthermore, Middelkoop *et al*. defined residential exposure to TB using notified TB patients over a 10-year period. This approach assumes that the notified TB patients and the adolescents and children lived on the same residential plot at the same time and that the risk of *M. tuberculosis* transmission for that residential plot was the same during the entire period including the time the TST surveys were conducted. However, this may not be the case as individuals are bound to migrate over time.

2.3.4. Description of studies reporting spatial distribution of TB disease

A separate systematic literature search to identify studies reporting spatial distribution of TB disease in sub-Saharan Africa was not carried out. Relevant titles reporting population based spatial distribution of TB disease using classical spatial methods from the search described above were selected and are summarised in Table 2-4. Among these, three studies were conducted in two zones in the South Eastern province, Ethiopia [64-66], two were conducted in the KwaZulu-Natal province, South Africa [67, 68] and one was conducted in Antananarivo, Madagascar [69]. In all these studies, spatial scanning methods were used to identify clustering (locations within the population that have more than expected number of

TB cases basing on the underlying population density), size of clusters and the relative risk of TB disease in the clusters. Smith *et al*. investigated spatial distribution of drug resistant TB (DR-TB) in rural KwaZulu-Natal. The studies from Ethiopia and Madagascar used notified patients from clinic records, while Smith *et al.* used records of patients admitted at a district hospital to identify DR-TB patients and geolocating them to their household within a well-defined DSA. Tomita *et al*. used self-reported TB diagnosis from adults (≥15 years) in the same well-defined DSA.

While Smith *et al.* (in KwaZulu-Natal) and Rakotosamimanana *et al.* (in Madagascar) investigated clustering of DR-TB and notified TB disease respectively at a single point in time while Dangiso *et al*., Tomita *et al.* and Tadesse *et al*. investigated temporal changes in the spatial distribution and clustering of TB disease between 2003-2013, 2009-2015 and 2007-2016 respectively.

The major limitation with these studies is that they relied on TB patients either identified from hospital records or participants self-reporting TB diagnosis in the previous year (Tomita *et al*.). Self-reported TB has not been verified and may not be a robust measure of TB disease. In addition, the participation rates for the yearly individual health survey which captures self-reported TB diagnosis in the DSA was below 50%, thus selection bias might have been introduced. For the studies relying on hospital records accuracy and completeness of the records would be the major issue as in most poor resource settings, hospital records are not verified in the same way as research or clinical trial databases [70]. This would make linkages to residential address difficult. Selection bias would be introduced if participants from one area are recorded with higher accuracy than participants from other areas.

Table 2- 2: Studies reporting prevalence of *Mycobacterium tuberculosis* infection among adolescents in sub-Saharan Africa

Author, year of study, Country (Province/city)	Participants (N), method of selection	Definition of TB Infection	Prevalence of TB infection (%)	Risk factors	Comments
Den Boon *et al.,* 2001, SA, WC province [71]	– Randomly selected general population children aged 0-14 years (n=1344, [439 were aged 10-14])	– TST induration ≥10mm	– 32.1	– Passive HH Smoking: OR: 1.35 (95% CI: 0.86–2.12) – Monthly income: ≥R2000 vs <R500, OR: 0.47 (95% CI: 0.28–0.77) – History of HH TB contact: OR, 2.01 (95% CI:1.46–2.77)	– Prevalence among 10-14-year olds was 44%. – In a sensitivity analysis restricted to HHs where there was a TB patient passive smoking was significant (OR: 4.6 [95% CI: 1.3-16.5])
Egwaga *et al.,* 2001-03, Tanzania [72]	– Nation TST survey of school-going children aged 6-14 years (N=96,228). – Estimates were obtained using children without BCG scars (N=10,239) – Cluster sampling of schools in each district was conducted.	– Mirror method at ≥17 mm	– 6.1 (for BCG scar negative participants)	– Not specified	– Participants with visible BCG scars: 88.8% – TB notification for Tanzania in 2002 180/100,000. – The authors obtained estimates using participants without BCG vaccination to overcome effect of cross-reactivity with BCG vaccination.
Elias *et al,* 2003, Ethiopia [73]	– Cross-sectional study in a rural community in Dendi district, Ethiopia. – Information about the study was given to the community and individuals were invited to be enrolled at a health facility within the study area. – 2,640 individuals (aged 13-54 years) were included in the study.	– TST induration ≥10 mm	– 29.7% for all the participants (13-54-year olds)	– Age: aOR: 1.4 (1.3–1.6) for every 10-year increase. – Male sex: aOR: 1.2 (1.01–1.5) – History of household TB contact: 1.8 (1.3–2.3). – Low SES: 1.5 (1.12–2.03) – Post high school education: aOR: 1.5 (1.12–2.03).	– Annual TB notification for Ethiopia in 2013 was 230 per 100,000 population. – 8.3% reported history of household TB contact. – Selection bias as the study recruited only those who could come to the recruitment point.

Author, year of study, Country (Province/city)	Participants (N), method of selection	Definition of TB Infection	Prevalence of TB infection (%)	Risk factors	Comments
Den Boon *et al.,* 2003, SA, WC province [74]	- Randomly selected adults ≥15 years) (N=2,401)	- TST ≥10 mm	- 76% for all ages ≥15 years - 66% for 15-24-year olds.	- Smoking (ever smoked for one year): OR, 2.0 [95% CI: 1.6-2.5]	- New smear positive TB notification for the study area in 1998: 238 per 100,000 population. - No information on BCG vaccination or HIV status of participants was provided. - HIV prevalence for the study area in 2002: 12.4%
Addo *et al.,* 2004-6, Ghana [75]	- Nation-wide TST survey children 6-10 years (N=21,861)	- TST ≥15 mm	- 5.4	- Not specified	- Participants with visible BCG scars: 89.3%
Kwamanga *et al.,* 2004-7, Kenya [53]	- National survey of primary school children without visible BCG scars aged 6-14 years (N=12,107).	- TST ≥17 mm	- 10.2% (95%CI: 7.2–13.1).	- Not specified	- Participants with visible BCG scars: 84.0%. - Annual TB notifications for Kenya in 2006: 329 per 100,000population.
Shanube *et al.,* 2005, Zambia and SA, WC province [52]	- TST surveys among school-going children aged 6-11 years from Zambian and South African communities (N=8,776)	- TST induration ≥10mm - (Other definitions were also used)	- Zambia: 16.5 (95% CI: 12.0–21.1) - South Africa: 30.5 (95% CI: 22.9-38.2)	- Age: 20% among 6-year olds vs 40% among 11-year olds.	- 85% had visible BCG scars. - Prevalence by presence of BCG scar: 31.0 (95% CI: 22.8–39.2) vs 27.8 (95% CI: 11.0–44.7). wider difference in 6-year olds
Mahomed *et al.,* 2005-7, SA, WC province [40, 76]	- All adolescents (12-18 years) attending high school in a densely populated township (N=5,244)	- TST induration: ≥5mm, ≥10mm, ≥15mm and - QFT-GIT (≥0.35 IU/ml)	- 55.2 (53.8-56.5) - 42.2 (40.9-43.6) - 18.7 (17.7-19.8) - 50.9 (49.5-52.2) respectively for the different TST cut offs and QFT-GIT.	- Age: 15-18 vs 12-14, OR: 1.4 (1.2–1.5) - No difference by sex: male vs female sex OR: 1.2 (1.0–1.3) - Low monthly income: OR: 1.6 (1.3–1.9) - Chronic allergy-related conditions, OR: 0.4 (0.2–0.9) - Current or prior HH TB contact: OR: 1.9 (1.7–2.2)	- ORs are based on QFT results - HIV testing was not done, but the area had a lower HIV prevalence compared to the national prevalence i.e. 5-10% vs 30% among pregnant women - All TB notifications for the study area in 2004: 1400 per 100,000 population

Author, year of study, Country (Province/city)	Participants (N), method of selection	Definition of TB Infection	Prevalence of TB infection (%)	Risk factors	Comments
Wood *et al.,* 2006, SA, WC province [56]	– School-going children (5-17 years) in a densely populated township (N=832)	– TST ≥10 mm	– 36.0% among 10-16-year olds.	– Older age: 28.0% among 10-11-year olds vs 41.7 among 11-15-year olds.	– All TB notifications for the study area in 2006: 1,500 per 100,000 population. – The estimate including adults (10-40 years) was 45.0%, but these were enrolled from an HIV testing facility.
Middelkoop *et al.,* 2006-2007, SA WC province [77]	– School-going children aged 5-17 years (N=831)	– TST ≥10 mm and – TST ≥17 mm	– 37.4 at ≥10 mm – 20.6 at ≥17 mm	– Age (26.2% for 5-8-year-olds vs 52.5% for 14-17-year-olds)	– Annual TB notifications for the area changed from 942-2,140/100,000 between 1999-2006
Middelkoop *et al.,* 2009, SA WC province [78]	– Randomly selected adolescents and young people (13-22 year) attending secondary school in a township (N=620)	– TST ≥10 mm	– 53.9	– Age 47.8% among 13-16-year olds vs 63.2% among 19-22-year olds.	– BCG scar 20% – 34 (5.6%) HIV positive participants were excluded from the analyses. In a sensitivity analysis there was weak evidence of an association between *M. tuberculosis* and HIV infection: (OR = 0.53; 95% CI: 0.27-1.05; p = 0.07).
Mumpe-Mwanja *et al.,* 2009-11, Uganda [51, 79]	– Randomly selected adolescents (aged 12-18 years) from a DSA (N=4,981)	– TST ≥10 mm if HIV negative (or TST ≥5 mm if HIV positive)	– 16.1 (15.1-17.2)	– Age (15-16 years vs 12-14 years), aOR: 1.25 (1.07-1.46) – Age (17-18 years vs 12-14 years). aOR: 1.46 (1.24-1.71) – BCG Scar, aOR: 1.29 (1.12-1.48) – Male sex, aOR: 1.37 (1.21-1.56) – Not attending school, aOR: 1.31 (1.05-1.62) – HH TB contact, aOR: 1.91 (1.55-2.35)	– 6 (0.12%) participants were HIV positive.
Adetifa *et al*., 2011, The Gambia [80]	– Nation-wide TST survey of school going children (6-11 years) from randomly selected schools (N=13,386)	– TST ≥10 mm, – mixture method	– 11.5 for TST ≥10 mm – 6.9 for Mixture	– Age (10 vs 6 years), aOR: 2.01 (1.24–3.42) – Age (11 vs 6 years). aOR: 1.98 (1.19–3.31)	– BCG scar 72.8% – No SES information

Author, year of study, Country (Province/city)	Participants (N), method of selection	Definition of TB Infection	Prevalence of TB infection (%)	Risk factors	Comments
Minime-Lingoupou *et al.*, 2011, CAR [81]	- School-going children aged 6-12 years selected from cluster sampling of school from Bangui and Ombella-M'Poko regions. (n=2,710)	- TST≥5 mm - TST≥10 mm - TST≥15 mm	- 23 (23.6-25.3) - 18.4 (16.8-20.1) - 8.9 (7.8-10.0)	- The odds of infection were not different at TST induration ≥5 mm and ≥10 mm but were lower at ≥15 mm (crude OR: 0.7 [0.5–1.0])	- BCG scars: 65.9% and was similar for the two regions. - Reported BCG vaccination: 83.8% was higher in Bangui (Urban) region. - Significant digit preference was observed at 10 mm and 15 mm. Mirror or mixture methods should have been used to estimate prevalence. - Annual TB notification for Bangui and Ombella-M'Poko regions in 2011 were 284 and 134 per 100,000 population respectively.
Ncayiyana *et al*., 2013-14, SA, Guateng province [55]	- Randomly selected individuals of all ages from a densely populated community (N=446)	- TST≥5 mm if HIV+ or TST≥10 mm if unknown or HIV negative	- 34.3 (30.0-38.8)	- Age: 18.8% (0-14-year olds) vs 45.2% (≥45-year olds) - Male sex: OR, 2.70 (1.55–4.70) - HIV: OR, 0.85 (0.49–1.46) - HH TB contact: 2.27 (0.76–6.82) - High HH SES: 2.11 (1.04–4.31)	- Population density of the study area: 11,357 per km^2 - 18% of the participants were HIV positive
Bunyasi *et al*. 2014-15, Western Cape, South Africa [41]	- Adolescents (12-18 years) attending state schools screened for a TB vaccine trial (n=1,968)	- QFT-GIT (≥0.35 IU/ml)	- 48.5 (41.1–55.8)	- Combined the sample of adolescents enrolled in 2005 in the same school to determine associations with M. tuberculosis infection. The associations may not reflect the 2015 cohort.	- TB notifications for the area in 2014: 710 per 100,000 population. This was lower than in 2015 when the authors conducted another survey among school-going children in the same area, but M. tuberculosis infection prevalence had slightly increased.

Author, year of study, Country (Province/city)	Participants (N), method of selection	Definition of TB Infection	Prevalence of TB infection (%)	Risk factors	Comments
Teklu *et al.* 2015-16, Ethiopia [42]	– Cross sectional study of adults (>18 years) in six districts in South Omo Zone, Ethiopia (n=497). – Sample size for each district was determined proportion to its size. Simple random sampling was used to select households. – Selected houses were visited, and participants were selected among those present during the visit.	– QFT-GIT (≥0.35 IU/ml)	– 50.5 (46.00-55.00) for all the participants	– Residing in a district with very low TB detection rate (14.3%): aOR: 2.89, [1.09-7.66]	– There was no difference by age, sex or history of household TB contact. – Prevalence was lower among individuals from households with more than five residents compared households with less than five residents, aOR: 0.65, [0.42-0.99]. – Selection bias might have been introduced as only individuals present during the home visit were enrolled.

aOR: adjusted odds ratio; ARI: annual risk of infection; BCG: Bacillus Calmette-Guérin; CAR: Central African Republic, DSA: demographic surveillance area; HH: household; HIV: human immunodeficiency virus; NS: not specified; OR: odd ratio; QFT-GIT: QuantiFERON gold in tube test; SA: South Africa; SES: socioeconomic status; TB: Tuberculosis; TST: tuberculin skin test; WC: Western Cape

Table 2- 3: Studies reporting spatial distribution of *Mycobacterium tuberculosis* infection among adolescents in sub-Saharan Africa

Author, Year of study, Country (Province/City)	Participants (N)	Geospatial methods used	Results	Comments
Middelkoop *et al*., 2007, South Africa (Western Cape) [57]	– TST survey among school-going children aged 5-17 years in a township (n=640) – Adults (≥15 years) notified with TB between 1997-2007 (n=1,212). These were extracted from clinic records and geolocated to their residential plot.	– Produced maps showing locations of residential plots of participants with TST positive results and adults notified with TB for the period 1997-2007. – The aim was to compare risk of *M. tuberculosis* infection between residential plots that have a history of exposure to TB disease and those without exposure.	– TST positivity was higher among participants living on residential plots with a history of a notified adult TB cases (65% vs 51%, p=0.001). – In a separate analysis, the authors restricted residential TB exposure to the last 12 months only (unlike the whole 10-year period). This showed that participants with residential plot exposure to TB were more likely to be TST positive if the notified TB patient was smear positive, but there was no difference if the notified adult with TB was smear negative.	– Since exposure to TB was defined for the whole 10-year period and TST prevalence was only measured in the 10th year, the participant and the adult TB patient may not have lived at the residential plot at the same time. – The lack of association if the notified TB patient was smear negative suggests the reduced infectiousness of the smear negative TB patients.
Middelkoop *et al.*, 2009, South Africa (Western Cape) [58]	– TST survey among school-going children and adolescents aged 5-22 years (n=1,100) – Adult (≥15 years) notified TB cases between 1997-2009 (n=1,604). – [same area as the above study, but with an addition of older participants]	– Maps for spatial distribution of TST positive school-going children and whether there was an adult notified with TB on the same residential plot. – Residential TB exposure was defined as living on a residential plot with the same address as a notified TB patient. – Comparisons were made by age group (<15 vs ≥15years)	– Overall, *M. tuberculosis* prevalence was higher among adolescents and children living on the same residential plot as an adult with notified TB disease whether any type of TB (OR: 1.6 [95% CI: 1.2-2.0]) or smear positive pulmonary TB (OR: 1.9 [95% CI: 1.4-2.4]). – *M. tuberculosis* infection was associated with residential plot exposure to TB for 5-9 and 10-14-year olds (OR: 2.0 [95% CI: 1.1-3.6] and OR: 1.5 [95% CI: 1.0-2.3] respectively), but not for ≥15-year olds (OR: 1.4 [95% CI: 0.9-2.0])	–

Author, Year of study, Country (Province/City)	Participants (N)	Geospatial methods used	Results	Comments
Khan *et al.*, 2012, Malawi (Karonga) [59]	– TST survey among children aged 2-4 years in a well-defined DSA (n=3,170) – Adults (≥15 years) smear positive pulmonary TB patients resident in the DSA diagnosed between 2007-2012 (n=108).	– The DSA is divided into geographically defined areas with about 450 households and the average annual TB notification was calculated for each area (community TB exposure). – Distance to the nearest known smear positive TB patient was calculated using the GPS coordinates of the TST children and smear positive TB patients. – Compared *M. tuberculosis* infection prevalence with distance to the nearest known adult smear positive TB case, population density and community exposure to smear positive TB.	– *M. tuberculosis* infection in children (2-4 years) was associated with: i) Decreasing distance to the nearest known adult smear positive TB case (aOR for trend: 1.6 ([95% CI: 1.1-2.4], p=0.03) and ii) Increased community exposure to SP TB (aOR: 2.2 ([95% CI: 1.1-4.6], p=0.03) (>30 vs ≤30 cases per 100,000 population)	– There was no difference by population density (aOR: 1.0 [95% CI: 0.5-2.1] for 250-1000 vs <250 residents per km^2 and 0.8 [95% CI: 0.3-2.0] for >1000 vs <250 residents per km^2, p=0.81)

aOR: adjusted odds ratio; CI: confidence interval; PTB: pulmonary tuberculosis; OR: odds ratio; SA: South Africa; SP: smear positive; TB: Tuberculosis; TST: tuberculin skin test

Table 2- 4: Studies reporting spatial distribution of tuberculosis disease in sub-Saharan Africa

Author, year of study	Country (Province/City)	Participants (N)	Geospatial methods used	Results
Rakotosamimanana *et al*., 2010-11 [69]	Madagascar (Antananarivo)	− Notified TB cases captured from TB registers (n=4,620)	− Used spatial methods to identify clustering of newly diagnosed TB patients	− Two clusters of newly diagnosed TB patients were identified in densely populated townships within the city with RR=1.7 and 1.6 respectively.
Dangiso *et al*., 2003-12 [64]	Ethiopia (Sidana Zone, SE)	− Notified TB cases over a 10-year period captured from TB registers (n=22,545)	− Spatial scanning methods (Global Moran's I and Getis-Ordi − statistics) were used to identify clustering of notified TB cases and temporal changes in clustering.	− Clusters of smear positive pulmonary TB (RR=2.0, p<0.001) were identified in urban, peri-urban and densely populated rural areas on the north western part of the study area. − The clusters were stable over the study period with smaller clusters appearing on the south eastern part towards the end of the study period.
Smith *et al*., 2011-15 [68]	South Africa (KZN)	− Individuals admitted at Hlabisa district hospital and recorded as having DR-TB (n=478).	− Compared the spatial distribution of DR-TB cases and patients admitted for other reasons. − Used spatial scanning methods to identify clustering of DR-TB patients in defined DSA population.	− A cluster with RR 2.5, p=0.057 was identified in a township with high HIV prevalence. The cluster contributed 55/111 (49.5%) of the DR-TB patients in the geographically defined DSA population.
Tomita *et al*., 2009-2015 [67]	South Africa (KZN)	− Individuals self-reporting TB diagnosis as part of surveillance at the AHRI DSA.	− Spatial scanning tools were used to identify the temporal distribution and clustering of self-reported notified TB cases. − Compared the distribution of TB patients with the distribution of ART coverage for the area.	− Spatial clusters (radius between 1.7-2.9 km and RR between 1.3-1.9) were identified in urban and peri-urban communities of the study area which are close to the national highway and are characterised with high HIV prevalence. The clusters in this area persisted throughout the duration of the study period. − A smaller persisting cluster was also identified north of the densely populated township along a main road with radius between 0.27-1.00 km and RR between 2.1-10.1 − Increase in community ART coverage was associated with decreasing odds of self-reported TB diagnosis (aOR: 0.98, 95% CI:0.97–0.99). .
Tadesse *et al*., 2007-16 [66]	Ethiopia (Gurage Zone, SE)	− Notified TB cases between 2007-2016 captured from TB registers (n=15,805)	− Spatial scanning methods (Global Moran's I, Getis-Ordi and Kulldorff's scan statistics) were used to identify clustering of notified TB cases and temporal changes in clustering.	− A cluster of radius 4.45 km with RR=4.16, p<0.01 was identified in an area characterised with frequent travel with zones in surrounding areas. − In each year significant clustering on was observed and the cases were clustered on the border areas of the zone.

AHRI: Africa health research institute; ART: antiretroviral therapy; DSA: demographic area site; DR: drug resistant; KZN: KwaZulu-Natal; PTB: pulmonary tuberculosis; RR: relative risk; SE: south eastern; SM: smear positive; TB: Tuberculosis; TST: tuberculin skin test

2.3.5. Estimates of prevalence of *Mycobacterium tuberculosis* infection

Overall, estimates of *M. tuberculosis* infection prevalence in studies conducted in South Africa ranged from 32.0% among individuals aged 0-14 years to 66.0% among individuals aged 15-24 years (using TST induration ≥10 mm). Among the studies conducted among school-going children and adolescent in countries other than South Africa, the prevalence ranged from 5.4% (using TST induration ≥15 mm) among individuals aged 6-10 years in Ghana to 18.4% (using TST induration ≥10 mm) among individuals aged 6-12 years in Central African Republic (Table 2-2). However, the frequency distribution for the participants in the Central African Republic study showed substantial digit preference at 10 mm. The prevalence obtained using TST induration ≥15 mm was 8.9%. The *M. tuberculosis* infection prevalence among individuals aged 13-54 years and ≥18 years in Ethiopian communities was 29.7% and 50.5% respectively (Table 2-2).

A plausible explanation for the higher prevalence in the South African studies could be due to increased exposure to potentially infectious individuals as reflected in the much higher annual TB notification rates in South Africa compared to the other countries (>800 per 100,000 population compared to <200 per 100,000 per population) at the time the studies were conducted.

Furthermore, the studies in South Africa were conducted in townships where individuals are likely to have wider and diverse social contacts thereby increasing the chance of contacting infectious individuals while the other studies were largely conducted in rural areas where individuals are likely to have fewer and less diverse social contacts. Within South Africa, the highest prevalence was reported in studies conducted in densely populated townships in the Western Cape province. The differences in prevalence are likely to be due to differences in social contacts and crowded living conditions between the different settings.

2.3.6. Risk factors for *Mycobacterium tuberculosis* infection prevalence

The risk factors for *M. tuberculosis* infection are shown in Table 2-2. The factors consistently shown to be associated with *M. tuberculosis* infection included older age, history of household contact with TB disease and low socioeconomic status. Furthermore, a nationwide TST survey in Gambia showed an increased risk of infection among participants from urban compared to rural communities

[80]. Two studies in the same study area in Western Cape province, South Africa reported the effect of smoking and passive household smoking (defined as living in the same house as a person who smokes) on *M. tuberculosis* infection [71, 74]. The authors, Den Boon *et al.,* did not find an association between passive smoking and *M. tuberculosis* infection. However, in a sensitivity analysis including participants with a history of household TB contacts only, the odds of *M. tuberculosis* infection were four times in households where there was a smoker (odds ratio [OR]: 4.6; [95% confidence interval (CI): 1.3-16.5]) [71]. In a study, investigating the effect of smoking on *M. tuberculosis* infection among adults (≥15 years), participants reporting having ever smoked had an increased odds of *M. tuberculosis* infection (OR: 2.0 [95% CI: 1.6-2.5]) [74].

The effect of HIV on *M. tuberculosis* infection was inadequately reported due to lack of information on the HIV status of participants or having a very small proportion of participants who were HIV positive. In a study in rural eastern Uganda only 6 (0.12%) participants were HIV positive [51] and a study among adolescents and young people in Western Cape province, South Africa, 34 (5.6%) participants were HIV positive. In the Western Cape study the authors found weak evidence of lower odds of *M. tuberculosis* infection among HIV positive compared to HIV negative participants (OR: 0.53; 95% CI: 0.27-1.05) [78]. This is possibly due to reduced reactivity to tuberculin due to the HIV infection [82].

2.3.7. Spatial distribution of *Mycobacterium tuberculosis* infection

Both Middlekoop *et al*. and Khan *et al*. showed that the odds of *M. tuberculosis* infection among young children (<5 years) and adolescents (10-22 years) were higher among those with a TB contact in their residential plots or among those residing in areas with higher community TB exposure (≤30 vs >30 TB patients per 100,000 population) respectively (Table 2-3). This reflects continued transmission within residential plots and the immediate community of the notified TB patients. Comparing the age of the participants and whether there was a TB patient on their residential plot, Middelkoop *et al*. reported that the odds of *M. tuberculosis* infection were significantly higher among individuals aged 5-9 and 10-14 years with a residential plot TB contact compared to those without a residential plot TB contact, but the evidence for a difference among older participants (15-22-year olds) with or without a residential plot TB contact was weak (OR: 1.4

[95% CI: 0.9-2.0]). Overall, the prevalence of *M. tuberculosis* infection was higher among the older adolescents compared to the younger children (28.6 % among 5-9-year olds vs 54.0% among 15-22 year olds [58, 83]). Khan *et al.* reported a population attributable fraction of a TB contact in the immediate community (within 200 m) of only 17.0%. This further highlights ongoing transmission in the wider community.

Overall, Middlekoop *et al.* showed that the odds of *M. tuberculosis* infection were higher for all forms of TB, pulmonary TB (PTB) and smear-positive TB, but there was no difference if the adult TB case on their residential plot had smear negative TB disease (OR: 0.95, 95% CI [0.59 - 1.54]) [57]. However, the authors did not show the spatial distribution of the different forms of TB to show whether they were clustered in the same parts of the study area. Khan *et al* assessed the association between population density (250-1000 vs <250 per km^2 and >1000 vs <250 per km^2) and *M. tuberculosis* infection but did not find a significant association (OR: 1.0 [95% CI: 0.5–2.1] and OR: 0.8 [95% CI: 0.3–2.0] respectively, p=0.18). This is possibly because the population density is low in this setting.

2.3.8. Spatial distribution of TB disease

Studies reporting spatial distribution of TB disease are summarised in Table 2-4. Significant clustering of notified TB and DR-TB disease was reported in all the studies. Nearly all the clusters were identified in townships or areas characterised with increased social contacts (Table 2-4). Tomita *et al.* and Smith *et al.* identified clusters of self-reported TB and DR-TB disease in the same area which is characterised by high HIV prevalence along a national highway in a rural district in the Kwazulu-Natal province, South Africa [67, 68] and Tadesse *et al.* identified clusters on the border areas of the Gurage zone which are characterised with regular movement to neighbouring zones in south eastern Ethiopia [66].

When looking at temporal changes, significant clustering over the duration of respective study periods was identified in both South Africa and Ethiopia [64, 66, 67]. Overall, larger persisting clusters (radius 2.9–4.5 km) were identified in the townships and smaller clusters appearing for shorter periods were identified in different parts of the studied areas. Tomita *et al.* identified smaller clusters (radius 0.3–1.0 km) in an area not characterised as having high HIV prevalence which

persisted the entire duration of the study period (2009-2015) [67]. This might reflect transmission among close contacts or recurrence of disease in the same individuals over time.

2.4. Conclusion

There is a paucity of data on prevalence of *M. tuberculosis* infection among adolescents in Sub-Saharan Africa. The available evidence largely comes from studies conducted in the Western Cape province, South Africa (Table 2-2). There is need for data on *M. tuberculosis* infection prevalence in other parts of Africa and South Africa, especially where the prevalence of HIV is still high, to help understand the effect of HIV on acquisition and transmission of *M. tuberculosis* infection.

The available evidence shows that prevalence of *M. tuberculosis* infection among adolescents in South Africa is high. Prevalence starts to pick up in adolescents and is highest in adults. This is largely due to increased number of social contacts [38] and cumulative exposure to potentially infectious individuals in the community.

The literature search for the spatial distribution of *M. tuberculosis* infection at population level in sub-Saharan Africa retrieved three studies. The studies revealed that household TB contact including living in the same residential plot and in the immediate community (within 200m) of known notified TB patients did not explain the majority of prevalent infections even among young children who are likely to be infected within their household. Information accurately characterising the spatial distribution of *M. tuberculosis* infection and local areas with higher burden (location of clustering) of infection is lacking in sub-Saharan Africa so that targeted inventions can be put in place.

The studies investigating spatial distribution of TB disease identified spatial clusters of disease which were persistent over durations of the study periods (7-10 years) and smaller clusters which were identified for shorter periods. These studies show areas in the general population which are disproportionally affected with disease. Targeting interventions in these areas would potentially have a significant impact in reducing the burden of disease. However, evidence for this approach is still lacking.

Chapter 3 Methods

3.1. Study Setting

The study was conducted from November 2017 to December 2018 in the southern part of the Africa Health Research Institute (AHRI) DSA which has been under surveillance since 2000. The area is situated in Mtubatuba local municipality within uMkhanyakude district in KwaZulu-Natal province, South Africa (Figure 3-1). In terms of healthcare management, the area falls under the Hlabisa health sub-district (Figure 3-2).

AHRI is a joint Wellcome Trust and Howard Hughes Medical Institute funded research institution affiliated to the University of KwaZulu-Natal. The institution was established in 2016 as a result of a merger of two institutions: KwaZulu-Natal Research Institute for TB-HIV (K-RITH) and Africa Centre for Health and Population Studies. The Africa Centre for Health and Population Studies was originally established in 2000 and had been conducting longitudinal population wide research investigating the epidemiology of HIV, understanding population dynamics and utilisation of healthcare and other services. In addition to the population surveillance, the DSA also provides a sampling frame for population-based studies and a platform for the implementation and evaluation of individual and population level interventions [84]. The population surveillance has continued after the merge with K-RITH.

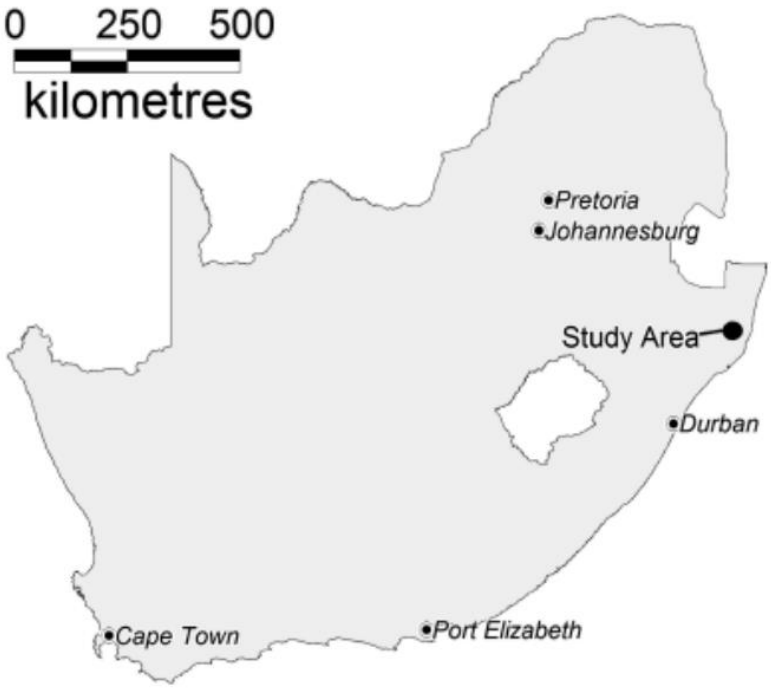

Figure 3- 1: Map showing location of the study area within South Africa [84]

The study area covers approximately 430 km^2 and is demarcated to the east by the N2 national highway, to the west by the Hluhluwe-iMfolozi game reserve and on the south by the Mfolozi river (Figure 3-2) [84]. The area has a population of around 90,000 and an adult (≥15 years) HIV prevalence estimated at 36.6% in 2016 [85]. The inhabitants are predominantly isiZulu speaking. The area is predominantly rural and includes a township (KwaMsane) on the south-eastern part and informal peri-urban settlements. In the township area, houses are close together and in the rural areas dwelling places are organised into multigenerational homesteads of varying sizes which are not clustered into clearly identifiable villages . For pragmatic purposes, a homestead is defined as a building, or a group of buildings, on a piece of land belonging to a single person or organisation and used for one main purpose. Most homesteads are primarily used for residential purposes. The study area is sub-divided into 45 geographic areas of varying population densities (25–2,000 population per km^2) called week-blocks (see Chapter 4 Figure 4–2). Week-blocks were designed such that a team of field staff can complete surveillance work within a five-day working week (taken from the unpublished 'Operational and Methodological Procedures of the Africa Centre Demographic Information System').

The study area includes seven primary healthcare clinics (PHC) which provide healthcare for the population. Six of these are located within the study area and one is located just outside at the Mtubatuba municipality. Hlabisa district hospital (about 25 km west of the DSA) acts as the main referral facility. At these facilities, data on clinic attendance are collected and the members of the DSA are linked in real time using their demographics surveillance identifier (DSID). The Somkhele field site, where the project office was located, is within the study area close to the Somkhele clinic (Figure 3-2).

3.2. Demographic surveillance activities

In the demographic surveillance, individuals were first registered when the DSA was set up in 2000, registered at birth or in-migration and exit at death or out-migration. Households are visited annually and information on births, immunizations, deaths, migration patterns of all members and household socioeconomic status is collected from a household informant (usually the head of the household) [84]. Resident individuals aged ≥15 years are invited to participate in interviews on general health,

sexual behaviour, and anonymised HIV testing. Information on self-reported TB diagnosis in the previous year is collected through these interviews.

3.3. Study design and population

This was a cross-sectional study of adolescents (aged 10-19 years) randomly selected from a sampling frame of about 15,000 resident adolescents in the study area. In the DSA, individuals are defined as resident if they report that they intend to sleep majority of nights at a homestead within the DSA.

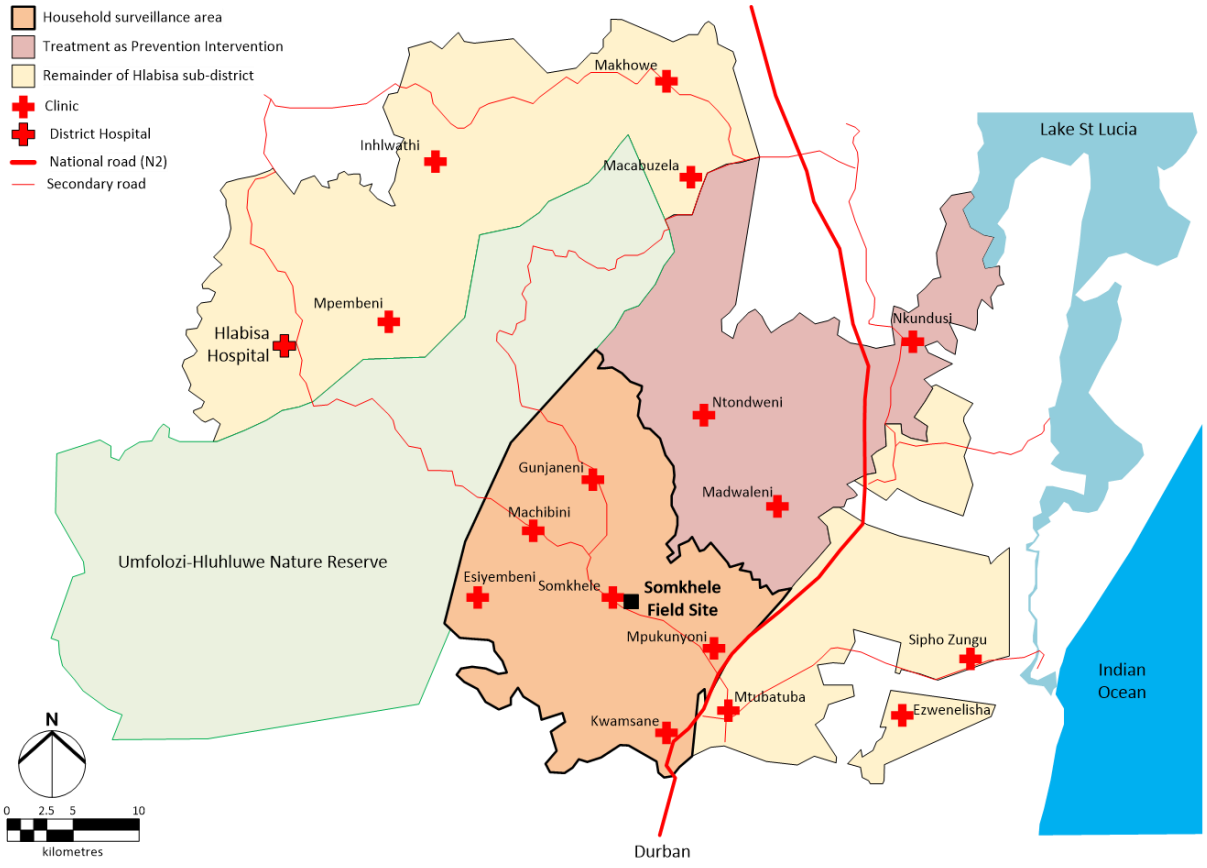

Figure 3- 2: Map of Hlabisa health sub-district showing location of the study area (light-brown area with a black border), the Somkhele field site and primary health care clinics within the study area and surrounding areas.

3.4. Data collection procedures

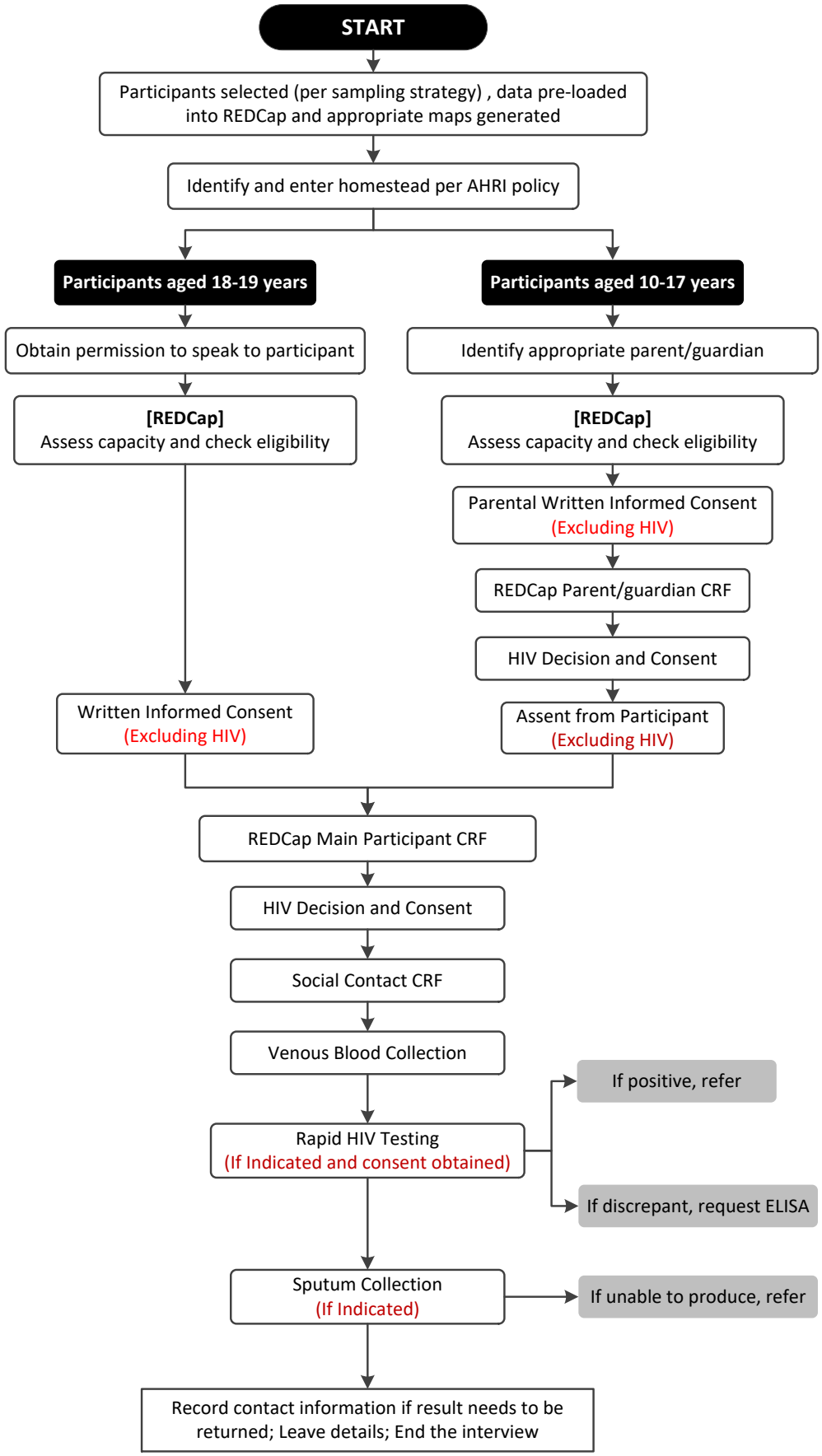

Figure 3- 3: Overview of the study procedures
AHRI: Africa health research institute; CRF: case report form; ELISA: enzyme-linked immunosorbent assay; HIV: human immunodeficiency virus; REDCap: Research electronic data capture system

3.4.1. Sampling and contacting selected individuals

The sampling was stratified by week-block, a fixed 14% proportion of resident adolescents was sampled from each week-block to reflect the population distribution of the study area. The number of individuals in each week-block were sampled in order to obtain the target sample size (1,100).

The selected individuals were approached at their homes by a team of research nurses (Figure 3-3). Since majority of individuals in this age group are school-going and are hard to find in homes during normal working hours (8.00 am–4.00 pm), up to 6 home visit attempts were made to find the selected individuals. In addition, field staff were working on weekends to maximise opportunity to find the selected individuals when they are not at school. During the home visit, permission to meet the selected individual or the parent/guardian of the selected individual was first sought from the owner of the homestead. With the help of the selected individual or the parent/guardian, a private space to conduct the interview within the homestead was identified (Figure 3-3). In very rare situations, if a private space could not be found, questions on more sensitive information like previous HIV test results were asked with caution or skipped altogether.

3.4.2. Screening and enrolment

During the interview with the selected individual or the parent/guardian, the field staff first cross-checked the individual's date of birth and whether they have ever been or are currently on TB treatment. Individuals self-reporting current or previous TB treatment were excluded from enrolment. Individuals who met the eligibility criteria (age 10-19 years and no previous or current TB treatment) and were willing to participate were taken through the informed consent process and were asked to sign consent forms (Appendices 1-3). For participants aged 10-17 years, consent was first sought from a parent or a designated guardian and assent was obtained from the participant. If the parent or designated guardian was not available, a suitable parental/guardian substitute was identified in line with the South African department of health (DOH) guidelines [86]. For participants and parents/guardians who indicated that they cannot read or write, a witness who was not a member of the research team was identified to attest to the informed consent process.

3.4.3. Questionnaire

A standard questionnaire (Appendices 9 and 10) including information on previous TB treatment, school attendance, BCG vaccination, contact with TB patients, admission to hospital, chronic illness, smoking, alcohol intake, attendance at indoor gathering places, road trips in closed vehicles, history of previous HIV testing and ART uptake was administered. Since some events might have happened when the participant was still young, a similar questionnaire (Appendix 11) including previous TB treatment, BCG vaccination, admission to hospital, HIV testing history, chronic illnesses and ART uptake was administered to parents/guardians of participants aged 10-17 years.

In October 2017, a draft version of the questionnaire was piloted on 50 adolescents from the study area and was updated following the experience. The pilot also served as part of training of the study procedures for the field staff.

3.4.4. TB symptom screen and sputum collection

For all participants a TB symptom screen incorporating cough for at least two weeks (any duration if HIV positive), fever, night sweats, and unexplained weight loss was performed. Two sputum samples were collected from participants with at least one of these symptoms. Participants who could not produce sputum were referred to their nearest PHC for further investigation.

3.4.5. Blood sample collation and HIV testing

For all participants a venous blood sample was collected in a generic 6 ml Lithium Heparin (LiHep) tubes. Participants without history of HIV testing, participants self-reporting a previous negative HIV test result more than three months before the enrolment date and participants with unknown previous HIV test results were offered a rapid HIV test by finger prick. The rapid test was done based on a parallel test algorithm using Abon HIV 1/2/0 Tri-line [Abon Biophrarm (Hangzhou) Co., Ltd] and Advanced Quality™ Rapid Anti-HIV [InTec Products Inc] test kits. Participants not wishing to test were given the option to have an anonymous HIV test on their venous blood for research purposes, or to decline HIV testing altogether.

In line with DOH guidelines [86, 87], participants aged 10–11 years were offered the rapid HIV test only if their parent/guardian consented to both the participant being offered rapid HIV testing and to

being present during the process of testing should the participant independently agree to test. Thus, for participants aged 10–11 years, the test was only done in the presence of the parent/guardian. Parents/guardians who knew the positive HIV status of their child but had not disclosed the status to their child were offered assistance for disclosure by a professional project nurse at their nearest PHC.

3.5. Laboratory procedures

3.5.1 Sputum samples

The sputum specimens were collected in sterile containers and were placed in cooler boxes with temperatures between 2–8^{o}C immediately after collection. At the end of each working day, the research nurses brought the samples to the project laboratory at the Somkhele field site for temporary storage.

On the next working day (within three days), the research nurse transported one of the sputum samples to Somkhele clinic where it was shipped using existing DOH procedures to the DOH laboratory at Hlabisa district hospital for testing TB disease using Xpert/MTB RIF. The second sample was sent to the project laboratory in Durban where it was cultured for *Mycobacteria* on liquid media. The results from the project laboratory were automatically integrated into the Laboratory Information Management System (LIMS). The individual reports for the results from Hlabisa Hospital were downloaded from the National Health Laboratory Service (NHLS) database and were stored in a secured repository on the project server. These were also made available to the collecting nurse after they were reviewed by a study doctor.

3.5.2 Venous blood samples

Venous blood specimens in the Lithium Heparin (LiHep) tubes were placed in cooler boxes with temperatures between 17–27^{o}C and transported to the project laboratory at the end of each working day. Upon arrival, the specimen was aliquoted into the four QuantiFERON TB Gold plus (QFT-plus) tubes (Nil, TB1, TB2 and Mitogen) and incubated immediately at 37^{o}C for 16–24 hours. After the incubation, the samples were sent to the project laboratory in Durban for testing immunological

responses to *M. tuberculosis* infection using the QFT-plus assay. The results were automatically integrated into the LIMS systems and were interpreted using manufacturer guidelines [20].

Participants who did not wish to have a rapid HIV test but had consented to have an anonymous HIV test done on their venous blood were tested for HIV using an enzyme-linked immunosorbent assay (ELISA) at the project laboratory in Durban. The ELISA was based on a two-test algorithm using HIV-1/HIV-2 ELISA (Vironostika HIV-1 Microelisa System: Biomérieux, Durham, NC, USA) which was followed by Wellcozyme HIV-1 + 2 GACELISA (Murex Diagnostics Benelux B.V., Breukelen, Netherlands). The results were also automatically integrated into the LIMS system, were not made available to investigators during the course of the study and were not fed back to participants.

3.5.3 Result feedback, referral and linkage to care

All results were reviewed by the study doctor. Results that were clinically relevant were made available to the collecting research nurse and were fed back to the participant or parent/guardian either by telephone or in person during a home visit (Figure 3-4 and Appendix 6). Two weeks after the date of referral to care, all participants who were referred to care for any reason and had not linked to care were contacted by phone to check if they had attended PHC (Appendix 7). Participants who had not linked to care were encouraged to attend the PHC.

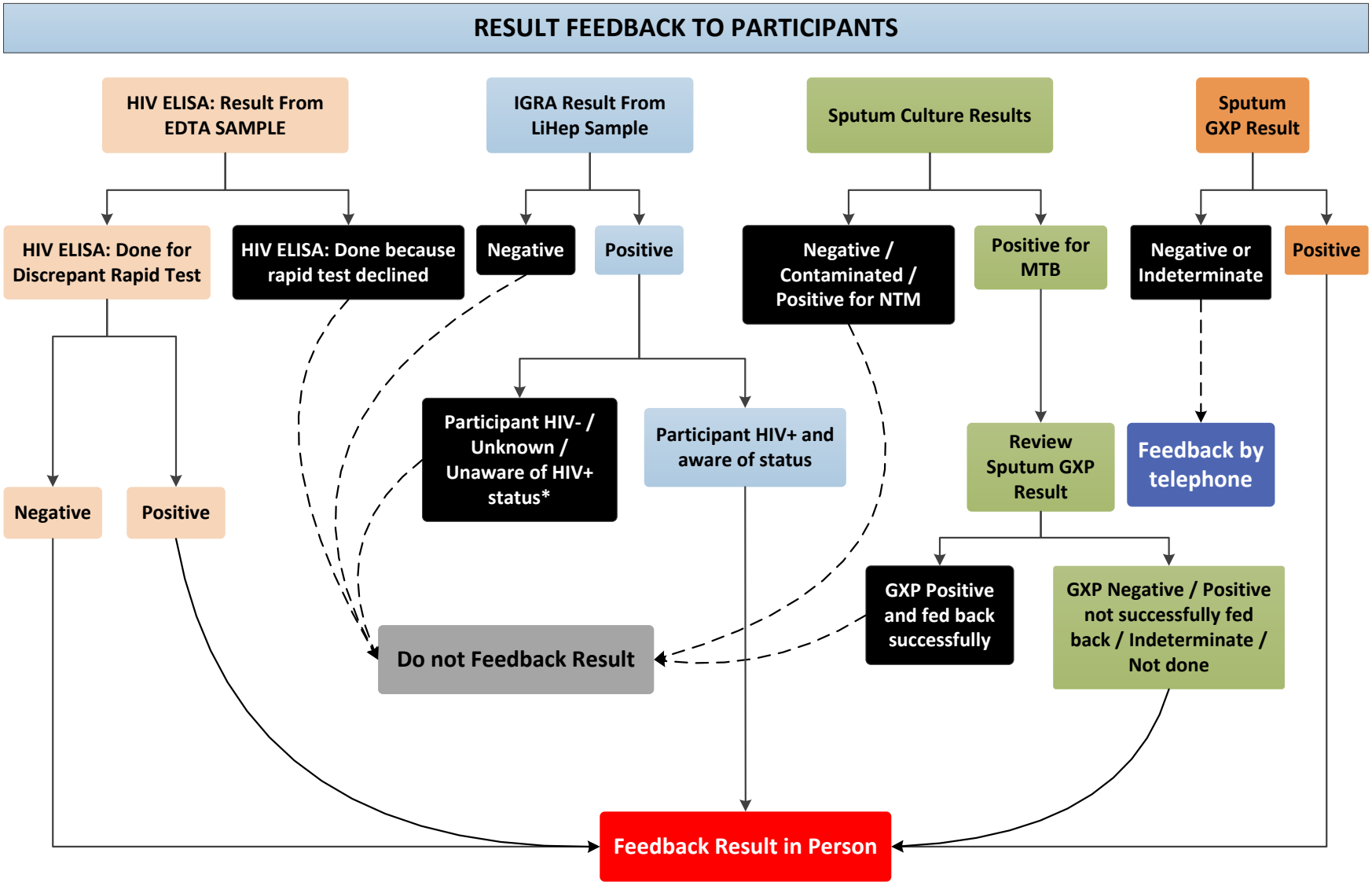

Figure 3- 4: Laboratory result feedback to participants
EDTA: Ethylenediaminetetraacetic acid; ELISA: enzyme-linked immunosorbent assay; GXP: Xpert MTB/RIF; HIV: human immunodeficiency virus; IGRA: interferon-gamma release assay; LiHep: Lithium heparin; MTB: *Mycobacterium tuberculosis*; NTM: non-tuberculous mycobacterium.

3.6. Data management

Data were managed by the AHRI Data Centre and were captured electronically on password protected tablets using the REDCap (Research Electronic Data Capture) Android application (REDCap Consortium Vanderbilt, Nashville) [88]. Automatic checks for invalid entries and internal consistency were pre-programmed into the questionnaire. At the end of each working day, the data from tablets was exported to the project database. Additional data validation checks were run after data were exported. Laboratory data was managed using the LIMS system. After testing, laboratory results were automatically integrated into the LIMS system. Programs written in Pentaho (Hitachi Vantara Corporation, Santa Clara) were used to merge participant data and the laboratory results.

3.7. Definitions

3.7.1. Outcome of interest

The outcome of interest was *M. tuberculosis* infection. This was defined as interferon-gamma (IFN-γ) concentration ≥0.35 IU/mL (calculated as either QuantiFERON TB1 or QuantiFERON TB2 antigen minus QuantiFERON nil) as per the manufacturer's guideline [20].

3.7.2. Exposures of interest

The potential risk factors for *M. tuberculosis* infection are shown in Table 3-1. Information on community HIV prevalence, urban/rural location, household socioeconomic status, number of household residents and distance to clinic were extracted from the DSA database. To come up with HIV status, information collected in the study and information captured in the surveillance was used. Information on passive smoking and the rest of the individual level factors was collected in during the interview.

Table 3- 1: Potential risk factors for *Mycobacterium tuberculosis* infection

Community level	Household level	Individual level
- Community HIV prevalence - Urban/rural location	- Household socioeconomic status - Number of household residents - Passive smoking. - Distance to clinic	- Age - Sex - BCG vaccination Status - Household TB contact - HIV status - Smoking - Alcohol intake - Education level - Admission to hospital - Contact hours with adult males - Contact hours with adult females - Attendance at church - Visits to other homesteads during the day - Sharing sleeping room with other people. - Visits to health facility

3.7.2.1. Definitions of community level factors

Community HIV prevalence: Data on community HIV prevalence were extracted from the DSA database where it was calculated based on previously-described methods using 2017 population data [89]. Briefly, every individual in the DSA was geo-located to a 30 m by 30 m grid superimposed at their homestead. Then a two-dimensional Gaussian kernel density of 3 km radius was passed across the grid to estimate HIV prevalence for the homestead based on the population and number of known HIV positive individuals within the search radius. The estimates of HIV prevalence for each household were categorised into four groups based on the distribution of HIV prevalence of the households of the selected individuals. The lowest category was coded '1' and included households with HIV prevalence below 25%. The highest category was coded '4' and included households with HIV prevalence at least 45%. The HIV prevalence was calculated for each homestead but was analysed as a community level factor since the estimated HIV prevalence reflects the HIV prevalence of the community surrounding the household and not just within the household.

Rural/urban location: Rural/urban location was defined as either urban or rural. In the DSA, communities are defined as urban if the population density is at least 400 per km^2.

3.7.2.2. Definitions of household level factors

Household socioeconomic status: information on household socioeconomic status was extracted from DSA database where it was calculated using principal component analysis. The analysis was performed using information collected from the annual household socioeconomic survey which collects information on ownership or access to the following assets or amenities: electricity, piped water in the homestead, flushing toilets, bed, bicycle, block maker, car, cattle, electric cooker with oven, electric hotplate, electric kettle, fridge, gas cooker, hoe/spade/fork, van/lorry/tractor, kitchen sink, motorcycle, other livestock (not cattle), primus cooker, radio, sofa, sewing machine, tables & chairs, telephone, cell phone, television, wheelbarrow, hot water geyser, washing machine, electric heater, paraffin heater, stereo or hi-fi, computer or laptop, tractor or farm vehicle, furnishings, jewellery and watches. A worth score for each household was created and was categorised into worth tertiles with the lowest coded as '1' and the highest coded as '3'.

Distance to the nearest PHC was extracted from the DSA database and was defined as the Euclidean distance from the homestead to the nearest PHC within the DSA. Passive smoking was defined as living in the same homestead as a person who smokes based on information collected during the survey.

3.7.2.3. Individual level factors

HIV status: Participants were classified as HIV positive if they either tested positive by the rapid test or by ELISA as part of the study; or previously tested positive as part of the DSA surveillance; or self-reported being HIV positive or being on ART. Participants were classified as HIV negative if they tested HIV negative as part of the study; previously tested negative as part of the surveillance within the previous two years before the interview or self-reported testing HIV negative in the previous 2 years. Participants were classified as HIV unknown status if they self-reported that they had never tested before and did not consent to HIV testing by either rapid test or ELISA; previously tested negative in surveillance more than 2 years before enrolment; or self-reported an HIV negative test more than 2 years before enrolment.

Contacts with adult males or females: Participants were asked about the frequency of visits to several indoor locations (school, church, bar, clinic, hospital other houses and closed vehicle), and the duration and number of individuals present at the last visit. Contact hours with adult males (≥12 years) on the last day of visiting an indoor location (school, church, hospital, closed vehicle, bar and other houses) were calculated as the maximum number of adult-males present multiplied by the duration (in hours) of the visit. Visits lasting less one hour were rounded off to one hour. This was multiplied by the number of visits to that indoor location in the previous month to obtain monthly contact hours for that location. Participants reporting not visiting a particular indoor location in the previous month were assigned zero contact hours for that location. To obtain the overall monthly contact hours, the monthly contact hours for all the indoor gathering places captured were summed up. Monthly contact hours with adult females were calculated in a similar manner.

Lifetime household TB contact: Participants were classified as having a lifetime household TB contact if they reported having ever lived in the same house as a person with TB disease for at least two weeks or had ever cared for a person with TB during their lifetime.

BCG vaccination: this was defined as either having documentary evidence of immunisation or scars consistent with BCG vaccination on the right deltoid insertion.

Smoking: Participants were classified as smokers if they reported ever smoking at least 100 cigarettes.

3.8. Sample size calculation

The sample size calculations were based on the following assumptions:

1. Estimated proportion adolescents with QFT-plus positive results to be 40-50% [90] (consistent with studies among adolescents in the Western Cape province, South Africa.
2. 60% of those approached to participate would be enrolled in the study [91].

The sample size required to estimate a prevalence of *M. tuberculosis* infection of 50% with a ±3% precision at 5% significance level was estimated to be 1,100. As this study was initially aimed at estimating cumulative *M. tuberculosis* infection incidence at 12 months, this sample would also enable estimation of a cumulative incidence of 5% at 12 months among participants with QFT-plus

negative results at baseline. In order to account for non-participation at baseline and loss follow up at 12 months, a total of 1,998 participants were initially selected.

3.9. Statistical analyses

3.8.1. Participant enrolment and baseline characteristics

All analyses were done using Stata software version 14.2 (College Station, TX, USA). A flowchart for participants from selection to inclusion in analyses was prepared. Characteristics (age, sex and age at TB diagnosis) of individuals self-reporting previous TB disease were tabulated using linked data from the DSA. Characteristics (age, sex, household SES, rural/urban location and community HIV prevalence) of individuals who were included in the analysis and those not included were tabulated, using linked data from the DSA. The Chi-squared test was used to make comparisons. The individuals not included in the analysis included those not approached, those approached but not contacted, those who refused participation those self-reporting previous TB treatment and those with missing or invalid QFT-plus results. Baseline characteristics of participants included in the analysis were tabulated.

3.8.2. *Mycobacterium tuberculosis* infection prevalence

The crude *M. tuberculosis* infection prevalence was defined as the proportion of participants with the outcome. In addition to the manufacturer cut-off (IFN-γ concentration ≥0.35 IU/mL), the *M. tuberculosis* infection prevalence was also estimated using a stringent (≥0.70 IU/mL) and a less stringent (≥0.20 IU/mL) cut off as suggested by Nemes *et al.* [92]. Finally, estimates for prevalence were obtained for all the three cut offs (0.20, 0.35 and 0.70 IU/ml) including individuals self-reporting previous TB treatment assuming they have *M. tuberculosis* infection. To account for non-participation, the weighted *M. tuberculosis* infection prevalence was calculated by multiplying the crude prevalence by the inverse of probability of participation in strata defined age, sex, and urban/rural residence.

3.8.3. Risk factors for *Mycobacterium tuberculosis* infection

Random effects logistic regression taking account of clustering within homesteads was used to obtain odds ratios (OR) and corresponding 95% confidence intervals (CI) for the association

between *M. tuberculosis* infection and the proposed risk factors (Table 3-1). Only participants with valid QFT-plus results were included in the analysis. Univariate models were fitted for all the proposed risk factors.

To account for inter-relationships between *M. tuberculosis* infection and the proposed risk factors, a hierarchical conceptual framework with three levels (community, household and individual level) was used to perform multivariable analyses (see Chapter 5, Figure 1) [93]. At the community level, the risk of *M. tuberculosis* is determined by exposure to infectious individuals which varies by community as reflected in differences in annual TB notification rates [7]. *M. tuberculosis* infection prevalence is expected to be high in communities with high TB notification rates. At the household level, the risk of exposure to *M. tuberculosis* is expected to be higher in households of people with TB disease and households with low socioeconomic status largely due to poor living conditions like overcrowding and poor access to medical care [94]. At the individual level, the risk of infection is expected to be higher among individuals with history of exposure to TB disease and older individuals due to increased long-term exposure to sources of *M. tuberculosis* infection and increased social contacts. This approach also assumes that the community level factors are the more distal, and individual level factors are the proximal.

Age, and household SES were considered as both *a priori* confounders and exposures of interest. First, community level factors that were associated with the outcome at p-value<0.20 in the univariate analyses were included in a multivariable model. The factors that remained associated at p-value<0.20 were retained in a core community-level model (model 1). Next, household factors were added to this core model one by one. Those that were associated with the outcome at p<0.20, after adjusting for community factors and socioeconomic status, were included in a multivariable model and retained if they remained associated with the outcome at p<0.20 (model 2). Associations with individual-level factors were determined in a similar way, adjusting for community-level and household-level factors, and age (model 3).

Logistic regression was used to obtain odds ratios (OR) and corresponding 95% confidence intervals (CI) for the association between *M. tuberculosis* infection and the proposed risk factors not taking account of clustering within households. The results are presented in Appendix 12.

3.8.4. Missing data

The proportion of participants with missing data for each variable was calculated and presented in the table of the baseline characteristics of participants. Since a small proportion (less than 5.0%) had missing information, imputations were not performed. A complete case analysis was performed.

3.8.5. Spatial distribution of *M. tuberculosis* infection and previous TB disease

All individuals assessed for enrolment in the study were geolocated to their week-block using linked information from the DSA. A map with gradients for the proportion of individuals self-reporting previous TB treatment in each week-block was produced. For participants with valid QFT-plus results, maps with gradients for the proportion of participants with QFT-plus positive results and those self-reporting history of household TB contact were produced. To compare the spatial distribution of *M. tuberculosis* infection prevalence and population density, a map showing gradients for the population density of each week-block was produced. The population densities for the week-blocks were categorised into quintiles with the lowest category coded as '1' and the highest coded as '5'.

3.10. Ethics approval and considerations

The study was approved by the Ethics Committee of the London School of Hygiene & Tropical Medicine, reference 10515 (Appendix 5), the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, reference BE483/15 (Appendix 4), and the KwaZulu-Natal Department of Health, reference 184/16 (Appendix 6). Approval for the study was also obtained from Hlabisa District Hospital and the AHRI Community Advisory Board. To compensate participants for their time, a food refreshment or airtime of about ZAR20.00 (about £1.00) was given. Parents/guardians were not compensated for their participation.

Chapter 4 Results

4.1. Participant selection and enrolment

A total of 1,998 individuals aged 10-19 years resident in the southern part of the AHRI-DSA between November 2017 and December 2018 were selected from the project database. By December 2018, a total 1,809 (90.1%) had an attempted home visit by field staff and 1,173 (60.8%) were assessed for eligibility (Figure 2-1). Among those assessed for eligibility, 35 (3.0%) had a history of TB treatment, 3 (0.2%) were not age eligible and 1,135 (96.8%) were enrolled. The QFT-plus test was successfully done, and results were available for 1,094 participants. These were included in the analyses.

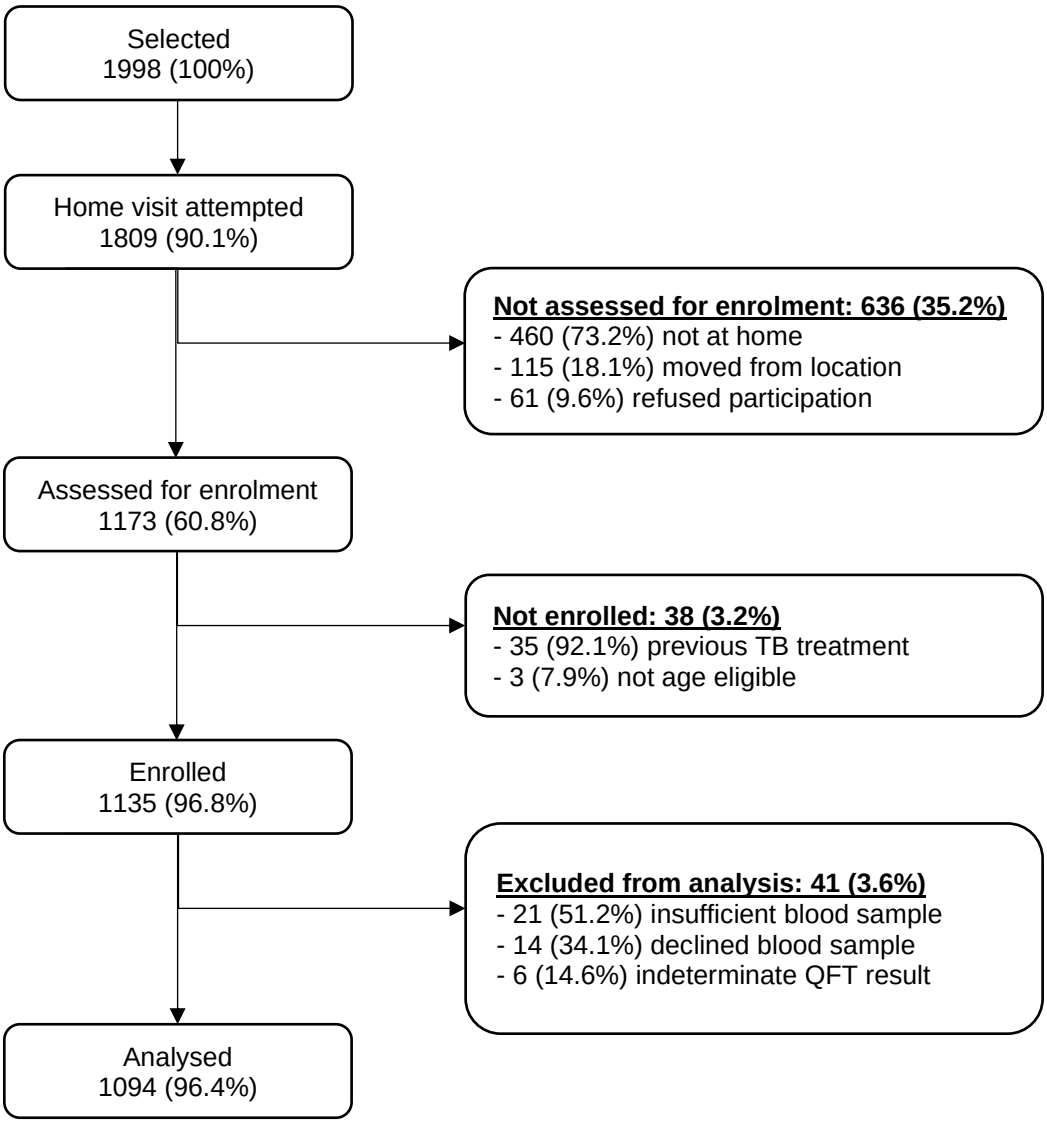

Figure 4- 1: Flow diagram showing participants from selection to analysis

4.2. Individuals reporting previous TB treatment

Overall, 35 (3.0%) individuals assessed for enrolment reported a history of TB treatment. Among these, 18 (51.4%) were female and the median age was 13 (IQR: 12-16) years. The reported median age at diagnosis was 4 (IQR: 2-7) years and 30 (85.7%) reported being diagnosed before they reached 12 years (table 4-1).

Table 4- 1: Characteristics of individuals self-reporting previous TB treatment (n=35)

	N (%)
Sex	
Female	18 (51.4)
Male	17 (48.6)
Age (years)	
10–11	6 (17.1)
12–14	15 (42.9)
15–17	9 (25.7)
≥18	5 (14.3)
Age at TB diagnosis	
<12 years	30 (85.7)
≥12 years	2 (5.7)
Missing	3 (8.5)

TB: tuberculosis

4.3. Comparison of analysed participants and those not analysed

Compared to individuals who were not included in the analysis, there were no differences by age and sex (Table 4-2). There was weak evidence of a difference by household socioeconomic index score, with participants included in the analysis slightly over-represented in the lowest tertile (29.1% vs 25.3%). The participants included in the analysis were less represented in urban communities (3.4% vs 9.5%) and were more likely to come from communities with HIV prevalence below 35.0% (Table 4-2).

Table 4- 2: Comparison of participants included in the analysis (i.e. with valid QFT-plus results) vs. selected individuals not included in the analysis

Variable	Total included in analysis (N=1,094) N (column %)	Total not included in analysis (N=904) N (column %)	p-value[a]
Sex			
Male	546 (49.9)	465 (51.6)	0.47
Female	548 (50.1)	438 (48.4)	
Age			
10–11 years	253 (23.1)	195 (21.6)	0.50
12–14 years	346 (31.6)	301 (33.3)	
15–17 years	309 (28.2)	239 (26.4)	
18+ years	186 (17.0)	169 (18.7)	
Socioeconomic index score (tertiles)			
Low	305 (29.1)	218 (25.3)	0.08
Medium	350 (33.4)	325 (37.7)	
High	393 (37.5)	319 (37.0)	
Number of household residents			
1-5	333 (31.1)	342 (38.5)	<0.01
6-7	252 (23.6)	208 (23.4)	
8-10	248 (23.2)	183 (20.6)	
>10	237 (22.2)	155 (17.5)	
Location			
Urban	37 (3.4)	86 (9.5)	<0.01
Peri-Urban	342 (31.3)	319 (35.3)	
Rural	715 (65.4)	499 (55.2)	
Community HIV prevalence (%)			
<25%	85 (8.1)	64 (7.4)	<0.01
25–34.9%	618 (59.1)	454 (52.2)	
35–44.9%	261 (25.0)	265 (30.5)	
≥45%	82 (7.8)	86 (9.9)	

[a]p-values obtained using Chi-squared distribution
HIV: human immunodeficiency virus

4.4. Characteristics of enrolled participants

Individual level characteristics of the participants included in the analysis are shown in table 4-3. The median age was 15 years (IQR: 12-18) and 548 (50.1%) were female. 43 (3.9%) were HIV positive and 196 (17.2%) had an unknown HIV status. Among those who were HIV positive 34 (79.1%) knew their HIV positive status. 266 (24.3%) reported having ever lived in the same house as a person with TB disease and 34 (3.1%) reported currently living in the same house as a person with TB disease. 984 (90.0%) had evidence of BCG vaccination (either a BCG vaccination scar or documentation). 18 (1.7%) reported ever smoked tobacco and 207 (18.9%) reported living in the same homestead with a person who smokes with 66 (6.0%) living in the same house. 121 (11.1%) reported having ever been admitted to hospital for any reason and 92 (8.4%) reported sleeping in other houses for (≥2 weeks in the previous year.

Household and community level characteristics of enrolled participants are presented in table 4-3. 715 (65.4%) were from rural communities and 34.4% were from communities with HIV prevalence ≥35.0%. 305 (27.9) were from households in the lowest socioeconomic index tertile. The median number of household residents was 7 (IQR: 5-10) and the median distance to the nearest clinic was 2.7 km (IQR: 1.7-4.2).

Details of frequency of attendance at selected indoor gathering places are presented in Table 4-5. 155 (14.2%) participants reported not attending school in the previous seven days. 675 (61.7%) reported not travelling in closed vehicles and 720 (65.8%) reported not making visits to other houses during the day in the previous seven days. 664 (60.7%) reported not attending church or prayer meetings in the previous month and 668 (61.1%) reported not visiting a clinic for any reason in the previous 12 months.

Table 4- 3: Individual level characteristics of participants included in the analysis (n=1,094)

	N (%)
Sex	
Female	548 (50.1)
Male	546 (49.9)
Age (years)	
10-11	237 (21.7)
12-14	349 (31.9)
15-17	297 (27.2)
≥18	211 (19.3)
HIV status	
Negative	855 (78.2)
Positive	43 (3.9)
Unknown	196 (17.9)
Lifetime household TB contact	
No	823 (75.2)
Yes	266 (24.3)
Missing	5 (0.5)
Current household TB contact	
No	1,055 (96.4)
Yes	34 (3.1)
Missing	5 (0.5)
BCG Vaccination [a]	
Vaccinated	984 (90.0)
Not vaccinated	101 (9.2)
Missing	9 (0.8)
Current alcohol intake	
No	1,040 (95.1)
Yes	35 (3.2)
Missing	19 (1.7)
Ever smoked tobacco [b]	
No	1,070 (97.8)
Yes	18 (1.7)
Missing	6 (0.5)
Reported smoker in homestead	
No	880 (80.4)
Yes	207 (18.9)
Missing	7 (0.6)
Reported smoker in household	
No	1,021 (93.3)
Yes	66 (6.0)
Missing	7 (0.6)
Education level	
None or primary	631 (57.6)
Secondary or above	459 (41.0)
Missing	4 (0.4)
Admission to hospital (ever)	
No	967 (88.4)
Yes	121 (11.1)
Missing	6 (0.5)
Sleep in other houses (≥2 weeks)	

	N (%)
No	990 (90.5)
Yes	92 (8.4)
Missing	12 (1.1)
Sharing sleeping room with other people	
None	363 (33.2)
1 person	336 (30.7)
≥2 people	389 (35.6)
Missing	6 (0.5)

[a] based-on BCG vaccination scars or documentation evidence of immunization

[b] ever smoked ≥100 cigarettes

BCG: Bacillus Calmette-Guérin; HIV: Human immunodeficiency virus; TB: Tuberculosis

Table 4- 4: Household and community characteristics of enrolled participants (n=1,094)

	N (%)
Location	
Urban	37 (3.4)
Peri-urban	342 (31.3)
Rural	715 (65.4)
Community HIV prevalence (%)	
<25%	85 (7.8)
25-34.9%	618 (56.5)
35-44.9%	261 (23.9)
≥45%	82 (7.5)
Missing	48 (4.4)
Distance to nearest clinic (quartiles)	
<1.85	301 (27.5)
1.85-3.41	403 (36.8)
3.42-5.36	259 (23.7)
>5.36	131 (12.0)
Household Social economic status (tertiles)	
Low	305 (27.9)
Medium	350 (32.0)
High	393 (35.9)
Missing	46 (4.2)
Number of household members	
<6	333 (30.4)
6-7	252 (23.0)
8-10	248 (22.7)
>10	237 (21.7)
Missing	24 (2.2)

Table 4- 5: Frequency of attendance at indoor gathering places (n=1,094)

	N (%)
Attendance to school (seven days)	
0 times	155 (14.2)
1-3 times	203 (18.6)
≥4 times	704 (64.4)
Missing	32 (2.9)
Road trips (previous seven days)	
0 trips	675 (61.7)
1 trip	158 (14.4)
≥2 trips	245 (22.4)
Missing	16 (1.5)
Visiting other houses during the day (seven days)	
None	720 (65.8)
1-2 houses	227 (20.8)
≥3 houses	136 (12.4)
Missing	11 (1.0)
Attendance to church (four weeks)	
0 times	664 (60.7)
1-2 times	176 (16.1)
≥3 times	233 (21.3)
Missing	21 (1.9)
Visit to bars (four weeks)*	
No	1,059 (96.8)
Yes	25 (2.3)
Missing	10 (0.9)
Attendance to hospital (12 months)*	
No	1,071 (97.9)
Yes	16 (1.5)
Missing	7 (0.6)
Clinic attendance (12 months)*	
No	668 (61.1)
Yes	419 (38.3)
Missing	7 (0.6)

*At least once

4.5. Prevalence of *Mycobacterium tuberculosis* infection

The proportion of participants with positive QFT-plus results using a less stringent (≥0.20 IU/ml), more stringent (≥0.70 IU/ml) and manufacturer cut off (≥0.35 IU/mL) are shown in Table 4-6. The crude prevalence ranged from 19.9% to 25.1%. Assuming individuals self-reporting previous TB disease have *M. tuberculosis* infection, the crude prevalence ranged from 22.4% using the more stringent cut off to 27.4% using the less stringent cut off. The *M. tuberculosis* infection prevalence weighted for non-participation by age, sex and rural/urban residence was 23.0% (95% CI:20.6-25.6%). Detailed results for *M. tuberculosis* infection prevalence and risk factors are presented in Chapter 5.

Table 4- 6: *Mycobacterium tuberculosis* infection prevalence using different criteria and including and excluding self-reported previous TB treatment

Criteria (Cut off)	Positive participants (n/N)	Prevalence % (95 % CI)
≥0.20 IU/mL	275/1094	25.1 (22.7-27.8)
≥0.35 IU/mL	249/1094	22.7 (20.4-25.3)
≥0.70 IU/mL	218/1094	19.9 (17.7-22.4)
≥0.20 IU/mL plus previous TB	310/1129	27.4 (24.9-30.1)
≥0.35 IU/mL plus previous TB	284/1129	25.2 (22.7-27.8)
≥0.70 IU/mL plus previous TB	253/1129	22.4 (20.1-24.9)

4.6. TB symptoms and chronic illnesses

Among the participants who were included in the analysis, 13 (1.2%) reported having a cough of at least two weeks (any duration if HIV positive), 6 (0.6%) reported fever, 6 (0.6%) reported night sweats and 11 (1.0%) reported weight loss (Table 4.7). Overall, 13 (1.2%) had least one TB symptom and were asked to submit sputum. Sputum samples were successfully collected in eight participants and one participant tested Xpert MTB/RIF positive. 21 (1.9%) participants reported having asthma, 34 (3.1%) reported being HIV positive and none reported being diabetic.

Table 4- 7: TB symptoms and chronic illnesses among enrolled participants (n=1,094)

	N (%)
Cough [a] (n=1,092)	13 (1.2)
Weight loss (n=1,088)	11 (1.0)
Fever (n=1,090)	6 (0.6)
Night sweat (n=1,090)	6 (0.6)
TB symptoms (≥1 symptom) [n=1,092]	13 (1.2)
Sputum collection (n=1,092) [b]	13 (1.2)
Xpert MTB/RIF positive results (n=1,092) [c]	1 (0.1)
MTB Culture positive (n=1,092)	0 (0.0)
Diabetes (n=1,087)	0 (0.0)
Asthma (n=1,089)	21 (1.9)
HIV	31 (2.8)

[a] Cough of at least two weeks if HIV negative and any duration if HIV positive.
[b] Five participants with TB symptoms were unable to produce sputum and were referred to their nearest clinic for further management.
[c] Among those who submitted sputum samples

4.7. Spatial distribution of *M. tuberculosis* infection prevalence

4.7.4 Population density

The gradients of the population density for the study area by week-block are shown in Figure 4–2. The median area for the week-blocks was 8.9 (IQR: 1.8-15.5) km^2 and the median population density was 128.1 (IQR: 48.6-527.9) per km^2. The highest population density (>1,000 individuals per km^2) was observed in week-blocks in the south eastern part of the study area.

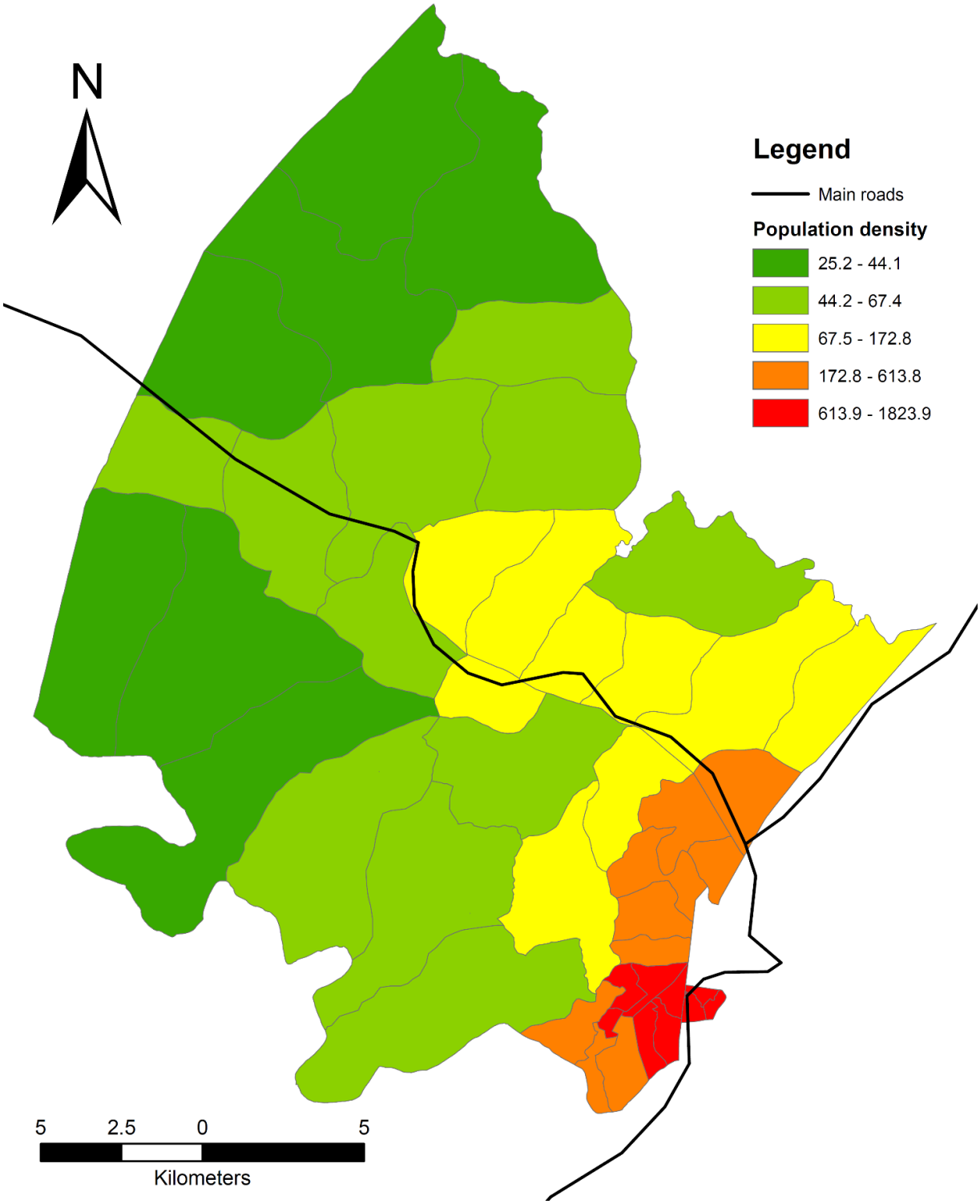

Figure 4- 2: Population density by week-block (sub-divisions of the study area)

4.7.5 Spatial distribution of *M. tuberculosis* infection prevalence

A total 249 participants had QFT-plus positive results giving a crude *M. tuberculosis* infection prevalence of 22.8% (95% CI: 20.4–25.3). The median *M. tuberculosis* infection prevalence in the week-blocks was 21.4% (IQR: 16.0–29.2). Among participants reporting history of household TB contact 78 (31.7%) had QFT-plus positive results. The map of the study area with gradients for *M. tuberculosis* infection prevalence for each week-block is shown in Figure 4–3 panel A. Overall, the distribution of *M. tuberculosis* infection prevalence was similar across rural areas of the study area with 30 (66.7%) week-blocks having *M. tuberculosis* infection prevalence below 25.0%. Eight week-blocks had intermediate prevalence (25.1–35.0%). Among these, seven were either along the national highway on the eastern part of the study area or along the R618 main road from Mtubatuba to Hlabisa Municipality across the middle of the study area (east to west). The highest prevalence (35–66.7%) was observed in four week-blocks on the south-eastern part of the study area (where the population density is higher than other parts of the study area), in one week-block at the centre of the study area and in another week-block on the north-western part of the study area (where only six participants were included in the analysis).

The spatial distribution for *M. tuberculosis* infection and self-reported previous TB diagnosis among adolescents did not show similarities. Only one week-block close to the centre of the study area had high prevalence for both *M. tuberculosis* infection (12/31 [38.7%]) and self-reported previous TB treatment (4/40 [10.0%]) among the adolescents (Figure 4–3 panels A and B).

The spatial distribution for the prevalence of self-reported household TB contact shows week-blocks with intermediate (25–45%) prevalence largely along the R613 main road (Figure 4–3 panel C) but these are not exactly the same week-blocks with intermediate *M. tuberculosis* infection prevalence. The distribution also shows week-blocks with household TB contact prevalence >45% on the south-eastern part of the study area where high prevalence of *M. tuberculosis* infection was also observed.

A. *Mycobacterium tuberculosis* infection prevalence	B. Previous TB disease prevalence among adolescents	C. Reported household TB contact

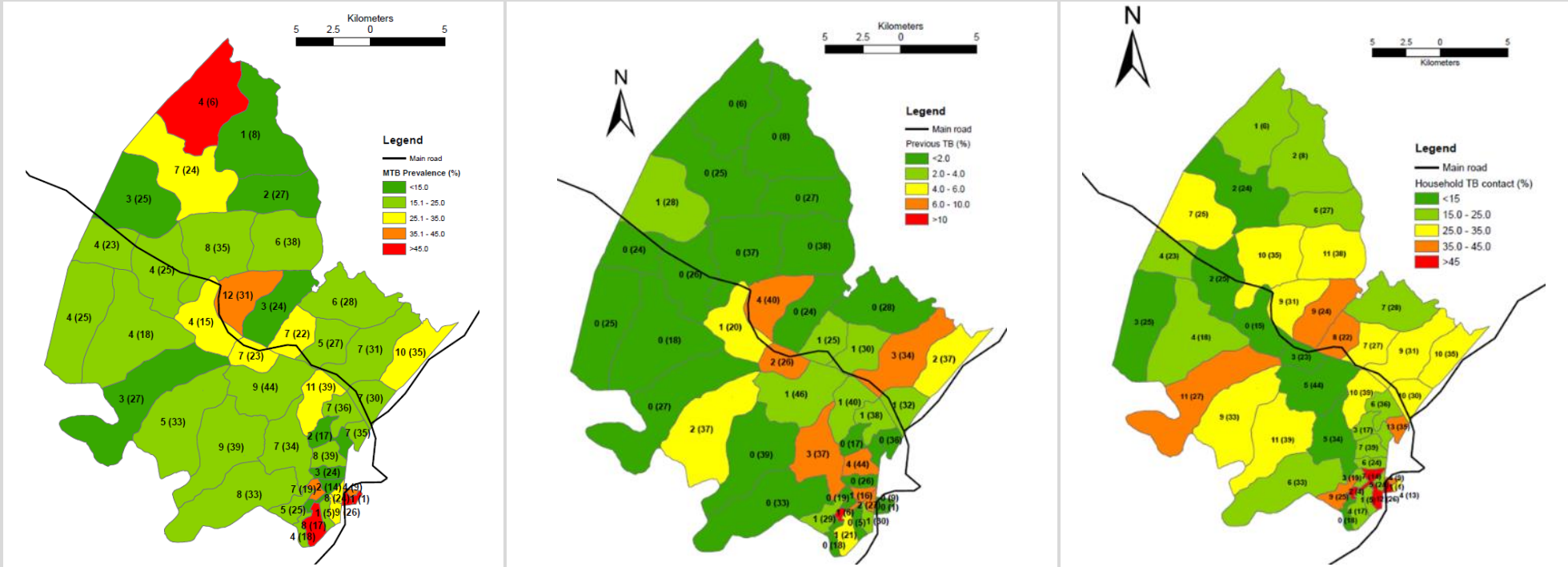

Figure 4- 3: Map of the study area showing spatial distributions:
A: gradients of *Mycobacterium tuberculosis* prevalence by week-block. The number shown in week-block is the number of enrolled participants with positive QFT-plus results and the number in brackets is the total number of enrolled participants.
B: self-reported lifetime TB prevalence among adolescents by week-block. The number shown in each week-block is the number of individuals self-reporting previous TB treatment and the number in brackets is the individuals assessed for enrolment.
C: gradients for prevalence of self-reported lifetime household TB contact by week-block. The number shown in each week-block is the number of enrolled participants self-reporting lifetime household TB contact and the number in brackets is the total number of enrolled participants.
TB: tuberculosis.

Chapter 5: Prevalence of and risk factors for *Mycobacterium tuberculosis* infection among adolescents in rural KwaZulu-Natal, South Africa

This chapter presents results for the analysis of prevalence and risk factors for *Mycobacterium tuberculosis* infection among adolescents. The chapter is presented in the format of a Journal article to be submitted to the International Journal of Tuberculosis and Lung Disease (IJTLD)

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SECTION A – Student Details

Student	Themba Mzembe
Principal Supervisor	Prof Alison Grant
Thesis Title	Prevalence of Mycobacterium tuberculosis infection among adolescents in rural KwaZulu-Natal South Africa

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	International journal of tuberculosis and lung disease (IJTLD)
Please list the paper's authors in the intended authorship order:	Themba Mzembe, Palwasha Khan, Aaron Karat, Safiyya Randera-Rees, Anita Edwards, Richard Lessells, Dickman Gareta, Frank Tanser, Kathy Baisley, Alison Grant
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I collected the data, did the analysis and wrote the manuscript

Student Signature: [redacted] **Date:** 25/10/2019

Supervisor Signature: [redacted] Date: 25/10/15

Prevalence of and risk factors for *Mycobacterium tuberculosis* infection among adolescents in rural KwaZulu-Natal, South Africa

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Abstract

Background: We aimed to estimate the prevalence and explore risk factors for *Mycobacterium tuberculosis* infection among adolescents in a high tuberculosis (TB) and HIV prevalence setting.

Methods: A cross-sectional study of adolescents (10–19 years) randomly selected from a demographic surveillance area (DSA) in rural KwaZulu-Natal, South Africa. We determined *M. tuberculosis* infection status using the QuantiFERON-TB Gold-plus assay. We used HIV data from the DSA to estimate community-level adult HIV prevalence and random effects logistic regression to identify risk factors for TB infection.

Results: We enrolled 1,094 adolescents (548 [50.1%] female); The *M. tuberculosis* infection prevalence (weighted for non-response by age, sex, and urban/rural residence) was 23.0% (95% confidence interval [CI]: 20.6–25.6%). *M. tuberculosis* infection was associated with older age (adjusted odds ratio [aOR]: 1.37; 95% CI: 1.10–1.71, for increasing in age-group [12–14, 15–17, 18–19 years vs. 10–11]), ever (vs. never) having a household TB contact (aOR: 2.13, 95% CI: 1.25–3.64) and increasing community-level HIV prevalence (aOR: 1.43, 95% CI: 1.07–1.92, for every increase in community-level HIV prevalence category [25–34.9%, 35–44.9%, ≥45% vs. <25%]).

Conclusion: Our data support prioritising TB prevention and care activities in TB-affected households, and high HIV prevalence communities.

Key words: latent *Mycobacterium tuberculosis* infection, IGRA, risk factors

Introduction

As an airborne infection, the risk of *Mycobacterium tuberculosis* infection is determined, in part, by the risk of contact with individuals with infectious tuberculosis (TB) disease [1]. *M. tuberculosis* infection in young children (<10 years) is used as a marker of recent transmission and to make inferences about transmission in the population [2, 3]. Compared to older children and adults, young children have limited social contacts and are more likely than older children and adults to be infected within the household [4-7]. However, empirical evidence from both epidemiologic and molecular studies in high TB prevalence settings has shown that household transmission accounts for only between 8%–20% of all transmission [8-12].

Throughout adolescence, young people have increasing social contact with the wider community and thus increased risk of *M. tuberculosis* exposure and infection [7, 13, 14]. This suggests that *M. tuberculosis* infection in adolescents might be a more representative measure of community-wide transmission, but there are few population-based studies from sub-Saharan Africa. We aimed to determine the prevalence of and risk factors for *M. tuberculosis* infection among adolescents in a high TB and HIV prevalence setting.

Methods

Study setting

The study was conducted in the southern part of the Africa Health Research Institute's (AHRI) demographic surveillance area (DSA), in uMkhanyakude district, KwaZulu-Natal, South Africa, which has a resident population of around 60,000 and an adult HIV prevalence estimated at 36.6% in 2016 [15]. The annual notification rate of all TB cases was 394 per 100,000 population in 2018 [personal communication from Jacqueline Ngozo].

Study participants and procedures

We randomly selected adolescents (aged 10–19 years) from the complete sampling frame of all residents (individuals reported as intending to spend the majority of nights at a household within the DSA). Between November 2017 and December 2018, the selected individuals were visited at home and invited to take part. Individuals reporting any history of treatment for active TB were excluded

from the main study. A standard questionnaire was administered that included questions on education level, Bacillus Calmette-Guérin (BCG) vaccination, history of lifetime household TB contact, admission to hospital, smoking (and passive smoking), alcohol intake and history of HIV testing. All participants were examined for presence of BCG scars (documentation of immunisations was also checked) and were asked history of attendance (including frequency of attendance in the previous month; duration and number of people present at the last visit) at relevant indoor gathering places (school, church, health facility, and public transport). Data were collected on electronic tablets using the REDCap application (Vanderbilt University, Nashville, Tennessee) [16].

Participants who were not known to be HIV positive, or whose most recent negative HIV test was more than three months previously, were encouraged to check their HIV status via a rapid HIV test on a fingerpick blood sample. Those who declined rapid testing were offered the option of undergoing anonymised laboratory enzyme-linked immunosorbent assay (ELISA) for research purposes only (further details on HIV testing are presented in the supplementary material Section 1). Participants newly testing HIV positive and those previously diagnosed with HIV but not on antiretroviral therapy (ART), were referred to initiate ART [17]. Participants with TB symptoms (any of cough [≥two weeks, or any duration if HIV positive], fever, night sweats, and weight loss) were asked to submit sputum for Xpert MTB/RIF testing. Those unable to produce sputum were referred to their nearest clinic for further management in accordance with national guidelines [18].

Information extracted from the DSA database included previous HIV test results (for those aged ≥15 years), and household data including urban/rural location, number of residents, socioeconomic status (SES), and distance to the nearest clinic. Community HIV prevalence (for ≥15-year-olds) was calculated using 2017 surveillance data by means of a two-dimensional Gaussian kernel density of 3 km search radius based on previously described methods [19].

Laboratory procedures

Details of laboratory testing are provided in the supplementary material Section 1. Briefly, venous blood was tested for *M. tuberculosis* infection using the QuantiFERON-TB Gold Plus (QFT-Plus) assay (QIAGEN, Hilden, Germany) according to the manufacturer's instructions [20]. Sputum

samples were tested using Xpert MTB/RIF (Cepheid, Sunnyvale, CA) at Hlabisa district hospital laboratory.

Definitions

M. tuberculosis infection was defined as interferon-gamma (IFN-γ) concentration ≥0.35 IU/mL (calculated as either TB1 or TB2 antigen minus nil) per the manufacturer's guideline [20]. Lifetime household TB contact was defined as either having lived in the same household as a person with TB disease for ≥two weeks or having cared for a person with TB during the participant's lifetime. Detailed definitions for exposures are provided in the supplementary material Section 2.

Statistical analysis

A sample size of 1,100 was sufficient to estimate the prevalence of *M. tuberculosis* infection of 50% with a precision of ±3% at 5% significance level. To account for non-participation (both inability to contact participants and refusal to participate), a total 1,998 adolescents were selected.

Prevalence was weighted to account for non-response, calculated as the inverse probability of participation in strata defined by age, sex, and urban/rural residence.

To account for non-participation, the weighted *M. tuberculosis* infection prevalence was calculated by multiplying the crude prevalence by the inverse of probability of participation in strata defined age, sex, and urban/rural residence. Characteristics of individuals included in the analysis were compared with those who were selected but not included (because of non-participation or missing results) using Chi-squared tests. Random effects logistic regression taking account of clustering within households was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association of *M. tuberculosis* infection with potential risk factors. To account for the interrelationships between the potential risk factors, a hierarchical approach [21] with three levels (community, household, and individual) was used to build a multivariable model. First, community factors associated with the outcome at $p<0.20$ on univariable analysis were retained in a core model. Next, household factors were added sequentially to the core model, and retained if they remained associated with the outcome at $p<0.20$, after adjusting for community factors and SES. Associations with individual-level factors were determined similarly, with age included in all the

models as an *a priori* confounder. A complete case analysis was performed. Analyses were performed using Stata version 14.2 (College Station, TX, USA).

Ethics approval

The study was approved by the London School of Hygiene & Tropical Medicine Ethics Committee (ref. 10515), the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (ref. BE483/15), and the KwaZulu-Natal Department of Health (ref. 184/16). Individual informed written consent was obtained from participants aged 18–19 years and from parents/guardians of participants aged 10–17 years, with informed assent from the participant. For participants or parent/guardians who could not read and/or write, a witness who was not a member of the research team attested to the informed consent procedure.

Results

Participant enrolment

Field workers successfully visited the homes of 1,809/1,998 (90.5%) selected individuals (Figure 2);1,173/1,809 (64.8%) were screened for eligibility, 575 (31.8%) were not found and 61 (3.4%) refused participation. Among those screened, 35 (3.0%) had a history of TB treatment, three (0.2%) were ineligible after crosschecking their date of birth, and the remaining 1,135 (96.8%) were enrolled. QFT-plus results were available for 1,094 participants (Figure 2).

Individuals included in the analysis compared to those not included were more likely to be from rural communities, and from communities with lower HIV prevalence (Supplementary Table 1). There were no differences by age, sex or SES.

Among 1,094 participants, 548 (50.1%) were female, 266 (24.4%) had a lifetime household TB contact, 379 (34.6%) were from urban communities, 965 (88.6%) had evidence of BCG vaccination, and 43 (3.9%) were HIV positive (Table 1). Overall, 898 participants had a known HIV status: 641 were through testing in the study, 103 through surveillance activities, and 154 through self-reporting.

M. tuberculosis infection prevalence

249 participants had IFN-γ values ≥0.35 IU/mL, giving a crude *M. tuberculosis* infection prevalence of 22.8% (95% CI: 20.4%-25.3%). The *M. tuberculosis* infection prevalence weighted for non-participation by age, sex and rural/urban residence was 23.0% (95% CI:20.6-25.6%). The distribution of IFN-γ values for all participants is presented in Supplementary Figure 1.

Risk factors for M. tuberculosis infection

At community level, there was evidence of an association between *M. tuberculosis* infection and community HIV prevalence (Table 2). The odds of *M. tuberculosis* infection increased with increasing community HIV prevalence (linear OR: 1.43 for each unit increase in community HIV prevalence category).

At the individual level, *M. tuberculosis* infection was positively associated with older age and having a lifetime household TB contact (Table 2). The odds of *M. tuberculosis* infection increased with increasing age (linear OR: 1.37 for each unit increase in age group) and were 2.1 times higher among participants with history of a household TB contact compared to those without. There was no evidence of association between *M. tuberculosis* infection and BCG vaccination or HIV infection after adjusting for community, household, and individual level factors (Table 2). *M. tuberculosis* infection was inversely associated with number of visits to church in the previous month, and houses visited during day hours in the previous week. There was no evidence of an association with sharing a sleeping room with other people, or with other estimates of social contacts (Supplementary Table 2).

Discussion

In this high TB/HIV prevalence setting, the prevalence of *M. tuberculosis* infection (23.0%) among adolescents was lower than found in the Western Cape province, South Africa [22, 23] . To our knowledge, this is the first study reporting a strong evidence of an association between *M. tuberculosis* infection and increased community-level HIV prevalence.

Recent data on *M. tuberculosis* infection among adolescents largely comes from two studies conducted in densely-populated townships in Western Cape province where prevalence (defined as

tuberculin skin test induration ≥10 mm) was much higher: 37% among individuals 5–17 year-olds [23] and 42.2% (95% CI: 40.9-43.6) 12–18 year-olds [22]. Possible explanations for this difference include differences in social contact patterns, as our study was conducted in a less densely-populated rural area. A second explanation could be differences in population prevalence of active TB; at the time of the studies in Western Cape, the annual TB notification was about 1,400 per 100,000 [22, 23] compared to 577 per 100,000 in 2015 for uMkhanyakude district (the setting of our study) [24]. A third possible explanation is differences in HIV prevalence among notified TB patients. For example, in 2015 the HIV prevalence among people notified with TB was 64.3% in uMkhanyakude district compared to 44.6% in Cape Town [24]. At individual level, HIV-positive individuals are likely to be less infectious due to cavitary lung disease being less common [25].

A 2013 TST survey among school-going children aged 6–8 years in our setting reported a *M. tuberculosis* infection prevalence of 12.4% (95% CI: 10.2%–15.0%) using TST ≥10 mm [26]. The study did not find an association between age and community-level HIV prevalence. However, the odds of *M. tuberculosis* infection for participants living in households with at least two HIV-positive individuals were slightly higher (adjusted odds ratio: 1.8, 95% CI: 1.1–3.1). The higher *M. tuberculosis* infection prevalence and the association with increased age in our study (in older individuals) reflects longer cumulative exposure to people with infectious TB, increased social contact of older adolescents with the wider community [7, 14]. In addition, the older adolescents in our study would also have experienced a higher risk of TB infection in their early lives, because TB notification rates in KwaZulu-Natal have fallen over the last decade [24].

Similar to the Western Cape study [22], we found increased odds of *M. tuberculosis* infection among participants with a lifetime household TB contact. Thus, transmission within households of individuals with TB disease remains an important consideration for TB prevention and care programmes and highlights the need for enhancing household TB contact tracing to reduce transmission. Despite this, 63% of our participants with *M. tuberculosis* infection reported to have never lived in the same house as an individual with TB disease.

The DSA setting of our study allowed us to investigate the effect of the participant's community HIV prevalence on *M. tuberculosis* infection. Though ART reduces the risk of TB disease following

infection and ART access has improved over the years [27], HIV-positive individuals remain at elevated risk of TB disease [28, 29]. Through long-term population-based surveillance, we have shown that HIV prevalence has remained consistently high in certain communities within the DSA over a number of years [19, 27]. We have also shown that active TB, and specifically drug-resistant TB, are associated with those high HIV prevalence areas [30, 31]. The association between higher *M. tuberculosis* infection prevalence among adolescents with higher community HIV prevalence suggests continuing transmission in these communities. Targeted efforts to find and treat TB in such communities could be effective in reducing TB transmission.

The odds of *M. tuberculosis* infection were lower among participants who reported visiting at least three houses during day hours in the previous week and those who attended at least three prayer meetings in the previous month. This might reflect limitations with our retrospective method for social contacts.

This study has several limitations. First, participants from urban communities and communities with high HIV prevalence were under-represented. Since *M. tuberculosis* infection prevalence was higher in communities with HIV prevalence ≥45%, our overall estimate for *M. tuberculosis* infection prevalence may have been slightly underestimated.

Another limitation is that social contact information was captured retrospectively by asking participants about their attendance at indoor gathering places and details of the last visit. Though knowledge of attending an indoor gathering place would still be in memory, reporting errors might have been introduced concerning the frequency and duration of visits, and numbers of people present, resulting in misclassification that may have obscured associations.

Strengths of this study include a large sample size allowing us to estimate prevalence with high precision, and power to detect important associations with potential risk factors. We believe that our estimate is reflective of *M. tuberculosis* infection prevalence among adolescents in this setting. Secondly, the QFT-plus test was used, which is a more specific test for *M. tuberculosis* infection than the TST. Furthermore, we experienced a very low proportion of indeterminate results. Another strength is that this study was nested within in a well-defined DSA which provided a comprehensive

sampling frame and allowed us to determine the effect of non-participation on the estimate for prevalence.

Conclusion

In this high TB and HIV burden setting, the prevalence of *M. tuberculosis* infection among adolescents was lower than reported from the Western Cape in South Africa. Community-level HIV prevalence, age, and lifetime household TB contact were associated with increased odds of *M. tuberculosis* infection. Enhancing TB household contact tracing and targeted case finding in high HIV prevalence communities has potential to reduce the burden of TB in this setting.

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Author contributions

ADG, RL and KB designed the study. TM, ASK, AE, KB and SRR collected the data. TM performed the statistical analyses with oversight from KB, PK and ADG. AT and FT performed analyses for

community-level HIV prevalence. TM wrote the manuscript with input from all the authors. All the authors reviewed the manuscript and approved the final version.

Conflicts of interest: None declared.

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Tables and Figures

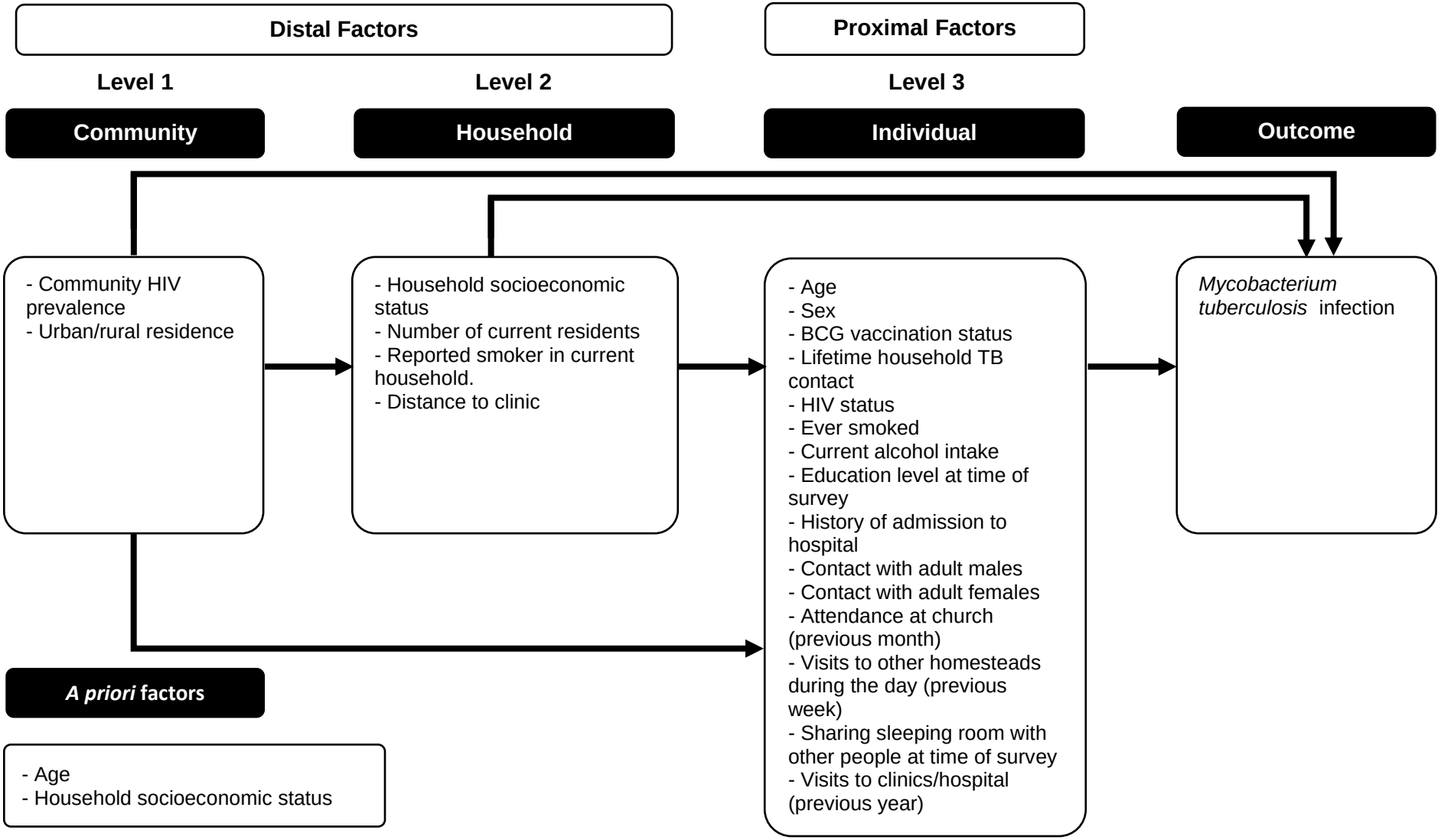


Figure 1: Conceptual framework for the hierarchical risk factor analysis for *Mycobacterium tuberculosis* infection among adolescents

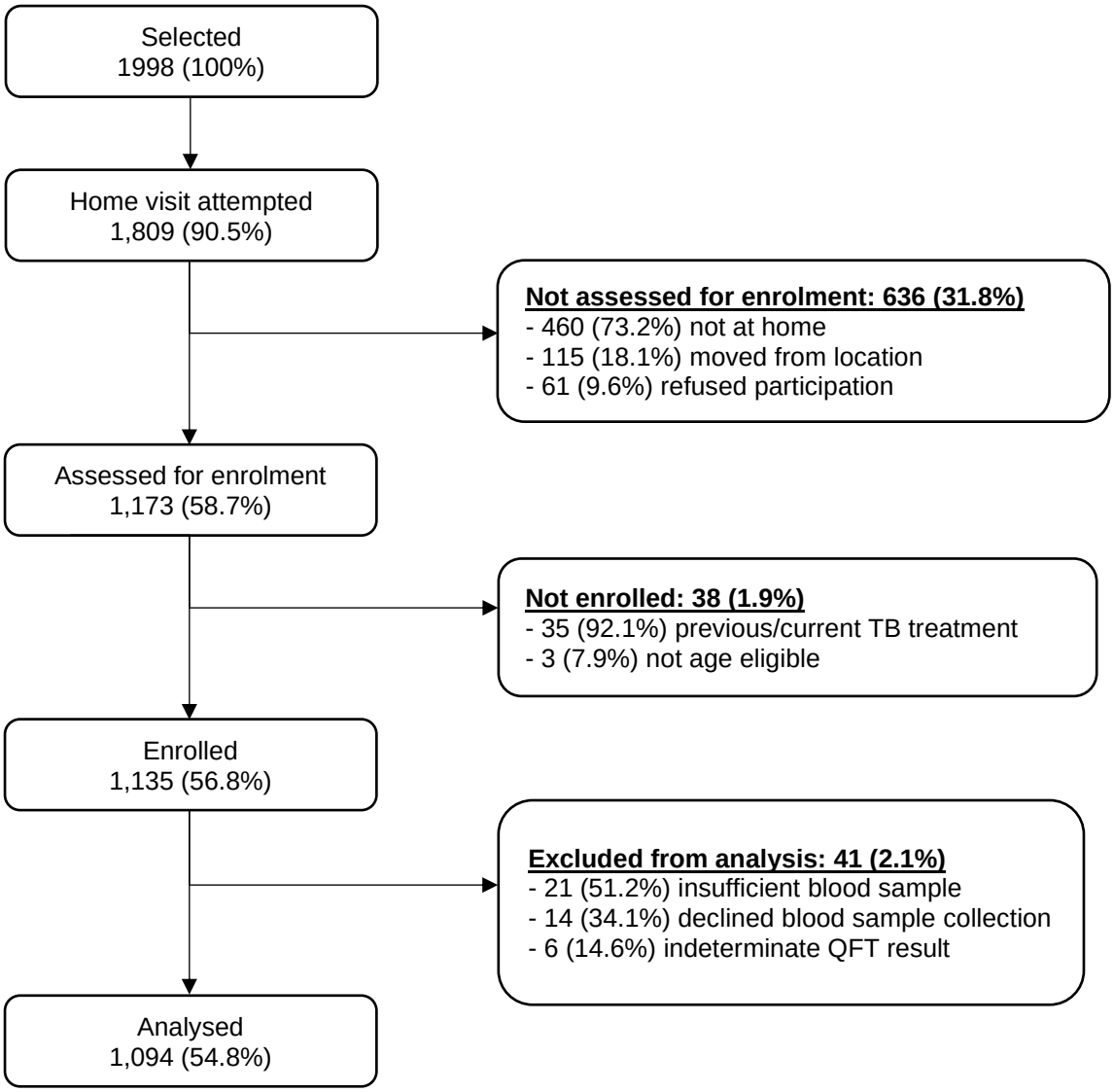


Figure 2: Flow diagram showing participants from selection to analysis

Table 1 : Characteristics of study participants (n=1,094)

	N (%)
Sex	
Female	548 (50.1)
Male	546 (49.9)
Age (years)	
10–11	237 (21.7)
12–14	349 (31.9)
15–17	297 (27.2)
≥18	211 (19.3)
Lifetime household TB contact (n=1089)	
No	823 (75.6)
Yes	266 (24.4)
HIV status	
Negative	855 (78.2)
Positive	43 (3.9)
Unknown	196 (17.9)
BCG vaccination (n=1085)	
Vaccinated	984 (90.7)
Not vaccinated	101 (9.3)
Education level (n=1090)	
Primary	631 (57.9)
Secondary or above	459 (42.1)
Location	
Rural	715 (65.4)
Urban	379 (34.6)
Household socioeconomic index tertiles (n=1048)	
Low	305 (29.1)
Middle	350 (33.4)
High	393 (37.5)
Number of household residents (n=1070)	
<6	333 (31.1)
6–7	252 (23.6)
8–10	248 (23.2)
>10	237 (22.2)
Church attendance in previous 4 weeks (n=1073)	
None	664 (61.9)
1–2 times	176 (16.4)
≥3 times	233 (21.7)

BCG: Bacillus Calmette-Guérin; HIV: Human immunodeficiency virus; TB: tuberculosis

Table 2: Risk factors for *Mycobacterium tuberculosis* infection

Hierarchical level		QFT Positive n/N (%)	Crude OR (95% CI)	*p*-value	Adjusted OR[1] (95% CI)	*p*-value
Community	Community HIV prevalence (%)					
	<25%	12/85 (14.1)				
	25–34.9%	133/618 (21.5)				
	35–44.9%	61/261 (23.4)	1.43 (1.07–1.92)*	0.02*		
	≥45%	26/82 (31.7)				
Household	Household social economic index score (tertiles)					
	Low	74/305 (24.3)	1	0.72	1[a]	0.75
	Middle	79/350 (22.6)	0.87 (0.51–1.47)		0.81 (0.46–1.41)	
	High	83/393 (21.1)	0.81 (0.49–1.36)		0.89 (0.52–1.54)	
Individual	Sex					
	Female	123/548 (22.4)	1	0.85	1[b]	0.80
	Male	126/546 (23.1)	1.04 (0.72–1.50)		0.95 (0.62–1.45)	
	Age					
	10–11 years	49/237 (20.7)				
	12–14 years	62/349 (17.8)	1.32 (1.09–1.59)*	<0.01*	1.37 (1.10–1.71) [b]	0.01*
	15–17 years	71/297 (23.9)				
	≥18 years	67/211 (31.8)				
	Lifetime household TB contact					
	No	168/823 (20.4)	1	<0.01	1[b]	0.01
	Yes	78/266 (29.3)	1.90 (1.20–3.01)		2.13 (1.25–3.64)	
	HIV status					
	Negative	193/855 (22.6)	1	0.88	1[b]	0.35
	Positive	9/43 (20.9)	0.91 (0.34–2.40)		0.65 (0.20–2.09)	
	Unknown	47/196 (24.0)	1.11 (0.69–1.81)		1.43 (0.80–2.56)	
	BCG vaccination					
	Vaccinated	216/984 (22.0)	1	0.24	1[b]	0.65
	Not vaccinated	28/101 (27.7)	1.43 (0.78–2.65)		1.19 (0.32–2.98)	
	Church attendance in previous month					
	None	165/664 (24.8)	1	0.08	1[b]	0.04
	1–2 times	37/176 (21.0)	0.75 (0.44–1.26)		0.59 (0.32–1.10)	
	≥3 times	41/233 (17.6)	0.58 (0.35–0.95)		0.49 (0.27–0.89)	

Hierarchical level		QFT Positive n/N (%)	Crude OR (95% CI)	*p*-value	Adjusted OR[1] (95% CI)	*p*-value
	Visiting other houses during the day (previous week)					
	None	169/720 (23.5)	1	0.02	1[b]	0.01
	1–2 houses	59/227 (26.0)	1.20 (0.76–1.88)		1.00 (0.60–1.69)	
	≥3 houses	17/136 (12.5)	0.38 (0.19–0.78)		0.28 (0.12–0.66)	

BCG: Bacillus Calmette-Guérin; CI: confidence interval; HIV: Human immunodeficiency virus; OR: odds ratios; QFT: QuantiFERON TB-Gold plus; TB: tuberculosis

* Odds ratios modelled as a linear trend across the categories; n and % QFT positive in each category shown for information only.

[1]The adjusted odds ratios were obtained at each level (community, household and individual) of the hierarchical approach as described in the methods section.

[a] Adjusted for community HIV prevalence and household socioeconomic status.

[b] Adjusted for community HIV prevalence, household socioeconomic status, age, lifetime household TB contact, church attendance and visits to other households.

Supplementary material

Section 1: Laboratory procedures

Participants without history of HIV testing or with a previous negative HIV test result more than three months earlier were encouraged to have a rapid HIV test (parallel test using Abon HIV 1/2/0 Tri-line [Abon Biophrarm (Hangzhou) Co., Ltd] and Advanced Quality™ Rapid Anti-HIV [InTec Products Inc]) based on a fingerpick blood sample. Those not wishing to test were given the option to have an anonymised HIV enzyme-linked immunosorbent assay (ELISA) test using their venous blood sample, or to decline HIV testing altogether. The anonymised results were not fed back to participants or made available to investigators during the course of the study. The ELISA was done at the project laboratory based on a two-test algorithm using HIV-1/HIV-2 ELISA (Vironostika HIV-1 Microelisa System: Biomérieux, Durham, NC, USA) which was followed by Wellcozyme HIV-1 + 2 GACELISA (Murex Diagnostics Benelux B.V., Breukelen, Netherlands).

For participants aged 10–17 years, we sought consent from the parent/guardian to approach the child and encourage HIV testing as part of the study. In line with national guidelines, HIV testing for participants aged 10–11 years was done in the presence of the parent/guardian after the participant had independently assented to the test [1].

Participants with positive Xpert MTB/RIF results were referred to their nearest primary health care clinic (PHC) to initiate TB treatment. Participants with negative results but persistent symptoms were also referred for further assessment. All HIV-positive participants with QFT-plus positive results were referred for assessment to their nearest PHC to initiate TB preventive therapy, if this was not contraindicated [2].

Section 2: Definitions

HIV status: participants were classified as HIV positive if they either tested positive by rapid test or ELISA as part of the study; previously tested positive as part of the demographic

surveillance area (DSA) annual HIV surveillance (which includes individuals aged ≥15 years); or self-reported being HIV positive. Participants were classified as HIV negative if they tested HIV negative as part of the study; previously tested negative as part of the annual surveillance within the previous two years before enrolment, or self-reported testing HIV negative in the previous two years without evidence of being HIV positive. Participants were classified as HIV unknown status if they self-reported that they had never tested before and did not consent to HIV testing by either rapid test or ELISA in the study; previously tested negative in the annual surveillance more than two years before enrolment, or self-reported an HIV negative test result more than two years before enrolment. Details of HIV testing for participants with known HIV status are provided below.

BCG vaccination was defined as either the presence of a BCG scar or documentary evidence of immunisation.

Household: a group of individuals living on the same residential plot regardless of whether they lived in the same building or a different building.

Household socioeconomic status:

A household socioeconomic index score was calculated using principal component analysis (PCA) [3]. The PCA used ownership or access to the following assets or amenities, as measured in the annual DSA surveillance, to create worth index scores for each household: electricity, piped water in the homestead, flushing toilets, bed, bicycle, block maker, car, cattle, electric cooker with oven, electric hotplate, electric kettle, fridge, gas cooker, hoe/spade/fork, van/lorry/tractor, kitchen sink, motorcycle, other livestock (not cattle), primus cooker, radio, sofa, sewing machine, tables & chairs, telephone, cell phone, television, wheelbarrow, hot water geyser, washing machine, electric heater, paraffin heater, stereo or hi-fi, computer or laptop, tractor or farm vehicle, furnishings, jewellery and watches. The index score was categorised into household worth tertiles (low, medium and high).

Urban/rural residence:

This was based on the definition used in the DSA where areas with population density below 400 per km^2 are classified as rural, areas above 400 km^2 as peri urban and KwaMsane (a township on the south eastern part of the DSA) as urban. For the purposes of this analysis, urban and peri-urban were combined as only 37 (3.4%) participants were from urban areas.

Monthly contact hours with adult males and females:

Contact hours with adult males (aged ≥12 years) on the last day of visiting an indoor location were calculated as the number of people present times the duration (in hours) of the visit. Visits lasting less one hour were captured as one hour. This was multiplied by the number of visits to that indoor location in the previous four weeks to obtain monthly hours for that location. Participants reporting not visiting a location in the previous four weeks were assigned zero contact hours for that location. The monthly contact hours for the selected indoor gathering locations (school, church, hospital, closed vehicle, bar, and other houses) were summed to obtain overall monthly contact hours. The procedure was repeated to obtain contact hours with adult females.

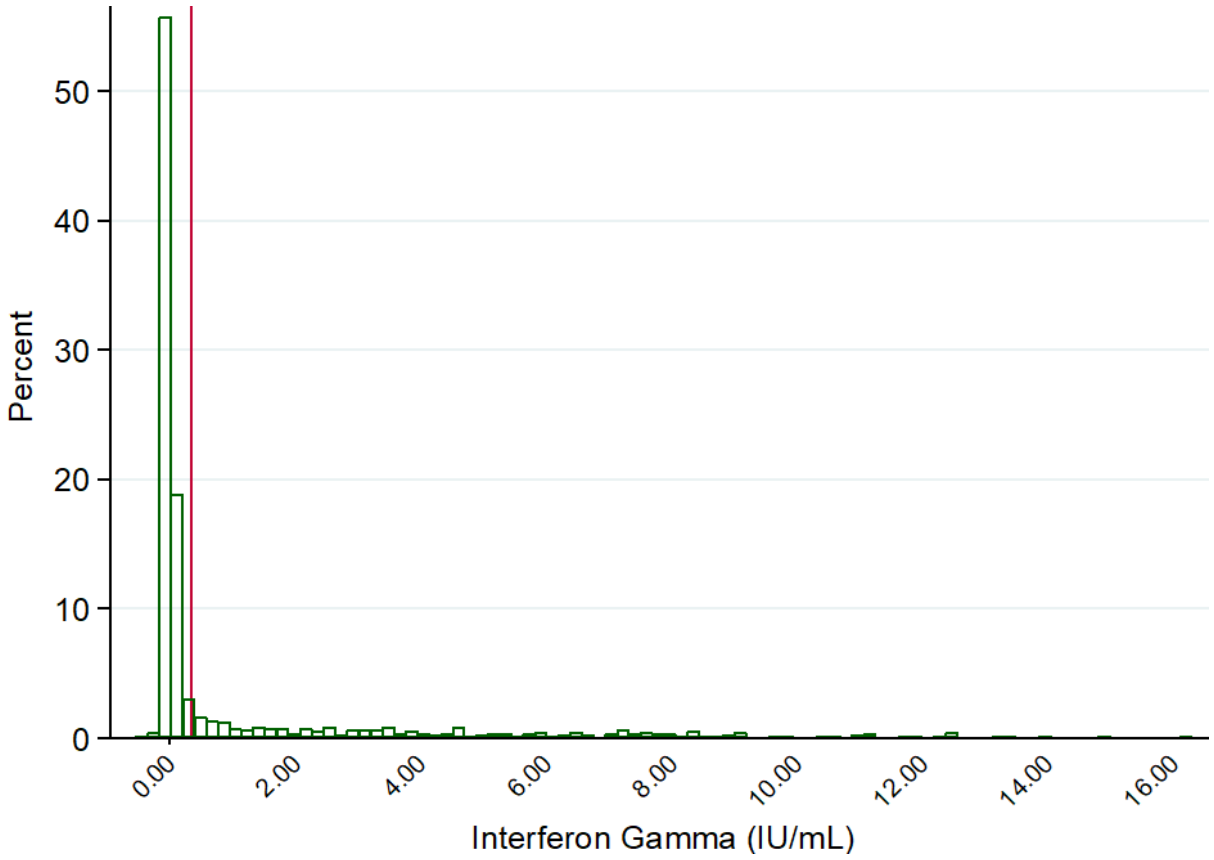
Section 3: Comparison of individuals included vs. not included in the analysis

Supplementary Table 1: Comparison of participants included in the analysis and individuals randomly selected but not included in the analysis

	Included in analysis (n=1,094) n (column %)	**Not included in analysis (n=904) n (column %)**	***p*-value***
Sex			
Male	546 (49.9)	465 (51.6)	0.47
Female	548 (50.1)	438 (48.4)	
Age (years)			
10-11	253 (23.1)	195 (21.6)	0.50
12-14	346 (31.6)	301 (33.3)	
15-17	309 (28.2)	239 (26.4)	
18+	186 (17.0)	169 (18.7)	
Location			
Urban	379 (34.6)	405 (44.8)	<0.01
Rural	715 (65.4)	499 (55.2)	
Social economic index score (tertiles)			
Low	305 (29.1)	218 (25.3)	0.15
Medium	350 (33.4)	325 (37.7)	
High	393 (37.5)	319 (37.0)	
Missing	46 (4.2)	42 (4.7)	
Community HIV prevalence (%)			
<25%	85 (7.8)	64 (7.1)	0.02
25–34.9%	618 (56.5)	454 (50.2)	
35–44.9%	261 (23.9)	265 (29.3)	
≥45%	82 (7.5)	86 (9.5)	
Missing	48 (4.4)	35 (3.9)	

* *p*-values were obtained from Chi-squared test
HIV: human immunodeficiency virus

Section 4: Interferon-gamma concentration distribution



Supplementary Figure 3: Interferon-gamma concentration distribution for all participants (n=1,094). The red line indicates the manufacturer defined cut-off for *Mycobacterium tuberculosis* infection (0.35 IU/mL).
IU: International units; mL: millilitres

Interferon-gamma (IFN-γ) concentration distribution for all participants are shown in Supplementary Figure 1. 361 (33.0%) had IFN-γ values ≤0.00 IU/mL; 56 (5.1%) had values between 0.20-0.70 IU/mL, 218 (19.9%) had values ≥0.70 IU/mL and 44 (4.0%) had IFN-γ values ≥7.59 IU/mL.

Section 5: HIV status and referral to care

43 participants were classified as HIV-positive. Among these, 20 were known through self-reporting being HIV positive and on antiretroviral therapy (ART), 13 though testing in the DSA, 8 through testing in the study (5 anonymised ELISA testing and 3 by rapid testing) and 2 through self-reporting being HIV-positive but not taking ART. 855 participants were classified as HIV-negative. Among these 633 were known through testing in the study (470 through rapid test and 163 through ELISA), 131 through self-report and 91 through testing in the DSA.

13 (1.3%) participants had least one TB symptom and were asked to submit sputum. Sputum samples were successfully collected in eight participants and one participant (0.1% of all participants) tested Xpert MTB/RIF positive. Overall, among all the participants with QFT-plus positive results, 7 (2.8%) were also HIV-positive and were referred for assessment for TB preventive therapy in line with national guidelines [2].

Section 6: Hierarchical risk factor analysis

Supplementary Table 2: Risk factors for *Mycobacterium tuberculosis* infection showing odds ratios obtained from the crude, partial and fully adjusted models at each level of hierarchical approach.

Variable	QFT Positive n/N (%)	Crude OR (95% CI)	*p*-value	Adjusted OR[1] (95% CI)	*p*-value	Adjusted OR[2] (95% CI)	*p*-value
Community Level factors							
Community HIV prevalence (%)							
<25%	12/85 (14.1)						
25–34.9%	133/618 (21.5)						
35–44.9%	61/261 (23.4)	1.43 (1.07–1.92)	0.02[3]				
≥45%	26/82 (31.7)						
Location							
Rural	156/715 (21.8)	1	0.34	1[a]	0.54		
Urban	93/379 (24.5)	1.21 (0.81–1.80)		0.84 (0.48–1.47)			
Household Level factors							
Distance to nearest clinic (km) (quartiles)							
<1.85	84/301 (27.9)	1	0.13	1[a]	0.21	1[b]	0.30
1.85-3.41	80/403 (19.9)	0.56 (0.34–0.92)		0.59 (0.35–1.01)		0.64 (0.36–1.14)	
3.42-5.36	55/259 (21.2)	0.64 (0.38–1.08)		0.78 (0.43–1.42)		0.82 (0.43–1.59)	
>5.36	30/131 (22.9)	0.71 (0.37–1.37)		1.02 (0.50–2.06)		1.21 (0.56–2.65)	
Household social economic index score (tertiles)							
Low	74/305 (24.3)	1	0.72	1[a]	0.75	1[b]	0.75
Middle	79/350 (22.6)	0.87 (0.5–-1.47)		0.81 (0.46–1.41)		0.81 (0.46–1.41)	
High	83/393 (21.1)	0.81 (0.49–1.36)		0.89 (0.52–1.54)		0.89 (0.52–1.54)	
Number of residents							
<6	87/333 (26.1)	1	0.25	1[a]	0.32	1[b]	0.41
6-7	58/252 (23.0)	0.83 (0.49–1.41)		0.83 (0.47–1.45)		0.89 (0.48–1.65)	
8-10	50/248 (20.2)	0.62 (0.35–1.09)		0.61 (0.34–1.11)		0.61 (0.32–1.18)	
>10	46/237 (19.4)	0.60 (0.34–1.07)		0.64 (0.35–1.14)		0.65 (0.34–1.24)	

Variable	QFT Positive n/N (%)	Crude OR (95% CI)	*p*-value	Adjusted OR[1] (95% CI)	*p*-value	Adjusted OR[2] (95% CI)	*p*-value
Reported smoker in household							
No	197/880 (22.4)	1	0.96	1[a]	0.53	1[b]	0.58
Yes	47/207 (22.7)	0.99 (0.62–1.59)		0.85 (0.50–1.43)		0.85 (0.49–1.50)	
Individual Level factors							
Sex							
Female	123/548 (22.4)	1	0.85	1[c]	0.96	1[d]	0.80
Male	126/546 (23.1)	1.04 (0.72–1.50)		0.99 (0.64–1.52)		0.95 (0.62–1.45)	
Age (years)							
10-11	49/237 (20.7)						
12-14	62/349 (17.8)						
15-17	71/297 (23.9)	1.32 (1.09–1.59)	<0.01[4]	1.36 (1.09–1.71)	0.01[c,4]	1.37 (1.10–1.71)	0.01[d,4]
≥18	67/211 (31.8)						
Lifetime household TB contact							
No	168/823 (20.4)	1	0.01	1[c]	0.02	1[d]	0.01
Yes	78/266 (29.3)	1.90 (1.20–3.01)		1.90 (1.12–3.12)		2.13 (1.25–3.64)	
HIV Status							
Negative	193/855 (22.6)	1	0.88	1[c]	0.39	1[d]	0.35
Positive	9/43 (20.9)	0.91 (0.34–2.40)		0.65 (0.20–2.11)		0.65 (0.20–2.09)	
Unknown	47/196 (24.0)	1.11 (0.69–1.81)		1.41 (0.78–2.53)		1.43 (0.80–2.56)	
BCG vaccination							
Vaccinated	216/984 (22.0)	1	0.24	1[c]	0.99	1[d]	0.65
Not vaccinated	28/101 (27.7)	1.43 (0.78–2.65)		1.00 (0.47–2.15)		1.19 (0.32–2.98)	
Smoking							
No	240/1070 (22.4)	1	0.32	1[c]	0.81	1[d]	0.54
Yes	6/18 (33.3)	1.98 (0.52–7.54)		0.82 (0.17–4.07)		0.61 (0.12–2.26)	
Alcohol intake							
No	226/1021 (22.1)	1	0.21	1[c]	0.76	1[d]	0.75
Yes	16/54 (29.6)	1.59 (0.72–3.54)		1.17 (0.44–3.06)		1.17 (0.45–3.04))	
Education level							
Primary	130/631 (20.6)	1	0.06	1[c]	0.23	1[d]	0.36
Secondary or above	118/459 (25.7)	1.45 (0.98–2.13)		0.64 (0.30–1.37)		0.71 (0.34–1.49)	
Admission to hospital							
No	221/967 (22.9)	1	0.67	1[c]	0.40	1[d]	0.27
Yes	25/121 (20.7)	0.87 (0.48–1.60)		0.74 (0.37–1.48)		0.68 (0.34–1.36)	

Variable	QFT Positive n/N (%)	Crude OR (95% CI)	*p*-value	Adjusted OR[1] (95% CI)	*p*-value	Adjusted OR[2] (95% CI)	*p*-value
Social contact factors							
Contact hours with adult men							
<100	63/274 (23.0)	1	0.95	1[c]	0.76	1[d]	0.69
100-1047	63/275 (22.9)	0.99 (0.58–1.67)		1.30 (0.70–2.43)		1.47 (0.78–2.76)	
1048-2400	59/272 (21.7)	0.89 (0.53–1.52)		1.00 (0.53–1.87)		1.16 (0.62–2.18)	
>2400	64/273 (23.4)	1.04 (0.62–1.76)		1.24 (0.66–2.32)		1.24 (0.66–2.31)	
Contact hour with adult females							
<160	68/277 (24.5)	1	0.47	1[c]	0.83	1[d]	0.89
160-1216	53/274 (19.3)	0.69 (0.41–1.18)		0.80 (0.43–1.49)		0.91 (0.49–1.66)	
1216-2880	66/270 (24.4)	1.03 (0.61–1.74)		1.07 (0.57–2.00)		1.16 (0.63–2.16)	
>2880	62/273 (22.7)	0.89 (0.53–1.50)		0.99 (0.53–1.84)		1.09 (0.59–2.00)	
Church attendance in previous month							
None	165/664 (24.8)	1	0.08	1[c]	0.13	1[d]	0.04
1-2 times	37/176 (21.0)	0.75 (0.44–1.26)		0.66 (0.35–1.23)		0.59 (0.32–1.10)	
≥3times	41/233 (17.6)	0.58 (0.35–0.95)		0.58 (0.33–1.03)		0.49 (0.27–0.89)	
Health facility attendance (12 months)							
No	142/667 (21.3)	1	0.26	1[c]	0.88	1[d]	0.83
Yes	104/422 (24.6)	1.24 (0.85–1.81)		1.03 (0.66–1.61)		1.04 (0.68–1.62)	
Visiting other houses during the day							
None	169/720 (23.5)	1	0.02	1[c]	0.02	1[d]	0.01
1-2 houses	59/227 (26.0)	1.20 (0.76–1.88)		1.17 (0.69–1.91)		1.00 (0.60–1.69)	
≥3 houses	17/136 (12.5)	0.38 (0.19–0.78)		0.31 (0.13–0.72)		0.28 (0.12–0.66)	
Sharing sleeping room with other people							
None	84/363 (23.1)	1	0.88	1[c]	0.71	1[d]	0.56
1 person	78/336 (23.2)	0.97 (0.61–1.54)		1.24 (0.73–2.11)		1.33 (0.78–2.26)	
≥2 persons	84/389 (21.6)	0.89 (0.57–1.40)		1.08 (0.64–1.83)		1.13 (0.66–1.93)	

BCG: Bacillus Calmette-Guérin; CI: confidence interval; HIV: Human immunodeficiency virus; OR: Odds ratios; QFT: QuantiFERON TB-Gold plus; TB: tuberculosis

* Odds ratios modelled as a linear trend across the categories; n and % QFT positive in each category shown for information only.

[1] Partially adjusted by *a priori* confounders and variables remaining significant (p<0.2) at higher levels in the hierarchy

[2] Adjusted by *a priori* confounders, variables remaining significant (p<0.2) at higher levels in the hierarchy and variables remaining significant (p<0.2) that levels in the hierarchy.

[3] Assuming a linear trend across the categories.

[a] Adjusted by community HIV prevalence

[b] Adjusted by community HIV prevalence and socioeconomic status (*a priori* confounder at household level)

[c] Adjusted by community HIV prevalence, socioeconomic status and age (*a priori* confounder at individual level)
[d] Adjusted by community HIV prevalence, socioeconomic status, age, lifetime household TB contact, attendance to church and visiting other houses during the day

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Chapter 6: Discussion

The aim of this thesis was to describe the prevalence and determinants of *M. tuberculosis* infection among adolescents in a high TB and HIV prevalence setting. In this chapter, I summarize the main findings and discuss the implications for TB prevention and care. I highlight the limitations of the research work carried out and make recommendations for TB prevention and care policy and future research.

Main findings

In this high TB and HIV prevalence setting the weighted *M. tuberculosis* infection prevalence was 23.0% (95% CI:20.6–25.6%). Older age, having a lifetime household TB contact and living in communities with high HIV prevalence were associated with increased odds of *M. tuberculosis* infection There was no evidence of association between *M. tuberculosis* infection and urban/rural residence, household socioeconomic status, household number of residents, passive smoking, sex, HIV status, BCG vaccination, smoking, alcohol intake and increased cumulative monthly contact hours with either adult males or adult females during visits to indoor gathering places. The spatial distribution of participants with *M. tuberculosis* infection showed geographical variations with *M. tuberculosis* infection prevalence above 35% observed in week-blocks in the south-eastern part of the study area.

Comparison with previous studies

The estimate for *M. tuberculosis* infection prevalence obtained in this study (the AHRI study) (23.0%) was higher than the prevalence among school-going children aged 6-14 years in a national TST survey in Kenya where a *M. tuberculosis* infection prevalence of 10.2% was reported (using TST induration ≥10 mm) [53]. The prevalence was similar to the prevalence reported among school-going children aged 6-11 years from 16 communities in Zambia (16.5%) [52] and among adolescents (12-18 years) in two rural districts in eastern Uganda (16.1%) [51] (both using TST induration ≥10 mm). As mentioned in chapter 2, the higher prevalence in South Africa reflects the higher annual TB notifications [1, 7].

Within South Africa, recent data on *M. tuberculosis* infection prevalence among adolescents largely comes from studies conducted in the Western Cape province, where much higher estimates of *M. tuberculosis* infection prevalence have been reported. In one township close to Cape Town, Middelkoop *et al.* reported a *M. tuberculosis* infection prevalence of about 53.9% among school-going adolescents aged 13–22 years (using TST≥10 mm) [78] and in another community, Mohamed *et al.* reported a prevalence of 50.9% among school-going children aged 12-18 years (using IGRA) [40]. However, these studies were conducted among adolescents attending primary and secondary schools in the study areas. Though school enrolment is high among individuals aged 10–15 years in South Africa (95-97%), school attendance rapidly drops when individuals finish secondary school (after 17 years) [95]. Thus, the prevalence of *M. tuberculosis* infection might have been underestimated as the prevalence was higher among the older participants. In addition, adolescents not attending school are more likely to be from households with low socioeconomic status where the risk of *M. tuberculosis* is usually high due to poor living conditions [94, 96-98]. The plausible explanation for the difference in *M. tuberculosis* infection prevalence between the AHRI study and the studies in the Cape Town could be differences in social contact patterns as the studies in Cape Town were conducted in townships where homesteads are usually close together unlike in the AHRI study area (in rural KwaZulu-Natal) where homesteads are scattered except for the communities in the township on the south eastern part of the study area which is a smaller part of the study area. The other difference could be the difference in infectiousness of the TB patients in the two provinces. Between 2012-2015, the annual TB notifications for all TB patients in Western Cape and KwaZulu-Natal provinces remained similar (from 776.5 in 2012 to 681.4 per 100,000 population in 2015 for Western Cape province and from 1,060.4 to 685.2 per 100,000 population for KwaZulu-Natal). However, in uMkanyakude district within KwaZulu-Natal province about 64.3% of all TB patients in 2015 were HIV positive while only 44.6% were HIV positive in Cape Town [99]. Thus, the TB epidemic in the current setting is largely driven by the very high HIV prevalence. Immune suppressed HIV positive individuals are likely to be less infectious as they normally present with sputum smear negative TB disease compared to HIV negative individuals [100].

The finding of increased odds of *M. tuberculosis* infection with increasing age are compatible with findings from the above mentioned studies among adolescents in the Western Cape province and

rural districts in Uganda [40, 55, 56, 58, 77, 78, 101, 102]. In a study investigating the effect of passive smoking in a township in Western Cape, Den Boon *et al.* reported the odds of *M. tuberculosis* infection was 3.6 (95% CI: 2.6-5.0) times higher among 10-14-year olds compared to 0-4-year olds [103]. Mahomed *et al.* reported an odds of *M. tuberculosis* infection of 1.3 (95% CI: 1.20–1.5) times among adolescents aged 16-18 years compared to 12-15-year olds. The increased odds of infection among older individuals reflects both overall increased cumulative exposure to sources of *M. tuberculosis* infection and increase in social contact as individuals grow older [104]. This is further reflected in studies which enrolled participants of all ages which reported much higher *M. tuberculosis* infection prevalence among older individuals (>18 years). In Western Cape province, Den Boon *et al*. reported a *M. tuberculosis* prevalence of 80.4% among individuals aged >25 years compared to 66.4% among 15-24-year olds [105]. In the same province, Wood *et al.* reported a prevalence of 74.7% among 25-40-year olds compared to 41.7% among 11-15-year olds. However, the estimates in the older participants (>18 years) were obtained using individuals attending an HIV testing clinic which may not be representative of the general population [56] while Den Boon *et al.* enrolled participants using a listing of residential addresses of two communities [105]. However, the completeness of this listing was at the time of the study is not clear.

Since *M. tuberculosis* transmission is determined by risk of contact with infectious individuals, household contacts of individuals with TB disease are at increased risk of infection [106]. Consistent with previous studies, we found increased odds of *M. tuberculosis* infection among individuals with a lifetime household TB contact [40, 55, 58, 101]. Though household TB contact was important as also seen in the previous studies, only 78 (31.7%) of all the participants with QFT-plus positive results reported to have ever lived in the same house as a person with TB disease during their lifetime. This might indicate transmission in the wider community as well as transmission from undiagnosed household contacts [107]. Thus, there is need for a better understanding the locations where transmission occurs in the wider community in this setting.

Since majority of transmission are likely to occur in the wider population and not just within households of people diagnosed with TB, there has been interest in understanding social contacts with potential for *M. tuberculosis* transmission. Contacts with adult men (≥15 years) are of interest

as globally the prevalence of TB disease is higher among men. In a recent meta-analysis of 56 population based TB prevalence surveys from middle and low income countries, Horton *et al.* reported an estimated male to female prevalence ratio of 2.2 (95% CI 1.9–2.5) for bacteriologically-confirmed TB disease [108]. In the AHRI study, we did not find an association between *M. tuberculosis* infection and increased cumulative monthly contact hours with either adult males or adult females. This possibly reflects limitations with the social contact diary method in measuring social contacts and teasing out the social contacts with potential for increased risk of *M. tuberculosis* transmission. Furthermore, a recent mathematical modelling analysis combining of data on social contacts, TB disease incidence among adults and *M. tuberculosis* infection incidence among children (6-11 years) suggested that though household and non-household repeated contacts contribute about 50% of contact time, they only contribute about 13% and 8% of disease transmissions respectively and that majority of transmissions (about 79%) possibly occurs from non-repeated contacts [109]. This potentially further explains the lack of association with increased contact hours with adult males or females as the social contacts measured in this study (for example contacts at church or school) are more of repetitive nature.

The exploratory analyses for the spatial distribution of *M. tuberculosis* infection showed geographical variations with the highest prevalence (above 35%) observed in week-blocks on the south eastern part of the study area. Long-term surveillance has shown that HIV prevalence has consistently remained high in this part of the DSA [89, 110]. Previous work in the DSA has also shown that both self-reported and drug resistant TB are associated with the high HIV prevalence communities [67, 68]. The high *M. tuberculosis* infection prevalence in these communities suggests continuing transmission. Targeted efforts to find and treat TB in these communities could be effective in reducing TB transmission. There were no clear similarities between the spatial distribution for *M. tuberculosis* infection and self-reported lifetime household TB contact. This possibly reflects movements within the DSA as the participants might have had a household TB contact in a different house other than the current one. This also possibly reflects transmission in the wider population and not just within households of individuals with TB disease.

Tobacco smoking has long been shown to increase the risk of progression to TB disease as well as death from TB disease [111-113]. Though definitive evidence of the effect on *M. tuberculosis* infection is lacking, tobacco control is an important component in the control of the TB epidemic [114-116]. A recent large study investigating *M. tuberculosis* infection among school-going children (aged 6-13 years) in Mongolia reported an increasing odds of *M. tuberculosis* infection with increase in the number of reported smokers in the living in the same house [102]. The lack of association with passive smoking in the AHRI study possibly reflects differences in the prevalence of smoking. In 2010, the prevalence of current smoking among men in Mongolia was estimated at 46.1% [117] compared to 29.2% among South African men in 2012 [118]. However, this may not reflect the prevalence of smoking in the study area as the prevalence among black South African men was estimated to be lower than other races like coloureds and Indians who are not members of the AHRI study area. The lack of association may also reflect differences in levels of exposure to tobacco smoking within the household in Mongolia and the AHRI study area. In the rural communities of the AHRI study are homesteads are organised into separate dwellings within a bounded residential plot. Thus, individuals of the same household may not share the same indoor space for extended periods.

Estimates for *M. tuberculosis* infection prevalence from TST surveys among young children (<10 years) are complicated by among other things cross-reactivity with BCG vaccination [52, 53, 119, 120]. This was less likely the case in this study as the QFT-plus test was used, which is a more specific test for *M. tuberculosis* infection as it contains antigens which are specific to *M. tuberculosis* and are not present in the strains used in BCG vaccination [13, 16, 19]. Similar with previous studies using QuantiFERON TB tests among adolescents (10-19-year olds) in the Western Cape province, South Africa and Mongolia, [40, 102] we found no association between BCG vaccination and *M. tuberculosis* infection. This may reflect the fact that absence of visible scars does not mean that the participants were not vaccinated as scars are not visible in other individuals [121, 122]. A re-analysis of three nationwide TST surveys conducted in Tanzanian between 1990-2002 including 215,739 participants with visible BCG scars and 61,849 without visible scars found a 11.0% protective effect of BCG vaccination on *M. tuberculosis* infection at ≥19 mm TST induration cut off with higher protective effect at higher cut offs [123]. In addition, a meta-analysis of 14 studies of children and

adolescents (<16 years) exposed to TB disease from high and low TB prevalent settings reported a protective effect of BCG vaccination of about 19% [124]. This has rekindled interest on the protective benefit of BCG vaccination. A recent phase II trial evaluating the protective effect of BCG revaccination among adolescents who were BCG vaccinated at birth and without evidence of *M. tuberculosis* infection in a high TB prevalence setting reported that BCG revaccination reduced rates of sustained QFT conversion from <0.35 IU/ml to ≥0.35 IU/ml with an efficacy of 45.4% [26]. However, the clinical relevance of conversion at this cut off is not established as values around this cut off may represent variation due to repeat testing [92].

Limitations

There are some limitations with the research work presented in this thesis. Firstly, as mentioned in chapter 5, participants from urban week-blocks which have a higher population density and HIV prevalence were underrepresented. Since the odds of *M. tuberculosis* infection were higher in communities with HIV prevalence, the estimate for *M. tuberculosis* infection prevalence may have been underestimated. Though the urban communities in this study area are have a higher prevalence of both *M. tuberculosis* and HIV infection, they form a smaller part (about 12%) of the DSA population [89]. In addition, the estimate for *M. tuberculosis* infection prevalence takes account of non-participation by age, sex and urban/rural residence. Thus, the estimate is less likely influenced by the poor participation in urban communities. We believe that our estimate is reflective of this population. The results are also applicable to other rural communities in KwaZulu-Natal province where dwelling places are organised into multigenerational homesteads and not clustered into villages. Furthermore, the analysis for risk factors for *M. tuberculosis* infection took account of clustering within households. Thus, the estimates for the odd ratios obtained for the associations with *M. tuberculosis* infection were more robust compared to the analysis not adjusted for clustering within households. Overall, adjusting for clustering within households resulted in increased odds ratios and wider confidence intervals as would be expected.

Secondly, with the cross-sectional design, we could not establish causal relationships between the outcome and the exposures of interest. We also could not establish whether the outcome happened after the exposure. For example, the *M. tuberculosis* infection might have occurred before the

participant had a household TB contact. The other limitation is with the QFT-plus test, though it is more specific to *M. tuberculosis* infection than the TST, it does not inform whether the infection occurred recently (for example in the last year) or further in the past [11, 19, 21, 125]. In addition, it has a low predictive value for progression to TB disease. Using a cohort of adolescents (aged 18-20 years) in a high TB prevalence setting in Western Cape province, Nemes *et al.* reported that adolescents converting from IFN-γ values <0.2 IU/ml to IFN-γ >0.7 IU/ml were 10.3 (2.4–93.6) times more likely to progress to TB disease. However, there were only two participants who developed TB disease among the non-convertors, the risk ratio might have been overestimated as shown by the very wide confidence interval [92]. With the cross-sectional design, we could not verify this finding.

The other limitation is with the social contacts data. Social contacts are classically measured by collecting detailed information (including, age, sex, duration of the contact and whether the contact occurred indoor) on each individual that the participants made on a selected day (usually a day before the interview) [126-132]. As mentioned in chapter 5, in the AHRI study, participants were asked history of attendance at selected indoor gathering places (including school, church, health facility and travel in closed vehicles) and details of the last visit (including duration and number of people present). Reporting errors were likely introduced in estimating actual frequency of attendance, duration and number of people present at the last visit resulting in the obscuring of the associations between *M. tuberculosis* infection and the estimated increased monthly contact hours with either adult males or females. In addition, we defined adults as individual at least 12 years old as individuals with TB disease at this age are more likely to present with pulmonary TB disease which is more infectious than disseminated TB disease in younger children [133, 134]. However, the prevalence of notified disease is highest among older (>18 year) individuals [1]. This may explain the lack of association with increased monthly contact hours with adult males or females.

A major limitation with the geospatial analyses was that participants were geo-located to their week-blocks which are wide geographic areas. The estimates for *M. tuberculosis* infection prevalence obtained in the week-blocks were prone to random error, especially in week-blocks with a small number of participants. This may explain the high *M. tuberculosis* prevalence observed in one week-block on the north-western part of the study area where only six participants were enrolled and

among them, four (66.7%) had *M. tuberculosis* infection. Secondly, the analysis did not take account of time spent at the "current" household as regular mobility within the DSA is common and individuals may concurrently belong to more than one household. Thirdly, data on the underlying spatial distribution of active TB disease in the area were not available. Comparison with spatial distribution of active TB disease was based on self-reported information may not be a robust measure of adult TB disease.

Strengths of the study

There are several strengths of the results presented in this thesis. Firstly, the sample size was large enough to estimate *M. tuberculosis* infection prevalence with a high precision and the study was powered to detect associations with potential risk factors. However, the estimated prevalence (23.0%) was lower than an anticipated prevalence of 50% based on studies among adolescents in the Western Cape province [40, 83]. The precision would be lower than initially intended.

Secondly, the QFT-plus test which is a more specific test (compared to the TST) was used to measure *M. tuberculosis* infection. The tests were performed in a specialised project research laboratory with highly trained and experienced staff. A batch of the first 100 samples was sent to an external accredited laboratory as a quality control measure and a 100% agreement was achieved. Thus, the measure for *M. tuberculosis* infection was both objective and more specific.

Thirdly, reporting bias was reduced by interviewing parents/guardians of the younger participants (10-17 years) on questions about BCG vaccination, previous TB treatment and household TB contact. The parent/guardian was more suited to give accurate information as the exposure might have happened when the participant was still too young to recall. In addition, where available immunization records were cross-checked to ascertain the BCG vaccination status of the participants and the proportion of participants where the immunization records were available was reasonably high (756 [69.1%]).

The other strength of this study was that it was conducted in a DSA setting. Unlike in many situations where the sampling frame is not available, the DSA provided a complete sampling frame of all eligible individuals in the study area and we were able to make comparisons between

individuals who participated and those who did not. Thus, we were able to determine the effect of non-participation on the estimate for prevalence. Information on household socioeconomic status, household number of residents, urban/rural residence and individual HIV status (for ≥15-year olds) is routinely collected in a standardised way during the surveillance and was available. In addition, we collected individual information on a wide range of exposures including, chronic illnesses, smoking, admission to hospital, lifetime household TB contact and attendance at indoor gathering places. This enabled testing associations with *M. tuberculosis* at community, household and individual level.

Recommendations

Our results show that adolescents reporting history of a household TB contact had higher odds of *M. tuberculosis* infection. This suggests that adolescents who are household contacts of people with TB disease in this area remain at increased risk of infection regardless of when the contact occurred during the adolescent's lifetime. The current national South African TB prevention and care guidelines recommend contact investigation of individuals with laboratory confirmed TB disease. However, this is hardly done in practice largely due to low yield of new confirmed TB patients identified with the increased intensified effort [135]. Thus, opportunity to identify individuals at risk of disease is missed. For example, in a high HIV prevalence setting like this study area, HIV positive household contacts who are latently infected with *M. tuberculosis* can benefit from preventive therapy even though they do not have TB disease at the time of the screening.

Our results also suggest that majority of infections in this area might be clustered in communities with very high HIV prevalence. Spatially targeting these communities with interventions like ACF (active case finding) has potential to make an impact in identifying individuals with disease early. However, evidence for this approach is lacking. Research work is needed to explore how best spatially targeted interventions can be implemented and their benefit.

Since the risk of *M. tuberculosis* infection is determined by risk of contact with infectious individuals [106], research work is needed to understand better social contacts and locations with potential for *M. tuberculosis* transmission in the wider community so that effective interventions can be put in place. Information on social contacts is largely captured using social contact diaries which are prone

to recall and reporting biases [136]. To eliminate biases with social contact diaries, more recent studies have collected social contact data using wireless proximity sensors which detect presence of other devices within a defined distance [129, 137-139]. However, this approach is not robust enough as it is limited to contacts with other study participants only. Another approach was used in Cape Town where wearable carbon dioxide (CO_2) monitors were used to measure ventilation during visits at indoor gathering places [140]. Future studies can explore the effect of increased indoor exposure to CO_2 on the risk of *M. tuberculosis* infection.

Measuring *M. tuberculosis* infection remains a challenge as there is no gold standard test. Both TST and IGRA are indirect markers of *M. tuberculosis* exposure and do not discriminate latent *M. tuberculosis* infection from active disease [11, 13, 19]. TST is cheaper and has been mass administered in different populations [54, 141, 142]. However, TST is prone to false positive results due to BCG vaccination and infection with other NTMs as such interpretation of TST data is complicated [54, 143]. Though IGRAs are more specific to *M. tuberculosis* than TSTs, they have limited use in low income settings due to high cost and the need for extensive laboratory infrastructure [11, 19]. There is need for development of better simpler tests for *M. tuberculosis* infection. The C-Tb (Statens Serum Institut, Copenhagen, Denmark) is a newer skin test containing *M. tuberculosis* specific antigens ESAT-6 and CPF-10 [18]. The C-Tb promises to offer the specificity of IGRAs without the requirement for extensive laboratory infrastructure. However, it still needs to be validated.

Nemes *et al*. suggested that IGRA conversion from low IFN-γ values to higher values may determine progression to TB disease [92]. Further research is required to validate this finding. Further research is also required to determine the predictive value of high baseline IFN-γ values on the progression to TB disease.

Conclusion

The prevalence of *M. tuberculosis* infection among adolescents in this high TB and HIV prevalence area was lower than the prevalence in Western Cape province. Increased community HIV prevalence, older age and having a lifetime household TB contact were associated with increased odds of *M. tuberculosis* infection. This reflects continued transmission both within households of

people with TB disease and in the wider community. Enhancing household contact tracing will help identify household contacts who are infected and at risk of developing TB disease early. Spatially targeting worst affected communities especially those with high HIV prevalence with interventions like ACF has potential to make an impact. However, evidence of the benefit of this approach is still lacking.

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Appendices

Appendix 1: Parent/guardian Consent forms

INFORMATION SHEET AND PARENTAL CONSENT FOR RESEARCH V3.0 30 NOVEMBER 2017

INCIDENCE OF TB INFECTION IN ADOLESCENTS IN RURAL KWAZULU-NATAL, SOUTH AFRICA

The researchers doing this study are:
Africa Health Research Institute (AHRI): Alison Grant, Richard Lessells, Olivier Koole, Kathy Baisley, Frank Tanser, Kobus Herbst, Safiyya Randera-Rees
Hlabisa Hospital: Martin Tshipuk
London School of Hygiene & Tropical Medicine, UK: Themba Mzembe, Rashida Ferrand, Katharina Kranzer, Janet Seeley, Chris Grundy, Naomi Walker, Aaron Karat
Liverpool School of Tropical Medicine, UK: Peter MacPherson

Introduction
Good day, my name is [*name of researcher*], and I am a researcher at AHRI. We would like to ask your permission to invite your child to take part in a research study about tuberculosis (TB). Research is the process to learn the answers to questions. This information sheet explains our study. You are free to decide whether you wish your child to take part, and before you decide, it is important that you understand why the research is being done and what it will involve. Please ask me if there is anything which is not clear. If you decide to give us permission to approach your child, to show that you understand the study and agree to this, we will ask you to sign or make your thumbprint on a consent form. You can withdraw your child from the study at any time. Your decision will not affect your or your child's health care in any way.

Why are we doing this study?
TB is a major health problem in South Africa, and particularly in KwaZulu-Natal. TB is passed from one person to another by breathing in TB germs when a person with TB coughs, sneezes or spits. In South Africa, people often breathe in TB germs when they are a young child or teenager. For most people, their body controls the TB germs, and the TB germs do not cause illness or do any harm. This is called TB infection. In a few people, the TB germs do cause illness, usually in the chest. This illness can start months or years after breathing in the TB germs. When TB does cause illness, it can be completely cured by taking a combination of medicines, usually for six months. In this research we want to learn how common TB infection is in young people in this area. TB infection can also be treated; treatment for TB infection is offered to people at highest risk of developing TB disease. We also want to learn why and where TB is passed from one person to another. We hope this will help us find better ways to find people who are ill with TB and start treatment earlier.

Why has my child been chosen for this study?
Your child has been chosen because he/she is between 10 and 19 years old, and he/she lives in the area where AHRI carries out its surveillance and research activities. We hope to include about 1100 adolescents in this study.

If my child takes part in this study, what will happen?
First, we would like to ask you a few questions about your child such as whether he/she has been vaccinated against TB; and whether he/she has ever been in contact with a person who was treated for TB.

Questionnaire: If your child agrees to take part in this study, in a private space we will ask him/her some questions about himself/herself and his/her health. We will ask how we can contact him/her (for example by phone). We will ask whether anyone he/she knows has had TB. We will ask about people he/she has been in contact with. We will check whether he/she has any symptoms that might suggest they are unwell with TB, such as cough, fever, night sweats and weight loss. The questions will take about 45-60 minutes. We will look at his/her arm to check whether he/she had the TB vaccine (BCG) when younger.

If your child says he/she has symptoms which suggest he/she might have TB, we will ask him/her to give us two samples of sputum (spit from the chest). We will take one sample to your local clinic for testing for TB.

We will let your child know the result of this test. If the sputum test is positive, your child will need treatment for TB, and we will help him/her get this treatment at a local clinic. If the sputum test is positive, the law says that we must give this result to the local TB treatment team, and they may contact you or your child if your child has not started TB treatment. The second sample of sputum will go to our lab in Durban for testing for TB. This is only for research purposes, and we will not give you or your child the result. If we think your child needs other tests for TB we will refer him/her to the clinic to get properly checked.

Blood sample: If your child agrees, we will take a blood sample from one of their arms. This will involve putting a tight band around their upper arm, cleaning the skin, then putting a thin needle through the skin and into a vein. We will take 10mls (two teaspoons) of blood, then take out the needle and press on the skin to stop a bruise developing. The blood sample will be sent to our laboratory in Durban and tested for signs that your child has TB infection. This test is used in research studies but is not usually used to measure TB infection in public clinics in South Africa. We will only report the result of this test to your child if the result shows that treatment is recommended. This result might take a month or more to come back. We will also test your child's blood for possible new markers for TB, which might help develop new tests for TB in the future.

If you give permission, and your child agrees, we would like to offer your child a test for HIV. We can give your child the result of the test today, after about 15 minutes. If the result is positive we will discuss with your child how to get care for HIV, if he/she is not already getting HIV care at a clinic. We encourage everyone to get their HIV test result. However, if your child prefers not to get the HIV test result, we can test his/her blood anonymously in the laboratory. That way we could link the HIV test result to the TB test results, which would help us a lot to understand TB in this community. We would keep the result confidential and not tell anyone (including you and your child) the result. As a third choice, if you are happy for your child to take part in the study but you do not want to have your child's blood tested for HIV, we will respect your choice, and no HIV test will be done.

Referral for care: if any of the questions we ask or tests we do suggest that your child may need further tests or health care, we will explain what he/she or you need to do, and give you a referral to your local health centre.

[*Paragraph for parents/guardians of adolescents in the RFID sub-study only, to be skipped for others]*: We would like your child to help us test out a new way to measure how long people spend in different public places in the community. We will give your child a wrist band and we would like him/her to wear it over the next two weeks, while he/she is filling in the contact diary. If your child goes through the doors of [*name of relevant health centre and any other local public building with sensors]*, the wrist band will record that your child was there. At the end of two weeks we will collect the diary and the wrist band. At this time, we will ask your child a few questions about where he/she went, and find out how easy or difficult it was for your child to fill in the diary, and what your child thought about wearing the wrist band.

Follow-up: We would like to see your child again after 12 months (one year). At this visit, we will ask some questions to see whether he/she has had any health problems during the year and whether he/she has been in contact with anyone with TB. We will again check whether he/she has any symptoms that suggest they might be unwell with TB. With their agreement, we will draw blood again from their arm, in the same way as the first visit, to do the same tests.

With your permission, we would like to check on your child's health from time to time over the next five years, particularly to see if he/she needs to start treatment for TB. We will do this by asking you or your family when we come to your home for surveillance visits; by checking clinic and laboratory records, and if needed by phoning your child.

We would also like to connect this information about TB to other information that AHRI has collected about your child and your family over the years. We will be very careful to be sure that all information you give us is kept confidential.

What are the possible risks of taking part?
There might be some pain or discomfort from having blood taken from the arm. We can reduce this by putting some cream on the arm that helps to numb the area and makes it less likely to be painful. There is also the risk of a bruise developing at the site where the blood was taken but this will be reduced by pressing on the area after the needle is taken out.

What are the possible benefits of taking part?
If your child reports symptoms that suggest he/she might be unwell with TB, we will take a sputum sample to the clinic to be tested for TB, and contact him/her with the result. If necessary, we will also refer him/her to the clinic to get properly checked for TB. If your child is unwell with TB, and your child starts treatment quickly, the TB is less likely to cause serious illness.

If your child is HIV-positive and his/her TB blood test is positive, we will refer him/her to the clinic for further checks and possibly to get some medication (isoniazid), if he/she is not already taking this. Taking isoniazid reduces the risk of becoming unwell with TB.

We hope that this research will help us to find ways to prevent people like your child getting TB infection, so that it may help people like your child in the future.

To thank your child for taking part in the study, we will give him/her an airtime voucher of about ZAR 20 or a food refreshment, and another ZAR 20 airtime voucher or food refreshment after the 12-month visit.

What happens if I (or my child) do not agree to take part in this study?
Your child does not have to take part in this study: if he/she does not take part, this will not affect them or you in any way. Your child can stop taking part in the study at any time, without giving a reason.

How will the information collected during this study be kept confidential?
All information collected during the course of this study will be kept securely and confidentially at AHRI: *[name of study manager]* is responsible for this. We will store information on a secure computer system. The information we collect will be identified on forms and computer files only by a study number or barcode, not your name. This means that your information remains private.

Study information may be looked at by the Ethics Committee, and authorised independent monitors, to check that the research has been done correctly and that the information is accurate. Your child's information will remain confidential, unless we are required by law to release information. Reports about the study will never include information which allows your child to be identified.

Results from the study will be presented to people working in the TB programme, the Department of Health, and other researchers as presentations and publications in medical journals. In all these presentations and reports, it will not be possible to identify people who took part.

Will blood samples that my child gives for the study be stored?
Yes, if there is blood remaining after the TB tests have been done, we will store it in our laboratories in Durban for up to 20 years. It may be used by approved researchers, for example to develop new tests for TB. Further studies using your child's blood sample could only be done if they were approved by the ethics committees who oversee this research. If you or your child decide later that you do not want his/her blood sample used for research, you can contact us and we will destroy any remaining blood sample. Once all tests have been done, the remaining sample will be disposed of by the laboratory in Durban.

Who is organising and funding the research?
This research study is being organised by AHRI through the Healthy Adolescents and Young Adults (HAYA) research unit. The study is being funded by Viiv HealthCare, a drug company that has funded the HAYA research unit.

Who has reviewed the study?
All research at AHRI is looked at by an independent group of people, called a Research Ethics Committee. This study has been approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal. As this study involves researchers from the UK, the study has also been approved by the Research Ethics Committee of the London School of Hygiene & Tropical Medicine. The study has also been approved by AHRI Community Advisory Board and by the Department of Health.

What if I have questions about this study?
If you have any questions, please feel free to ask me now. If you have questions about the study later you may contact the main researcher:
Professor Alison Grant
Professor of International Health
c/o Africa Health Research Institute
PO Box 198, Mtubatuba 3930
Tel: 035-500-7500; fax: 035-550-7565; email: agrant2@ahri.org

If you have concerns about the study, you can contact the UKZN Biomedical Research Ethics Committee:

Biomedical Research Ethics Administration
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001, Durban 4000
Tel: 031-260-4769; Fax: 031-260-4609; Email: BREC@ukzn.ac.za

We will give you a copy of this information sheet which explains the study to take away with you.

PARENTAL CONSENT FOR RESEARCH V2.0 30 NOVEMBER 2017

INCIDENCE OF TB INFECTION IN ADOLESCENTS IN RURAL KWAZULU-NATAL, SOUTH AFRICA

Study ID

The researchers doing this study are:

Africa Health Research Institute: Alison Grant, Richard Lessells, Kathy Baisley, Frank Tanser, Kobus Herbst, Safiyya Randera-Rees

Hlabisa Hospital: Martin Tshipuk

London School of Hygiene & Tropical Medicine, UK: Themba Mzembe, Rashida Ferrand, Katharina Kranzer, Janet Seeley, Chris Grundy, Naomi Walker, Aaron Karat

Liverpool School of Tropical Medicine, UK: Peter MacPherson

I have read the information sheet about this study (or the information sheet about this study has been read to me). I understand what will be required of my child and what will happen if my child takes part in the study.

My questions concerning this study have been answered by:

Research staff name (printed)	Signature	Date

I understand that I may withdraw my child from this study at any time without giving a reason, and without affecting my normal care and management.

I agree that my child's blood can be tested for HIV as part of this study *(initial box to show agreement)*

I give permission for you to approach my child regarding this study

Name of parent or guardian (printed)	Signature/ thumbprint	Date

If the parent or guardian has given verbal consent, indicated by a thumbprint, ask the independent person who witnessed the consent to write their name, signature and date here:

Witness name (printed)	Signature	Date

Appendix 2: Participant consent forms (10-17 years)

INFORMATION SHEET AND ASSENT TO PARTICIPATE IN RESEARCH V3.0 30 NOVEMBER 2017
INCIDENCE OF TB INFECTION IN ADOLESCENTS IN RURAL KWAZULU-NATAL, SOUTH AFRICA

The researchers doing this study are:
Africa Health Research Institute (AHRI): Alison Grant, Richard Lessells, Kathy Baisley, Frank Tanser, Kobus Herbst, Safiyya Randera-Rees
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Liverpool School of Tropical Medicine, UK: Peter MacPherson

Introduction

Good day, my name is [*name of researcher*], and I am a researcher at AHRI. We would like to invite you to take part in a research study about tuberculosis (TB). Research is the process to learn the answers to questions. This information sheet helps us to explain the study. If there is anything you don't understand, please ask me. It is fine if you decide you don't want to take part. You can stop taking part at any time. Your decision to take part or not will not affect your health care or your family in any way.

Why are we doing this study?

TB is a big health problem in South Africa, and particularly in KwaZulu-Natal. TB is passed from one person to another by breathing in TB germs when a person with TB coughs, sneezes or spits. In South Africa, people often breathe in TB germs when they are a young child or teenager. For most people, their body controls the TB germs, and the TB germs do not cause illness or do any harm. This is called TB infection. In a few people, the TB germs do cause illness, usually in the chest. This illness can start months or years after breathing in the TB germs. When TB does cause illness, it can be completely cured by taking the right medicines. TB infection can also be treated. In this research we want to learn how common TB infection is in young people. We also want to learn why and where TB is passed from one person to another. We hope this will help us find better ways to find people who are ill with TB and start treatment earlier.

Why have I been chosen for this study?

You have been chosen because you are between 10 and 19 years old, and you live in the area where AHRI carries out its research.

If I take part in this study, what will happen?

Questionnaire: In a private space, we will ask you some questions about yourself and your health. We will take your contact details. We will ask whether anyone you know has had TB, and people you have been in contact with. We will check whether you have any symptoms that might suggest you are unwell with TB. The questions will take about 45-60 minutes. We will look at your arm to check whether you had the TB vaccine (BCG) when you were younger.

If you have symptoms that might be due to TB, we will ask you to give us two

sample of sputum (spit from the chest). One sample will be tested for TB at the clinic. We will let you know the result. If the result is positive, we will help you get TB treatment at a local clinic. If the result is positive, we will also have to tell the local TB treatment team about it. The other sample will go to our lab in Durban for research.

Blood sample: If you agree, we will take a blood sample from one of your arms. We will put a tight band around your upper arm, clean your skin, then put a thin needle through the skin and into a vein. We will take 10mls (two teaspoons) of blood, then take out the needle and press on your skin to stop a bruise developing. The blood will be tested for signs of TB infection in a lab in Durban. If the result shows you need treatment, we will let you know. The result might take a month or more to come back. We will also use your blood for research which might help to develop new tests for TB in the future.

[*omit if parent/guardian has declined permission for HIV testing. Researcher will first establish the child's understanding of HIV, and give age- and maturity-appropriate counselling]* If you agree, we will take a fingerprick blood sample to test for HIV. We can give you the result of the test today, after about 15 minutes. If the result is positive we will discuss with you how to get care for HIV, if you are not already getting HIV care at a clinic. Everyone should know their HIV status, and we encourage you to get the HIV test result today. However, if you prefer not to get the HIV test result, we can test your blood anonymously in the laboratory, and not give anyone the result. As a third choice, if you are happy to take part in the study but you do not want to have your blood tested for HIV, that's fine too.

Referral for care: if any of the questions we ask or tests we do suggest that you may need further tests or health care, we will explain what you need to do.

[*Paragraph for people in the RFID substudy only, to be skipped for others]*: We would like you to help us test out a new way to measure how long people spend in different public places in the community. We will give you a wrist band and we would like you to wear it over the next two weeks. If you go through the doors of [*name of relevant health centre and any other local public building with sensors]*, the wrist band will record that you were there. This information will be kept confidential. At the end of two weeks we will collect the diary and the wrist band. At this time, we will ask you a few questions about where you went, and find out from you how easy or difficult it was to fill in the diary, and what you thought about wearing the wrist band.

Follow-up: We would like to see you again after 12 months (one year). At this visit, we will ask about any health problems and whether you have been in

contact with anyone with TB. If you agree, we take another blood sample from your arm, in the same way as the first visit. to check on your health from time to time over the next five years, particularly to see if you need to start treatment for TB. We will do this by asking you or your family when we come to your home for surveillance visits; by checking clinic and laboratory records, and if needed by phoning you.

We would also like to connect this information about TB to other information that AHRI has collected about you and your family over the years. We will be very careful to be sure that all information you give us is kept confidential.

What are the possible risks of taking part?
There might be some discomfort from having blood taken from your arm. We can reduce this by putting some cream on your arm that helps make the skin numb, so it hurts less. You might get a bruise where the blood was taken.

What are the possible benefits of taking part?
If you have symptoms which suggest you may be sick with TB, we will help you get checked for TB. If TB is treated early, the TB has less chance to make you unwell.

If you know you are HIV-positive and your TB blood test is positive, we will refer you to the clinic for further checks and possibly to get some medication (isoniazid), if you are not already taking this. Taking isoniazid reduces the risk of you becoming unwell with TB.

We hope this research will help us to find ways to stop people like you getting TB infection, so that it may help people like you in the future.

To thank you for taking part in the study, we will give you an airtime voucher of about ZAR 20 or a food refreshment after we finish the questions and tests, and another ZAR 20 airtime voucher or food refreshment after the 12-month visit.

What happens if I do not agree to take part in this study?
You do not have to take part in this study: if you do not take part, this will not affect you in any way. You can also stop taking part in the study at any time, without giving a reason.

How will the information collected during this study be kept confidential?
All information collected during the course of this study will be kept private on a secure computer system at AHRI.

We would only release your information if a law says that we must do that. Reports about the study will never include information which allows you to be identified.

Will blood samples that I give for the study be stored?
Yes, if there is blood remaining after the TB tests have been done, we will store it in our laboratories in Durban for up to 20 years. It may be used, for example

to develop new tests for TB, but only if this was approved by the ethics committees who oversee this research. If you decide later that you do not want your blood sample used for research, you can contact us and we will destroy any remaining blood sample. Once the TB test has been performed on the blood sample, the remaining sample will be destroyed.

Who is organising and funding the research?

This research study is being organised by AHRI through the Healthy Adolescents and Young Adults (HAYA) research unit. The HAYA research unit is funded by Viiv HealthCare, a drug company.

What if I have questions about this study?

If you have any questions, please feel free to ask me now. If you have questions about the study later you may contact the main researcher:

Professor Alison Grant
Professor of International Health
c/o Africa Health Research Institute
PO Box 198, Mtubatuba 3930
Tel: 035-500-7500; fax: 035-550-7565; email: agrant2@ahri.org

If you have concerns about the study, you can contact the UKZN Biomedical Research Ethics Committee:

Biomedical Research Ethics Administration
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001, Durban 4000
Tel: 031-260-4769; Fax: 031-260-4609; Email: BREC@ukzn.ac.za

We will give you a copy of this information sheet which explains the study to take away with you.

ASSENT TO PARTICIPATE IN RESEARCH V3.0 30 NOVEMBER 2017

INCIDENCE OF TB INFECTION IN ADOLESCENTS IN RURAL KWAZULU-NATAL, SOUTH AFRICA

Study ID

The researchers doing this study are:
Africa Health Research Institute: Alison Grant, Richard Lessells, Kathy Baisley, Frank Tanser, Kobus Herbst, Safiyya Randera-Rees
Hlabisa Hospital: Martin Tshipuk
London School of Hygiene & Tropical Medicine, UK: Themba Mzembe, Rashida Ferrand, Katharina Kranzer, Janet Seeley, Chris Grundy, Naomi Walker, Aaron Karat
Liverpool School of Tropical Medicine, UK: Peter MacPherson

I have read the information sheet about this study (or the information sheet about this study has been read to me). I understand what the study is about and what I need to do if I take part in the study.

My questions concerning this study have been answered by:

Research staff name (printed)	Signature	Date

I understand that I may withdraw from this study at any time without giving a reason, and without affecting my normal care and management.

I agree that my blood can be tested for HIV as part of this study *(initial box to show agreement)*

I agree to take part in the study

Study participant name (printed)	Signature/ thumbprint	Date

If the participant gave verbal assent, indicated by a thumbprint, enter the name of the independent person who witnessed the assent here and their signature:

Witness name (printed)	Signature	Date

Appendix 3: Participant consent forms (18-19 years)

INFORMATION SHEET AND CONSENT TO PARTICIPATE IN RESEARCH V3.0 30 NOVEMBER 2017

INCIDENCE OF TB INFECTION IN ADOLESCENTS IN RURAL KWAZULU-NATAL, SOUTH AFRICA

The researchers doing this study are:

Africa Health Research Institute (AHRI): Alison Grant, Richard Lessells, Olivier Koole Kathy Baisley, Frank Tanser, Kobus Herbst, Safiyya Randera-Rees

Hlabisa Hospital: Martin Tshipuk

London School of Hygiene & Tropical Medicine, UK: Themba Mzembe, Rashida Ferrand, Katharina Kranzer, Janet Seeley, Chris Grundy, Naomi Walker, Aaron Karat

Liverpool School of Tropical Medicine, UK: Peter MacPherson

Introduction

Good day, my name is [*name of researcher*], and I am a researcher at AHRI. We would like to invite you to take part in a research study about tuberculosis (TB). Research is the process to learn the answers to questions. This information sheet explains our study. You are free to decide whether you wish to take part. Before you decide, it is important that you understand why the research is being done and what it will involve. Please ask me if there is anything which is not clear. If you decide to take part, to show that you understand the study and agree to take part, we will ask you to sign or make your thumbprint on a consent form. It is your right to withdraw from the study at any time. Your decision to take part or not will not affect your health care in any way.

Why are we doing this study?

TB is a major health problem in South Africa, and particularly in KwaZulu-Natal. TB is passed from one person to another by breathing in TB germs when a person with TB coughs, sneezes or spits. In South Africa, people often breathe in TB germs when they are a young child or teenager. For most people, their body controls the TB germs, and the TB germs do not cause illness or do any harm. This is called TB infection. In a few people, the TB germs do cause illness, usually in the chest. This illness can start months or years after breathing in the TB germs. When TB does cause illness, it can be completely cured by taking a combination of medicines, usually for six months. In this research we want to learn how common TB infection is in young people. TB infection can also be treated; treatment for TB infection is offered to people at highest risk of developing TB disease. We also want to learn why and where TB is passed from one person to another. We hope this will help us find better ways to find people who are ill with TB and start treatment earlier.

Why have I been chosen for this study?

You have been chosen because you are between 10 and 19 years old, and you live in the area where AHRI carries out its surveillance and research activities. We hope to include about 1100 adolescents in this study.

If I take part in this study, what will happen?

Questionnaire: If you agree to take part in this study, with your permission and in a private space we will ask you some questions about yourself and your health. We will ask you how we can contact you (for example by phone), and ask for the name of a family member or close friend who we could contact if we can't find you. We will ask whether anyone among your family, friends or neighbours has had TB. We will check whether you have any symptoms that might suggest you are unwell with TB, such as cough, fever, night sweats and weight loss. We will also ask you about people you have been in contact with; we will ask how many people, and their age and sex, but not their names. The questions will take about 45-60 minutes. We will look at your arm to check whether you had the TB vaccine (BCG) when you were younger.

If you have symptoms which suggest you might have TB, we will ask you to give us two samples of sputum (spit from the chest). We will take one sample to your local clinic for testing for TB. We will let you know

the result of this test. If the sputum test is positive, you will need treatment for TB, and we will help you get this treatment at a local clinic. If the sputum test is positive, the law says that we must give this result to the local TB treatment team, and they may contact you if you have not started TB treatment. The second sample of sputum will go to our lab in Durban for testing for TB. This is only for research purposes, and we will not give you the result. If we think you need other tests for TB we will refer you to the clinic to get properly checked.

Blood sample: If you agree, we will take a blood sample from one of your arms. We will put a tight band around your upper arm, clean your skin, then put a thin needle through the skin and into a vein. We will take 10mls (two teaspoons) of blood, then take out the needle and press on your skin to stop a bruise developing. The blood sample will be sent to our laboratory in Durban and tested for signs of TB infection. This test is used in research studies but is not usually used to measure TB infection in public clinics in South Africa. We will only report the result of this test to you if the result shows that treatment is recommended. This result might take a month or more to come back. We will also test your blood for possible new markers for TB, which might help develop new tests for TB in the future.

If you have not had a test for HIV in the last three months, we would like to offer you a test for HIV, using a fingerprick blood sample. We can give you the result of the test today, after about 15 minutes. If the result is positive we will discuss with you how to get care for HIV, if you are not already getting HIV care at a clinic. Everyone should know their HIV status, and we encourage you to get the HIV test result today. However if you prefer not to get the HIV test result, we can test your blood anonymously in the laboratory. That way we could link the HIV test result to the TB test results, which would help us a lot to understand TB in this community. We would keep the result confidential and not tell anyone (including you) the result. As a third choice, if you are happy to take part in the study but you do not want to have your blood tested for HIV, we will respect your choice, and no HIV test will be done.

Referral for care: if any of the questions we ask or tests we do suggest that you may need further tests or health care, we will explain what you need to do, and give you a referral to your local health centre.

[*Paragraph for people in the RFID substudy only, to be skipped for others]*: We would like you to help us test out a new way to measure how long people spend in different public places in the community. We will give you a wrist band and we would like you to wear it over the next two weeks, while you are filling in the contact diary. If you go through the doors of [*name of relevant health centre and any other local public building with sensors]*, the wrist band will record that you were there. This information will be kept confidential. At the end of two weeks we will collect the diary and the wrist band. At this time, we will ask you a few questions about where you went, and find out from you how easy or difficult it was to fill in the diary, and what you thought about wearing the wrist band.

Follow-up: We would like to see you again after 12 months (one year). At this visit, we will ask some questions to see whether you have had any health problems during the year and whether you have been in contact with anyone with TB. We will check whether you have any symptoms that suggest you might be unwell with TB. If you agree, we will take a blood sample from your arm, in the same way as the first visit, to do the same tests.
With your permission, we would like to check on your health from time to time over the next five years, particularly to see if you need to start treatment for TB. We will do this by asking you or your family when we come to your home for surveillance visits; by checking clinic and laboratory records, and if needed by phoning you.

We would like to connect this information about TB to other information that AHRI has collected about you and your family over the years. We will be very careful to be sure that all information you give us is kept confidential.

What are the possible risks of taking part?
There might be some pain or discomfort from having blood taken from your arm. We can reduce this by putting some cream on your arm that helps to numb the area and makes it less painful. There is also the risk of a bruise at the site where the blood was taken; this will be reduced by pressing on the area after the needle is taken out.

What are the possible benefits of taking part?
If you have symptoms that suggest you might be unwell with TB, we will take a sputum sample to the clinic to be tested for TB, and contact you with the result. If necessary, we will also refer you to the clinic to get properly checked for TB. If you are unwell with TB, and you start treatment quickly, the TB is less likely to cause serious illness.

If you are HIV-positive and your TB blood test is positive, we will refer you to the clinic for further checks and possibly to get some medication (isoniazid), if you are not already taking this. Taking isoniazid reduces the risk of you becoming unwell with TB.

More generally, we hope that this research will help us to find ways to prevent people like you getting infected with TB, so that it may help people like you in the future.

To thank you for taking part in the study, we will give you an airtime voucher or a food refreshment of about ZAR 20, after we finish the questions and tests, and another ZAR 20 airtime voucher or food refreshment after the 12-month visit.

What happens if I do not agree to take part in this study?
You do not have to take part in this study: if you do not take part, this will not affect you in any way. You can stop taking part in the study at any time, without giving a reason.

How will the information collected during this study be kept confidential?
All information collected during the course of this study will be kept securely and confidentially at AHRI: *[name of study manager]* is responsible for this. We will store information on a secure computer system. The information we collect will be identified on forms and computer files only by a study number or barcode, not your name. This means that your information remains private.

Study information may be looked at by the Ethics Committee, and authorised independent monitors, to check that the study procedures were done correctly, and the information is accurate. Your information will remain confidential, unless we are required by law to release information. Reports about the study will never include information which allows you to be identified.

Results from the study will be presented to people working in the TB programme, the Department of Health, and other researchers as presentations and publications in medical journals. In all these presentations and reports, it will not be possible to identify people who took part.

Will blood samples that I give for the study be stored?
Yes, if there is blood remaining after the TB tests have been done, we will store it in our laboratories in Durban for up to 20 years. It may be used by approved researchers, for example to develop new tests for TB. Further studies using your blood sample could only be done if they were approved by the ethics committees who oversee this research. If you decide later that you do not want your blood sample used for

research, you can contact us and we will destroy any remaining blood sample Once all tests have been done, the remaining sample will be disposed of by the laboratory in Durban.

Who is organising and funding the research?
This research study is being organised by AHRI through the Healthy Adolescents and Young Adults (HAYA) research unit. The study is being funded by Viiv HealthCare, a drug company that has funded the HAYA research unit.

Who has reviewed the study?
All research at the AHRI is looked at by an independent group of people, called a Research Ethics Committee. This study has been approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal. As this study involves researchers from the UK, the study has also been approved by the Research Ethics Committee of the London School of Hygiene & Tropical Medicine. The study has also been approved by the AHRI Community Advisory Board and by the Department of Health.

What if I have questions about this study?
If you have any questions, please feel free to ask me now. If you have questions about the study later you may contact the main researcher:
Professor Alison Grant
Professor of International Health
c/o AHRI
PO Box 198, Mtubatuba 3930
Tel: 035-500-7500; fax: 035-550-7565; email: agrant2@ahri.org

If you have concerns about the study, you can contact the UKZN Biomedical Research Ethics Committee:
Biomedical Research Ethics Administration
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001, Durban 4000
Tel: 031-260-4769; Fax: 031-260-4609; Email: BREC@ukzn.ac.za

We will give you a copy of this information sheet which explains the study to take away with you.

CONSENT TO PARTICIPATE IN RESEARCH V3.0 30 NOVEMBER 2017

INCIDENCE OF TB INFECTION IN ADOLESCENTS IN RURAL KWAZULU-NATAL, SOUTH AFRICA

Study ID | |

The researchers doing this study are:

Africa Health Research Institute: Alison Grant, Richard Lessells, Kathy Baisley, Frank Tanser, Kobus Herbst, Safiyya Randera-Rees

Hlabisa Hospital: Martin Tshipuk

London School of Hygiene & Tropical Medicine, UK: Themba Mzembe, Rashida Ferrand, Katharina Kranzer, Janet Seeley, Chris Grundy, Naomi Walker, Aaron Karat

Liverpool School of Tropical Medicine, UK: Peter MacPherson

I have read the information sheet about this study (or the information sheet about this study has been read to me). I understand what will be required of me and what will happen if I take part in the study.

My questions concerning this study have been answered by:

Research staff name (printed)	Signature	Date

I understand that I may withdraw from this study at any time without giving a reason, and without affecting my normal care and management.

I agree that my blood can be tested for HIV as part of this study *(initial box to show agreement)* | |

I agree to take part in the study

Study participant name (printed)	Signature/ thumbprint	Date

If the participant gave verbal consent, indicated by a thumbprint, enter the name of the independent person who witnessed the consent here and their signature:

Witness name (printed)	Signature	Date

Appendix 4: Biomedical Research Ethics Committee of the University of KwaZulu-Natal approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

11 July 2016

Professor A Grant
Africa Centre for Population Health
London School of Hygiene and Tropical Medicine
alison.grant@ishtm.ac.uk

Protocol: Incidents of tuberculosis infection in adolescents in rural KwaZulu-Natal, South Africa.
Degree: Non-degree
BREC reference number: BE483/15

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 06 November 2015.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 04 July 2016 to queries raised on 31 May 2016 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval.**

This approval is valid for one year from **11 July 2016.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee**'s** decision will be **RATIFIED** by a full Committee at its meeting taking place on **16 August 2016.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

[redacted]

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

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Appendix 5: London School of Hygiene & Tropical Medicine ethics approval

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

Observational / Interventions Research Ethics Committee

Professor Alison Grant
Professor of International Health
Department of Clinical Research (CRD)
Infectious and Tropical Diseases (ITD)
LSHTM

27 May 2016

Dear Alison

Study Title: Incidence of tuberculosis infection among adolescents in rural KwaZulu-Natal, South Africa

LSHTM Ethics Ref: 10515

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Investigator CV	Kranzer_UKZN BREC_CV15	28/10/2015	1.0
Protocol / Proposal	Study protocol	05/11/2015	1.0
Protocol / Proposal	Baseline case report form	05/11/2015	1.0
Protocol / Proposal	Social contact diary	05/11/2015	1.0
Information Sheet	Information sheet adult participant	05/11/2015	1.0
Information Sheet	Information sheet parent/guardian	05/11/2015	1.0
Information Sheet	Information sheet child	05/11/2015	1.0
Investigator CV	CV Richard Lessells	01/12/2015	1.0
Investigator CV	GrantCVshort_investigator_dec15	14/12/2015	1.0
Investigator CV	Baisley CV_15Dec2015	15/12/2015	1.0
Local Approval	BE483-15_Provisional approval_20jan2016	20/01/2016	1
Protocol / Proposal	Study protocol	10/05/2016	1.1
Information Sheet	Information sheet parent/guardian	10/05/2016	1.1
Information Sheet	Information sheet child	10/05/2016	1.1
Covering Letter	Cover letter	24/05/2016	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix 6: KwaZulu-Natal Department of Health approval for research

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

DIRECTORATE:
Health Research & Knowledge Management

Physical Address: 330 Langalibalele Street, Pietermaritburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3189/ 3123 Fax: 033 394 3782
Email: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

HRKM Ref: 184/16
NHRD Ref: KZ_2016RP7_35

Date: 24 June 2016
Dear Prof A. Grant
London School of Hygiene and Tropical Medicine

Approval of research

1. The research proposal titled **'Incidence of tuberculosis infection among adolescents in rural KwaZulu Natal, South Africa'** was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at the Africa Centre for Demographic Surveillance Centre, Esiyembeni, Gunjaneni, KwaMsane, Machibini, Mpukunyoni, Mtubatuba and Somkhele clinics

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

[redacted]

Dr E Lutge
Chairperson, Health Research Committee
Date: 01/07/16.

Fighting Disease, Fighting Poverty, Giving Hope

Appendix 7: Standard operating procedures for feeding back laboratory results

AHRI Standard Operating Procedure AHRI ATB09

HAYA TB Laboratory Results feedback to participants

Version:	**1.0**	
Issue date:	**13 April 2018**	
Author:	**Aaron Karat**	**Signature:** [redacted]
	Kathy Baisley	**Signature:**
	Themba Mzembe	**Signature:**
Approved by:	**Alison Grant (PI)**	**Signature:**
Approved by:	**Research Nursing Manager**	**Signature:**

1. Purpose

1.1. To define the procedures for feeding back results to participants in the HAYA TB Study. The procedures for handling the laboratory specimens are described in SOPs ATB08 (Procedures for collection and transportation of sputum specimens), ATB12 ('Procedure for processing and transportation of blood specimens). Procedures for describing the handling of the laboratory results before they are returned to the participant are described in the Laboratory Results Management manual.

2. Scope

2.1. This SOP applies to AHRI personnel who are required to collect and/or feedback lab results as part of the HAYA TB study.

3. Responsible personnel

3.1. The PI, Study Manager and Research Nurse Manager have overall responsibility for ensuring that this procedure is followed.

3.2. The Research Nurses are responsible for feeding back the results to the participants.

3.3. The designated Data Manager is responsible for producing lists of results to be fed back to participants, and for running checks of results that have not been returned, and of the time taken to return results.

3.4. The Study Manager and Study Doctor are responsible for overseeing the procedures on a day-to-day basis.

4. Overview

4.1. The HAYA Adolescent TB study will be testing participants for TB infection using an Interferon-Gamma Release Assay (IGRA). In addition, participants will be offered HIV rapid testing; those with discordant rapids will have an HIV ELISA test. Participants with symptoms of TB disease will have sputum collected for Xpert® MTB/RIF (at an NHLS lab) and culture (at the AHRI lab). Most results will be fed back to participants at their home.

5. Management of laboratory results before feed back

5.1. Individual reports of the Xpert® MTB/RIF results will be downloaded in PDF format from the NHLS website by the Study Doctor, using the NHLS barcode number (see section 6.7). The Study Doctor will save the PDF files on a secure folder on the shared drive (see Laboratory Results Management Manual).

5.2. The Study Doctor will check that the participant's details are correct and will print a copy of the result and give the printed copy to the relevant Research Nurse.

5.3. The Research Nurse will enter the result manually into REDCap.

5.4. All results from the Durban lab are imported directly into REDCap from the Laboratory Information System (LIMS) by the designated Data Manager (see Laboratory Results Management manual).

5.5. The Data Manager will use a validated, automated computer program to generate a list of participants whose LIMS results need to be fed back; the computer program will be checked and approved by the Study Statistician before it is released for use by the study.

5.6. The Durban laboratory will use the list generated in step 5.5 to produce an individual signed report that can be given to the participant. The report will be in PDF format and will be stored in a secure folder on the shared drive (see Laboratory Results Management manual).

5.7. The Research Nurse and the Study Doctor will be notified via email when the signed report becomes available. The Research Nurse will obtain a printed copy of the report to give to the participant. At the time of receiving the report, the Research Nurse and Study Doctor must check that the participant details are correct (DSID, PT-ID, name and date of birth), and notify the Study Manager if there are any errors.

5.8. The Research Nurse will use the Results Management instrument of the REDCap study database to support the process of feeding back the results. In this instrument, the Research Nurse will only have access to the results that need to be fed back.

5.9. The Data Manager will run a weekly check for any outstanding results that have not yet been fed back. In addition, the Data Manager will run a weekly check for any missing results from the Durban laboratory, or Xpert® MTB/RIF results that have not been downloaded from the NHLS website. The queries generated by these checks will be given to the Study Doctor to follow up.

5.10. The Study Doctor, or appropriate clinical delegate, will do a weekly review of the results that need to be fed back, to ensure that they have been acted on appropriately.

6. Results and Required Actions

6.1. An overview of the results that should be fed back, and to whom, is provided in this section. The process of feeding back the results is described in detail in Section 7, including the circumstances under which results can be fed back to the parent/guardian, if the participant cannot be contacted.

6.2. Negative IGRA

a. The Research Nurse will not be notified about negative IGRA results.

b. No action is required.

6.3. Positive IGRA

a. The Research Nurse will be notified about all positive IGRA results that need to be fed back (see section 5 and Laboratory Results Management manual).

b. If the participant is aged 15 years and above, positive IGRA results will only fed back if the participant is HIV positive, and is aware of his/her HIV positive status. If the participant is HIV negative, or their HIV status is unknown to them, the results will not be fed back.
c. If the participant is aged <15 years and HIV positive, ascertain who is aware of the HIV status:
   - Both parent/guardian and participant aware: IGRA results are fed back to both
   - Only the parent/guardian knows the participant's HIV positive status: IGRA results are fed back to parent/guardian alone
   - Only the participant knows his/her positive HIV status: IGRA results are fed back to participant alone.
   - Participant and parent/guardian are both unaware of participant's HIV status: results are not fed back.
d. The result should be fed back to the participant (or parent/guardian, as above) in person at the participant's home.
e. Referral to care is done during the home visit, with support of linkage to care, as described in SOP ATB10.
f. Results should be fed back at a time which is convenient to the Research Nurse and participant, within four weeks of receiving the positive IGRA result (see Section 7.2 for more information)

6.4. Negative HIV ELISA if rapid test was invalid or discordant

a. The Research Nurse will be notified about all negative HIV ELISA results that were requested because of a discordant or invalid rapid HIV test result (see Section 5).
b. If the participant is aged 10-11 years, results should be fed back to both the participant and parent/guardian.
c. If the participant is aged 12-14 years old, the result should be fed back to the participant; however, the parent can be present if the participant prefers.
d. If the participant is aged ≥15 years, the results should be fed back to the participant only.
e. All HIV ELISA results should be fed back in person at the participant's home, as far as possible. However, if this is not possible (e.g. if the family have moved away), but the phone number is still valid, after discussion with an appropriate senior clinician it may be agreed that negative results can be given over the phone.
f. All results should be fed back with appropriate post-test counselling (see ATB06).
g. As far as possible, the Research Nurse should aim to feed back the results within 2-3 working days of receiving the result
h. Referral to care is not required.

6.5. Positive HIV ELISA if rapid test was invalid or discordant

a. The Research Nurse will be notified about **all** positive HIV ELISA results that were requested because of a discordant or invalid rapid HIV test result (see Section 5.1).
b. If the participant is aged 10-11 years, results should be fed back to both the participant and parent/guardian.
c. If the participant is aged 12-14 years old, the result should be fed back to the participant; however, the parent can be present if the participant prefers.
d. If the participant is aged ≥15 years, the results should be fed back to the participant only.
e. All results should be fed back in person at the participant's home, with appropriate post-test counselling (see ATB06).

f. As far as possible, the Research Nurse should aim to feed back the results within 2-3 days of receiving the result

g. Referral and support of linkage to care are required and should be done during the visit (see SOP ATB10).

6.6. Positive or Negative HIV ELISA if rapid test was declined or not offered

a. The study team will not be notified of any HIV ELISA results that were not done in the context of HIV rapid testing.

b. No action is required.

6.7. Sputum Xpert® MTB/RIF results

a. The Study Doctor will download the individual report of the Xpert® MTB/RIF results from the NHLS website in PDF format; he/she will check the website for results 2 working days after the sputum sample was submitted (see Laboratory Results Management manual).

b. The Study Doctor will check each result carefully to confirm that it is for a HAYA TB study participant, and that the participant details are correct.

c. The Study Doctor will save the PDF file in a secure folder on the shared drive. He/she will print a copy of the results that need to be fed back to the participant in person and give it to the Research Nurse who collected the sample.

d. The Research Nurse will cross-check and enter the result details into REDCap (date and result of test).

6.7.1. Negative or Invalid Xpert® MTB/RIF

a. A negative or invalid result should be fed back to the participant by telephone, (see Section 7.1 for more information).

b. If the participant is aged 10-11 years, results should be fed back to the parent/guardian (see section 7.1)

c. As far as possible, the Research Nurse should aim to feed back the result within 2-3 working days of receiving it.

d. When feeding back the results, the Research Nurse should ask the participant (or parent/guardian) about current symptoms. Individuals with persistent or worsening symptoms should be visited at home and be issued with a copy of the negative result and referral slip (see SOP ATB10) to attend a clinic for further investigation.

6.7.2. Positive Xpert® MTB/RIF

a. A positive result should be fed back to the participant in person.

b. If the participant is aged 10-11 years, results should be fed back to both the participant and parent/guardian (see section 7.2).

c. As far as possible, the Research Nurse should aim to feed back the result within 2-3 working days of receiving it.

d. The participant should be given a copy of the positive Xpert® MTB/RIF result and referred to one of the 11 PIPSA clinics. The AHRI nurse at that clinic should also be provided with a copy of the positive result (see SOP ATB10 and Section 7.2).

6.8. Negative sputum culture

a. Sputum culture results will generally only be available two to six weeks after the sputum was collected.
b. A negative sputum culture result will not routinely be communicated to the Research Nurse.
c. No action is required.

6.9. Sputum culture positive for MTB

a. The Research Nurse will be notified of **all** sputum culture results which are positive for MTB (see Section 5).
b. Any action required will depend on the results of the participant's Xpert® MTB/RIF test (see SOP ATB10).
c. If the Xpert® MTB/RIF was positive, and if the result has been fed back, and the participant is confirmed as having linked to care, no further action is needed.
d. If the Xpert® MTB/RIF was positive, and the participant has not yet linked to care, please follow up accordingly.
e. If the Xpert® MTB/RIF was negative or invalid or not done, the result should be fed back to the participant (and parent/guardian, if aged <15 years) in person, and a copy of the result should be given to the participant. Referral to care, with linkage support, is required, and the AHRI nurse at the specified clinic should also be provided with a copy of the culture result and the Xpert® MTB/RIF result.
f. Please discuss with the Study Manager or the Study Doctor if you are unsure how to proceed.

6.10. Sputum culture positive for NTM

a. This result should **not** be fed back to the participant
b. No action is required.

7. Process of feeding back results

7.1. By Telephone – negative Xpert results

a. Only negative Xpert® MTB/RIF results will be fed back by telephone.
b. Results should be fed back using the telephone number that was given at the enrolment interview.
c. As far as possible, the Research Nurse who originally interviewed the participant will be responsible for feeding back the result.
d. Result feedback should be prioritised over participant recruitment and should aim to be fed back within 2-3 working days of receiving the result from the NHLS.
e. The phone call should be made any time during working hours, but preferably after 2 pm as participants might be at school.
f. If the participant is <18 years old, the negative Xpert® MTB/RIF results can be fed back to the parent/guardian, if the participant cannot be contacted.
g. Up to 4 attempts should be made to telephone the participant or parent/guardian. If no contact is made, the Study Nurse should inform the Study Manager. The Study Manager will discuss with an appropriate clinician and decide the appropriate action.

During the phone call about a negative Xpert® MTB/RIF result:

a. Confirm that this the right person that you are talking to.
b. Greet the participant. Remind them that you are a Research Nurse from AHRI who interviewed them a few days/weeks (as appropriate) before.

c. Ask the participant if this is an acceptable time for them to talk. If not, schedule a time to call again and make the call at the agreed time.
d. Inform the participant the purpose of the phone call.
e. Explain that the Xpert® MTB/RIF result for the sputum sample that you collected came back and that the result is negative (i.e. TB germs were not detected in the sputum).
f. Ask about persistent TB symptoms.
g. If the participant is still experiencing symptoms suggesting TB, advise them that a negative sputum Xpert® MTB/RIF does not imply that they do not have TB disease.
h. Make a record of any symptoms in the relevant section in REDCap.
i. Advise participant that they need to attend clinic for further investigation. Inform the participant that before going to the clinic, you will visit them in person and deliver the negative result and referral slip which they can take to the clinic with them (see SOP ATP10). Ask the participant about an acceptable date to visit and arrange an appointment.
j. Thank the participant for their time and address any further questions that they may have.

7.2. In Person

a. Only negative Xpert® MTB/RIF results can be fed back by telephone.
b. As far as possible, the Research Nurse who originally interviewed the participant is responsible for feeding back the result.
c. If the participant is aged 12 years or older, the Research Nurse should feed the result back to the participant directly; however, if the participant cannot be contacted, the following results can be fed back to the parent/guardian instead:
    - Positive Xpert® MTB/RIF or sputum culture results, if the participant is aged 12-17 years old
    - Positive IGRA results, if the participant is aged 12-14 years old AND the parent/guardian knows the participant's positive HIV status.
d. The following results must ONLY be fed back to the participant (i.e. cannot be fed back to the parent/guardian if the participant cannot be contacted):
    - ALL HIV ELISA results, if the participant is aged 12 years or older.
    - Positive IGRA results, if the participant is aged 15 years or older.
e. If the participant is 10-11 years old, the Research Nurse should attempt to feed the result back to both the parent/guardian and the participant, if possible. If the participant cannot be contacted, results can be fed back to the parent/guardian alone (except for positive IGRA results, unless the parent/guardian knows the participant's HIV positive status, as below). The following results should NOT be fed back to the participant alone - the parent/guardian should be present:
    - ALL HIV ELISA results.
    - Positive IGRA results (unless the parent/guardian does not know the participant's HIV positive status, then it must be fed back to the participant only).
    - Positive Xpert® MTB/RIF or sputum culture results.
f. As soon as the Research Nurse receives the result, he/she should telephone the participant (and/or parent/guardian, if appropriate) to make an appointment to visit the participant at home to feed back their results, and give them a printed copy of the report. The Research Nurse should record the date and outcome of the telephone conversation in REDCap.
g. During the phone call the Research Nurse should do the following:

- Greet the participant, confirm their identity and remind them that you are a Research Nurse from AHRI who interviewed them a few days/weeks (as appropriate) before
- Explain that you have received their lab results and would like to arrange a time to visit them to feed back the result
- Agree on an appointment date to visit them at home, and record the date in REDCap
- Thank the participant for their time.

h. At the home visit, the Research Nurse informs the participant of laboratory results with appropriate post-test counselling. If appropriate, the participant is referred to a preferred clinic for care (see SOP ATB10)

i. Up to 4 attempts should be made to telephone the participant (or parent/guardian, if appropriate) for an appointment to visit them at home. If the participant or parent/guardian cannot be contacted by telephone after 4 attempts, the Research Nurse should visit the participant's home in person. If no contact is made, the Study Nurse should inform the Study Manager.

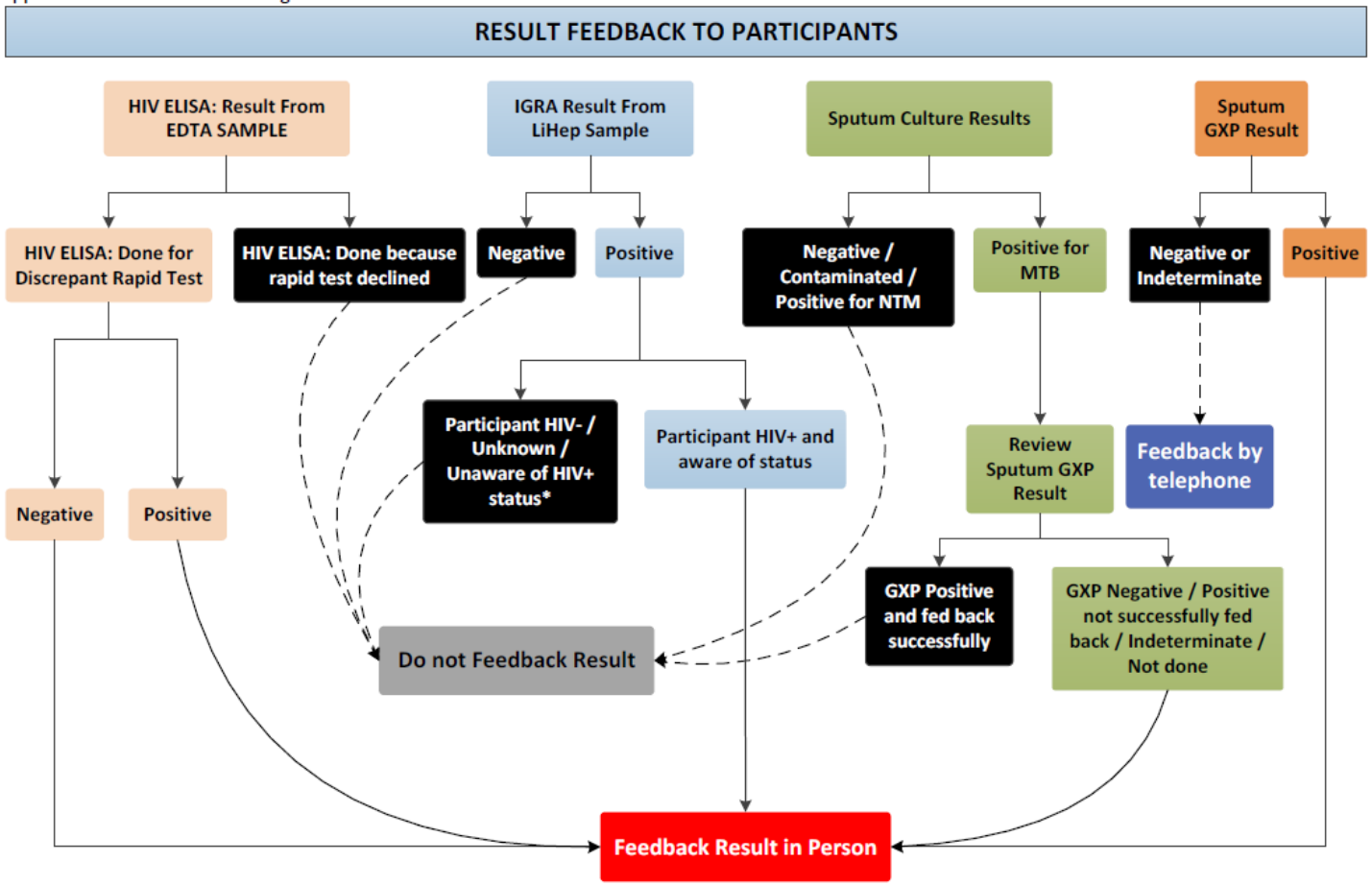
j. Once the result is available, the process of feeding back HIV ELISA (for discordant HIV rapid test), or Xpert® MTB/RIF or sputum culture positive, should not exceed 14 days. The process from IGRA positive result to feeding back to HIV positive participants should not exceed four weeks. The time span will be audited monthly, looking for both average time interval and outliers.

8. Definition/abbreviations

AHRI: Africa Health Research Institute; **DSID**: Demographic Surveillance Identifier; **DST**: Drug Sensitivity Testing; **MTB**: Mycobacterium tuberculosis; **NHLS**: National Health Laboratory Service; **NTM**: Nontuberculous mycobacteria; **SOP**: Standard Operating Procedure; **RN**: Research Nurse; **IGRA**: Interferon Gamma Release Assay

9. Attachments & appendices

Appendix 1: Result Feedback categories



*Unless parent/guardian aware of HIV+ status, then feedback to parent/guardian in person

Appendix 8: Standard operating procedures for referral and linkage to care

AHRI Standard Operating Procedure AHRI ATB10

HAYA TB Procedure for linking participants to care

(for HAYA nurses)

Version:	**1.0**	
Issue date:	**29 May 2018**	
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	Research Nursing Manager	**Signature:**

1. Purpose

1.1. The purpose of this SOP is to provide guidance to HAYA TB Research Nurses (RN) on procedures for participants who may need to be referred to health care services.

2. Scope

2.1. This SOP applies to AHRI nurses working in the HAYA adolescent TB study.

3. Responsible personnel

3.1. The PI, Study Manager and Research Nurse Manager have overall responsibility for ensuring that this procedure is followed.

3.2. The Research Nurse is responsible for referring participants to care and monitoring linkage to care.

3.3. The Study Manager and Study Doctor are responsible for overseeing the procedures on a day-to-day basis.

4. Policy and procedure

4.1. Study Overview

4.1.1. The HAYA TB Study aims to estimate the prevalence and incidence of tuberculosis (TB) infection (i.e., latent TB) among adolescents (aged 10–19 years), resident in the AHRI PIPSA.

4.1.2. To do this, Research Nurses will travel to the homes of ~1100 consenting young people, interview them, and take venous blood (at a minimum).

4.1.3. Blood will be tested using an Interferon-Gamma Release Assay (IGRA) called QuantiFERON-TB Gold Plus (QFT-Plus) which detects TB infection.

a. This test is currently not used as routine in South Africa and is therefore not included in any of the NDoH diagnostic or treatment algorithms.

b. However, it is a test that is commonly used in research settings, and increasingly used in high-income settings, and is useful for predicting an HIV-positive person's risk of developing active TB disease.
c. HIV-positive people with a positive IGRA and no evidence of active TB disease should be prioritised for Isoniazid Preventive Therapy (IPT), as long as this is not contraindicated.

4.1.4. Participants will also be offered HIV counselling and testing (HCT) as part of the study, and participants with symptoms of TB disease will have sputum collected for Xpert® MTB/RIF (at an NHLS lab) and culture (at the AHRI lab).

4.1.5. There are several reasons why participants may then be referred to a primary health clinic: an overview of study processes is presented in Figure 1.

4.1.6. This study will use REDCap and the ClinicLink system to coordinate and track referrals and linkage to care; as such, the AHRI nurses stationed at DoH clinics will act as the first point of contact for participants who are referred.

4.1.7. Participants aged <15 years who are newly diagnosed with HIV (via rapid or ELISA testing as part of HCT) will be referred to a clinic where they can be seen by an AHRI Paediatric Nurse at their first appointment.

4.1.8. The linkage of participants aged 15 years or older who are newly diagnosed with HIV will be supported using existing structures within the ClinicLink system. For other referrals, linkage to care will be monitored within the ClinicLink system but tracking of participants who have not linked will be done within REDCap.

4.1.9. Certain actions may be carried out in the clinics by the AHRI nurses themselves, but most will need referral to a DoH nurse.

4.2. Process of referral

4.2.1. Participants may be referred by the Research Nurse immediately after the enrolment interview, after HCT, or after a number of days/weeks, once test results are available.

4.2.2. As standard, at the time of making the referral, Research Nurses will ask participants which of the 11 clinics in the PIPSA (AHRI population study area) they would prefer to attend. This will be recorded in REDCap.

4.2.3. Most laboratory results will be fed back to the participant in person by the Research Nurse at the participant's home (see SOP ATB09). If referral to care is needed, the Research Nurse will initiate the referral during the home visit.

4.2.4. The Data Manager will generate a list of participants who need to be referred based on their laboratory results, using an automated computer programme (see SOP ATB09).

4.2.5. When making a referral, the Research Nurse will provide the participant with a referral letter to the AHRI nurse at that clinic (see Figure 2 for an example referral letter).

4.2.6. For participants aged <15 years who are newly diagnosed with HIV, every effort will be made to refer them to one of the clinics where there is an AHRI Paediatric Nurse (see SOP ATB17). However, if the participant cannot attend one of these clinics, he/she will be referred to their preferred PIPSA clinic.

4.2.7. The Research Nurse will ask the participant to agree on a target date for attending the clinic and will tell the participant that they will be contacted if they have not attended the clinic by that date.

4.2.8. Table 1 gives a summary of reasons for referring participants to care and respective referral codes.

4.2.9. When making referral, Research Nurses should write the reason for referral and the referral code on the referral letter.

4.2.10. If there is a concern about confidentiality, only the referral code should be used, and the reason for referral should not be included in the letter.

4.2.11. The list of referral codes and reasons for referral will also be available to the clinic-based AHRI nurse so that the appropriate action can be taken (SOP ATB11).

Table 1. List of reasons for referral to care and referral codes

Group/Code	Reason for referral
Group A	**HIV-related**
A1	New HIV diagnosis by rapid or ELISA testing as part of HCT
A2	Previous diagnosis of HIV; parent/guardian/caregiver requires assistance disclosing status to child
A3	Self-report of previous HIV diagnosis, not on ART
Group B	**TB disease**
B1	Symptoms of TB but unable to produce sputum
B2	Persistent TB symptoms with negative sputum Xpert® MTB/RIF
B3	Positive sputum Xpert® MTB/RIF
B4	Sputum culture positive for MTB after previous negative sputum Xpert® MTB/RIF; or Xpert® MTB/RIF results not available
Group C	**TB infection**
C1	New or previously known diagnosis of HIV with positive QuantiFERON (QFN) TB [IGRA]

4.3. Actions needed for any referral

4.3.1. The Research Nurse should explain carefully the reason for the referral and the importance of attending clinic.

4.3.2. All referrals must be recorded on the relevant section in REDCap on the tablet.

4.3.3. The Research Nurse should provide the participant and/or parent/guardian/caregiver with a referral letter to their preferred clinic.

4.3.4. The Research Nurse should consult the list of reasons for referral and complete the referral letter appropriately.

4.4. Actions needed for specific referrals

4.4.1. Code A1: New HIV diagnosis (via rapid or ELISA testing as part of HCT)

a. For an HIV diagnosis after rapid testing, the referral should be made immediately after the participant has received post-test counselling (i.e., during the enrolment visit).

b. For HIV ELISA testing (if rapid tests are discordant or invalid), the visit for arranging the referral to care should be made as soon as possible once the result is available (generally around one week after the blood is sent to the lab for testing).

c. This result should ideally be delivered to the participant in person and by the same Research Nurse that conducted the initial interview and took the blood – see SOP ATB09 'Collecting and feeding back results').

d. The Research Nurse should ensure that the participant and/or parent/guardian/caregiver has been provided with age-appropriate, post-test counselling (see ATB06 'Procedure for Rapid HIV testing' for details) including clear information about the benefits of treatment.
e. The Research Nurse should explain that he/she needs to make a referral to one of the 11 PIPSA clinics, where the participant (and/or parent/guardian/caregiver) will be met by an AHRI nurse who will give them further information about treatment.
f. If the participant is <15 years old, explain that the Research Nurse will need to refer them to one of the clinics where the AHRI Paediatric Nurse can meet with them on their first appointment (see SOP ATB16 'HIV referrals for participants <15 years'). If the participant cannot attend one of these clinics, then they should be referred to one of the other PIPSA clinics.
g. The Research Nurse should agree with the participant on a target date to attend the clinic (within two weeks) and explain that they will be contacted if they have not attended the clinic by that date.
h. The Research Nurse should complete the referral letter to the preferred clinic (see Figure 2) and provide contact details (work cell number).
i. The Research Nurse should enter the referral details (date, clinic, target date, contact details, etc.) into the relevant section in REDCap.

Documents that the participant and/or parent/guardian/caregiver should be provided with:

i. Referral letter to preferred clinic.
ii. A copy of the HIV positive test result for rapid or ELISA.
iii. If there are concerns about confidentiality, and only the referral code is written on the referral letter (see section 4.2.9), then a copy of the test results should NOT be provided.

4.4.2. Code A2: Previous HIV diagnosis; Parent/guardian/caregiver Requires Assistance Disclosing Status to Child

a. If a participant was diagnosed as HIV-positive when very young, they may not be aware of their status (even though they may be on ART); in these cases, the Research Nurse should encourage the parent/guardian/caregiver to disclose the child's status and offer to help them do so.
b. This referral should be made immediately (i.e. at the time of the enrolment interview).
c. Explain to the parent/guardian/caregiver that one of the AHRI Paediatric Nurses will be able to help them discuss their child's HIV status with her/him, and (if not already in care) assist with getting the child on ART.
d. If the child is already on ART, explain that disclosure should take place, ideally, at the clinic that has been caring for their child (i.e., providing ART). If the child is not on ART, ask which clinic (of the three PIPSA clinics with an AHRI Paediatric Nurse) they would prefer to attend (see SOP ATB17).
e. If the child is not already on ART, the Research Nurse should explain the benefits of treatment. The Research Nurse should answer, as far as possible, any questions the parent/guardian/caregiver has about the treatment and the need to attend clinic, and should try, in particular, to address any anxieties about attending clinics (provide reassurance and reiterate that they are free to contact whenever necessary).
f. The Research Nurse should complete the referral letter to the preferred clinic (see Figure 2 and provide contact details (work cell number).
g. The Research Nurse should enter the referral details into the appropriate section (parent/guardian/caregiver section) in REDCap.

Documents that the parent/guardian/caregiver should be provided with:

i. Referral letter to preferred clinic

4.4.3. Code A3: Self-report of previous HIV diagnosis not on ART

a. Participants who self-report previously being diagnosed with HIV but not on ART should be referred to care during the enrolment visit.
b. The Research Nurse should explain the benefits of starting treatment.
c. The Research Nurse should explain that he/she needs to make a referral to one of the 11 PIPSA clinics, where the participant (and/or parent) will be met by an AHRI nurse and be given further information about treatment.
d. The Research Nurse should answer, as far as possible, any questions the participant and/or parent/guardian/caregiver has about the process and should try, in particular, to address any anxieties about attending clinics (the Research Nurse should provide reassurance and reiterate that the participant and/or parent/guardian/caregiver are free to contact whenever necessary).
e. If the participant is <15 years old, the Research Nurse should explain that he/she needs to refer to one the 3 clinics where there is an AHRI Paediatric Nurse (see SOP ATB17).
f. The Research Nurse should agree on a target date to attend the clinic (within 2 weeks) and explain that they will be contacted if they have not attended the clinic by that date.
g. The Research Nurse should complete the referral letter to the preferred clinic (see Figure 2) and provide contact details (work cell number).
h. The Research Nurse should enter the referral details (date, clinic, target date, contact details, etc.) into the relevant section in REDCap.

Documents that the participant and/or parent/guardian/caregiver should be provided with:

i. Referral letter to preferred clinic

4.4.4. Code B1: TB Symptoms but unable to Produce Sputum

a. Participants with symptoms suggestive of active TB disease (especially those who are HIV-positive) and are unable to produce sputum during the enrolment interview should be referred to primary care for further assessment and investigation (see SOP ATB08 'Sputum collection').
b. This referral should be made immediately (i.e., during the enrolment interview).
c. The Research Nurse should explain to the participant and/or parent/guardian/caregiver that, because the participant has symptoms suggestive of TB and cannot produce sputum, they need to be reassessed at a clinic.
d. The Research Nurse should further explain that the nurse or doctor at the clinic may ask them, again, to try and produce sputum, and/or may do alternative tests.
e. The Research Nurse should answer, as far as possible, any questions the participant and/or parent/guardian/caregiver has about the process and try, in particular, to address any anxieties about attending clinics (provide reassurance and reiterate that they are free to contact you whenever necessary).
f. The Research Nurse should ask which clinic (of the 11 PIPSA clinics) they would prefer to attend and agree on a target date to attend clinic.
g. The Research Nurse should complete the referral letter to the preferred clinic (see Figure 2) and provide contact details (work cell number).

h. The Research Nurse should explain that he/she will need to contact them by phone to check if they have gone to the clinic for further assistance by the target date.
i. The Research Nurse should enter the referral details (date, clinic, target date, contact details, etc.) into the relevant section in REDCap.

Documents that the participant and/or parent/guardian/caregiver should be provided with:

i. Referral letter to preferred clinic

4.4.5. Code B2: Persistent TB symptoms with negative sputum Xpert® MTB/RIF

a. Participants with symptoms suggestive of active TB should have sputum collected at the enrolment visit (see SOP ATB08); sputum samples will be sent to an NHLS laboratory for testing with GeneXpert® MTB/RIF.
b. A negative Xpert® MTB/RIF result will be fed back to the participant by telephone (see SOP ATB09).
c. During the telephone conversation, the Research Nurse should refer to the details of the symptoms present at enrolment and ask the participant about these symptoms.

Who to refer:

a. All participants who are confirmed HIV-positive or of unknown HIV status with symptoms that are persistent or worse should be referred to a clinic for further assessment.
b. A participant who is 1) confirmed HIV-negative, 2) originally had one of the following symptoms, and 3) reports that the symptom has not resolved or has worsened should also be referred to care:
    1. Productive cough
    2. Persistent fever
    3. Night sweats
    4. Weight loss >1.5kg/month
    5. Haemoptysis

For those who need referral

a. The Research Nurse should make the referral in person.
b. The Research Nurse should explain to participant and/or parent/guardian/caregiver that even though they have one negative Xpert® MTB/RIF result, they still might have active TB, especially because their symptoms are persistent.
c. The Research Nurse should answer, as far as possible, any questions the participant and/or parent/guardian/caregiver has about the process and should try, in particular, to address any anxieties about attending clinics (provide reassurance and reiterate that they are free to contact you whenever necessary).
d. The Research Nurse should ask which clinic (of the 11 PIPSA clinics) they would prefer to attend and agree on a target date (within 2 weeks) for attending clinic.
e. The Research Nurse should complete the referral letter to the preferred clinic (see Figure 2), and provide contact details (work cell number).
f. The Research Nurse should explain that he/she will contact them if they have not attended the clinic by the agreed target date.
g. The Research Nurse should enter the referral details (date, clinic, target date, contact details, etc.) into the relevant section in REDCap.

Documents that the participant and/or parent/guardian/caregiver should be provided with:

i. Referral letter to preferred clinic
ii. A copy of the negative sputum Xpert® MTB/RIF result

4.4.6. Code B3: Positive Sputum Xpert® MTB/RIF

a. Participants with symptoms suggestive of active TB should have sputum collected at the enrolment visit (see SOP ATB08); sputum samples will be sent to an NHLS laboratory for testing with Xpert® MTB/RIF.
b. A positive Xpert® MTB/RIF result should be fed back to the participant in person (see SOP ATB09).
c. During the visit the Research Nurse should explain to the participant and/or parent/guardian/caregiver that the participant's sputum has tested positive for TB.
d. The Research Nurse should explain that this means the participant has active TB disease and requires urgent treatment at a health facility.
e. The Research Nurse should answer, as far as possible, any questions the participant and/or parent/guardian/caregiver has about the process and try, in particular, to address any anxieties about attending clinics (provide reassurance and reiterate that they are free to contact you whenever necessary).
f. The Research Nurse should ask which clinic (of the 11 PIPSA clinics) they would prefer to attend.
g. The Research Nurse should complete the referral letter to the preferred clinic (see Figure 2 and provide contact details (work cell number).
h. The Research Nurse should ask the participant to attend clinic the **within 3 days**, at the latest. The Research Nurse should explain that most clinics will only start TB treatment on Mondays-Fridays.
i. The Research Nurse should recommend that other members of the household (other children, in particular) also attend the clinic, as they are also at high risk of developing TB disease.
j. The Research Nurse should explain that he/she will contact them again after 3 working days to check if they have started TB treatment, and that if they have not attended the clinic, he/she will need to notify the clinic.
k. The Research Nurse should enter the referral details (date, clinic, target date, contact details, etc.) into the relevant section in REDCap.

Documents that the participant +/− parent/guardian/caregiver should be provided with:

i. Referral letter to preferred clinic.
ii. A copy of the positive sputum Xpert® MTB/RIF result.

4.4.7. Code B4: Sputum culture positive for MTB after previous negative sputum Xpert® MTB/RIF, or Xpert® MTB/RIF results not available

a. Participants who are able to produce enough sputum will also have a sample sent to the AHRI Durban lab for MGIT TB culture (SOP ATB08).
b. Culture results will be available after approximately six weeks of the sputum being sent to the lab, but action is only required in certain circumstances.

Who to refer

a. Refer only participants who:
    1. Are sputum culture positive for MTB; **AND**
    2. Are sputum Xpert® MTB/RIF was negative/indeterminate/not done; **AND**

3. Report that they have not been started on TB treatment.

b. Participants who are sputum culture positive for MTB who previously had a positive Xpert® MTB/RIF do not need this referral. Ideally, these should have been referred once the Xpert® MTB/RIF positive result is available. Confirm there is a record of the referral following positive Xpert® MTB/RIF result.

c. Participants who are sputum culture positive for MTB and a sputum Xpert® MTB/RIF negative who have since started TB treatment do not need referral.

d. Participants who are sputum culture negative do not need referral.

For those who need referral

a. The referral should be made in person at the participant's home.

b. During the visit, the Research Nurse should explain to the participant and/or parent/guardian/caregiver that the participant's sputum has tested positive for TB.

c. The Research Nurse should explain further that this means the participant has active TB disease and that they require urgent treatment at a health facility.

d. The Research Nurse should answer, as far as possible, any questions the participant and /or parent/guardian/caregiver has about the process and try, in particular, to address any anxieties about attending clinics (provide reassurance and reiterate that they are free to contact you whenever necessary).

e. The Research Nurse should ask which clinic (of the 11 PIPSA clinics) they would prefer to attend.

f. The Research Nurse should complete the referral letter to the preferred clinic (see Figure 2) and provide contact details (work cell number).

g. The Research Nurse should ask the participant to attend the clinic **within 3 days**, at the latest. The Research Nurse should explain that most clinics will only start TB treatment on Mondays-Fridays.

h. The Research Nurse should recommend that other members of the household (other children, in particular) also attend the clinic, as they are also at high risk of developing TB disease.

i. The Research Nurse should explain that he/she will contact them by phone after 3 working days to check if they have started treatment, and that if they have not attended the clinic, he/she will need to notify the clinic.

j. The Research Nurse should enter the referral details (date, clinic, target date, contact details, etc.) into the relevant section in REDCap.

Documents that the participant +/− parent/guardian/caregiver should be provided with:

i. Referral letter to preferred clinic.

ii. A copy of the negative/indeterminate sputum Xpert® MTB/RIF result, if available.

iii. A copy of the positive sputum culture result.

4.4.8. Code C1: New or Previously known HIV-Positive with Positive IGRA (QFT-Plus) Result

a. Only participants who know themselves to be HIV-positive need to be referred to clinics if they have a positive IGRA test result.

b. A positive IGRA in an HIV-positive person suggests that they are at high risk of developing active TB disease; these individuals should be screened for active TB, and if active TB is ruled out, prioritised for Isoniazid Preventive Therapy (IPT), as long as this is not contraindicated.

c. IGRA results should be available from the lab after approximately 4 weeks of the blood being sent for testing.

d. The Research Nurse should aim to deliver the result (when required) in person within 4 weeks of receiving the result from the lab.

Who to refer

a. Only refer participants who:
   1. Know themselves to be HIV-positive (i.e., newly diagnosed, self-report being HIV positive, or self-report being on ART for HIV treatment); **AND**
   2. Have a positive IGRA result; **AND**
   3. Are not receiving treatment for TB disease; **AND**
   4. Report that they are not already on IPT.

b. HIV-positive participants with a positive IGRA who do not know themselves to be HIV-positive (or do not have a parent/guardian/caregiver who knows they are HIV-positive), i.e., individuals with a positive HIV ELISA that was requested as part of anonymous testing, do not require referral.

c. For HIV-positive participants whose parent/guardian/caregiver knows the participant's status but has not disclosed it to them, the referral should be made through the parent/guardian/caregiver.

d. The Data Manager will generate a list of participants who are HIV positive, know their status (or parent/guardian/caregiver knows their status), and whose IGRA results are positive. Before making the referral, the Research Nurse will ask the participant if he/she is receiving TB treatment or is on IPT.

For those that require referral

a. The Research Nurse should explain to the participant and/or parent/guardian/caregiver that the test for latent TB infection (not TB disease) is positive, and, because the participant is HIV-positive, this means that they are at higher risk of developing TB disease.

b. The Research Nurse should explain that the South African Department of Health recommends that HIV-positive people should be screened for active TB, and if active TB is ruled out, receive IPT to reduce their chances of developing TB disease.

h. The Research Nurse should further explain that he/she needs to refer them to a clinic where a nurse will decide if they need to receive IPT or not.

i. The Research Nurse should answer, as far as possible, any questions the participant and/or parent/guardian/caregiver has about the process and try, in particular, to address any anxieties about attending clinics (provide reassurance and reiterate that they are free to contact you whenever necessary).

c. The Research Nurse should ask which clinic (of the 11 PIPSA clinics) they would prefer to attend.

d. The Research Nurse should complete the referral letter to the preferred clinic (see Figure 2) and provide contact details (work cell number).

e. The Research Nurse should explain that he/she will contact them if they have not attended the clinic by the agreed target date (within 2 weeks).

f. The Research Nurse should enter the referral details (date, clinic, target date, contact details, etc.) into the relevant section in REDCap.

Documents that the participant and/or parent/guardian/caregiver should be provided with:

i. Referral letter to preferred clinic.

ii. A copy of the positive IGRA result.

iii. IGRA information card.

4.4.9. Other reasons for referral

a. Participants may need referral to care for a range of other reasons apart from those specified above.
b. The Research Nurse should write the reasons for referral on the referral letter.
c. The Research Nurse should ensure that the reason(s) for referral and contact details on the referral letter are clear and correct, as the AHRI nurse in the PIP clinic (or anyone attending to the participant at the clinic) may need to ask for additional information.

4.5. Linkage to care

4.5.1. Overview

A standard procedure should be followed to support linkage to care, regardless of reason for referral. At the time of making the referral, the following should be agreed with the participant and/or parent/guardian/caregiver and recorded in REDCap:

a. Preferred clinic within PIPSA (due to AHRI nurse presence).
b. Target date for attending clinic.
c. Carefully checked phone number, with second option number if available, for contacting the participant and/or parent/guardian/caregiver.
d. For HIV referrals of participants aged 15 years and above, the reminder SMS message option to be used if participant and/or parent/guardian/caregiver has not attended clinic by the target date. The participant and/or parent/guardian/caregiver should further be informed that if they do not link into care within 30 days after the date of the referral, they will be contacted by phone.
e. For TB referrals (including positive IGRA result), the participant and/or parent/guardian/caregiver should be informed that they will be contacted by phone if they have not attended the clinic by the agreed target date.

4.5.2. Linkage for HIV referrals for participants aged 15+ years

a. For participants 15 years or older, tracking of linkage for HIV referrals will be managed within the existing ClinicLink system.
b. If the participant is not linked into care 14 days after the date of referral, a gentle text reminder will be sent using the message option which the participant/parent had chosen.
c. If the participant has still not linked into care 30 days from the date of referral, they will be contacted by phone.
d. Three phone call attempts should be made until they are contacted:
    i. The first attempt should be made anytime during working hours in the week.
    ii. The second attempt should be made after working hours during the week.
    iii. The third attempt should be anytime during the weekend.
e. The Research Nurse will record the date of each phone call attempt, and the final outcome in the relevant section in REDCap.
f. If the participant has not attended the clinic after a further 2 weeks (6 weeks from referral), or cannot be contacted, the Research Nurse will inform the Study Manager; the Study Manager will discuss each case with an appropriate AHRI clinician and agree on any action to be taken.

4.5.3. Linkage for HIV referral for participant aged <15 years

a. For participants <15 years, tracking of linkage for HIV referrals will be managed within REDCap by the Research Nurse.

b. On a weekly basis, the AHRI Paediatric Nurse will give a list of participants who have attended the clinic, and the date of visit, to the Study Doctor. The Study Doctor will give this information to the relevant Research Nurse, who will record it in REDCap.

c. Although every effort will be made to refer HIV positive participants aged <15 years to the AHRI Paediatric Nurse, some participants may elect to attend another clinic in PIP. In these cases, ClinicLink will be used to track whether the participant has linked to care.

d. The designated Data Manager will generate a list of participants who have not attended the clinic by the agreed target date. This list will be produced every 2-3 days and given to the Study Doctor, who will inform the relevant Research Nurse.

e. The Research Nurse will telephone the participant; up to four contact attempts will be made.

f. The Research Nurse will record the date of each contact attempt, and the outcome, in REDCap.

g. If the participant has still not attended the clinic after a further 4 weeks (6 weeks from referral), or cannot be contacted, the Research Nurse will inform the Study Manager. The Study Manager will discuss each case with an appropriate AHRI clinician and agree on any action to be taken.

4.5.4. Linkage for TB referrals

a. Tracking of linkage for TB referrals will be managed within REDCap by the Research Nurses.

b. The Data Manager will generate a list of participants who have not attended the clinic by the agreed target date; this list will be produced every 2-3 days and given to the Study Doctor, who will inform the relevant Research Nurse.

c. For participants with positive Xpert/sputum culture results, if the participant has not attended the clinic within 3 working days, the Research Nurse will telephone the participant, and will notify the AHRI nurse at the clinic to which the participant was referred. Up to four phone call attempts will be made. If the participant still has not attended the clinic within 2 weeks of referral, or cannot be contacted, the Research Nurse will inform the Study Manager. The result should be handed over to DoH TB Nurse at the participant's local clinic.

d. Participants with symptoms suggestive of active TB, whose Xpert results were negative or who could not produce a sputum sample, will be contacted by telephone if they have not attended the clinic within 2 weeks. Up to four phone call attempts will be made. The Research Nurse will ask the participant if he/she still has symptoms; this information will be recorded in REDCap. If the participant's symptoms have resolved, no further action will be taken. If the participant still has symptoms, the Research Nurse will encourage them again to go to the clinic for further testing. If the participant has not attended the clinic after a further 2 weeks (4 weeks from referral), or cannot be contacted, the Research Nurse will inform the Study Manager.

e. HIV positive participants with a positive IGRA result will be contacted by telephone if they have not attended the clinic within 2 weeks. Up to four phone call attempts will be made. If the participant has not attended the clinic after a further 4 weeks (6 weeks from referral), or cannot be contacted, the Research Nurse will inform the Study Manager.

f. The Research Nurse will record the date of each phone call attempt, and the final outcome in REDCap.

g. For participants who cannot be contacted or have not linked to care within the allocated time frame, the Study Manager will discuss each case with an appropriate AHRI clinician and agree on any action to be taken.

5. Figures

Figure 1. Overview of study processes

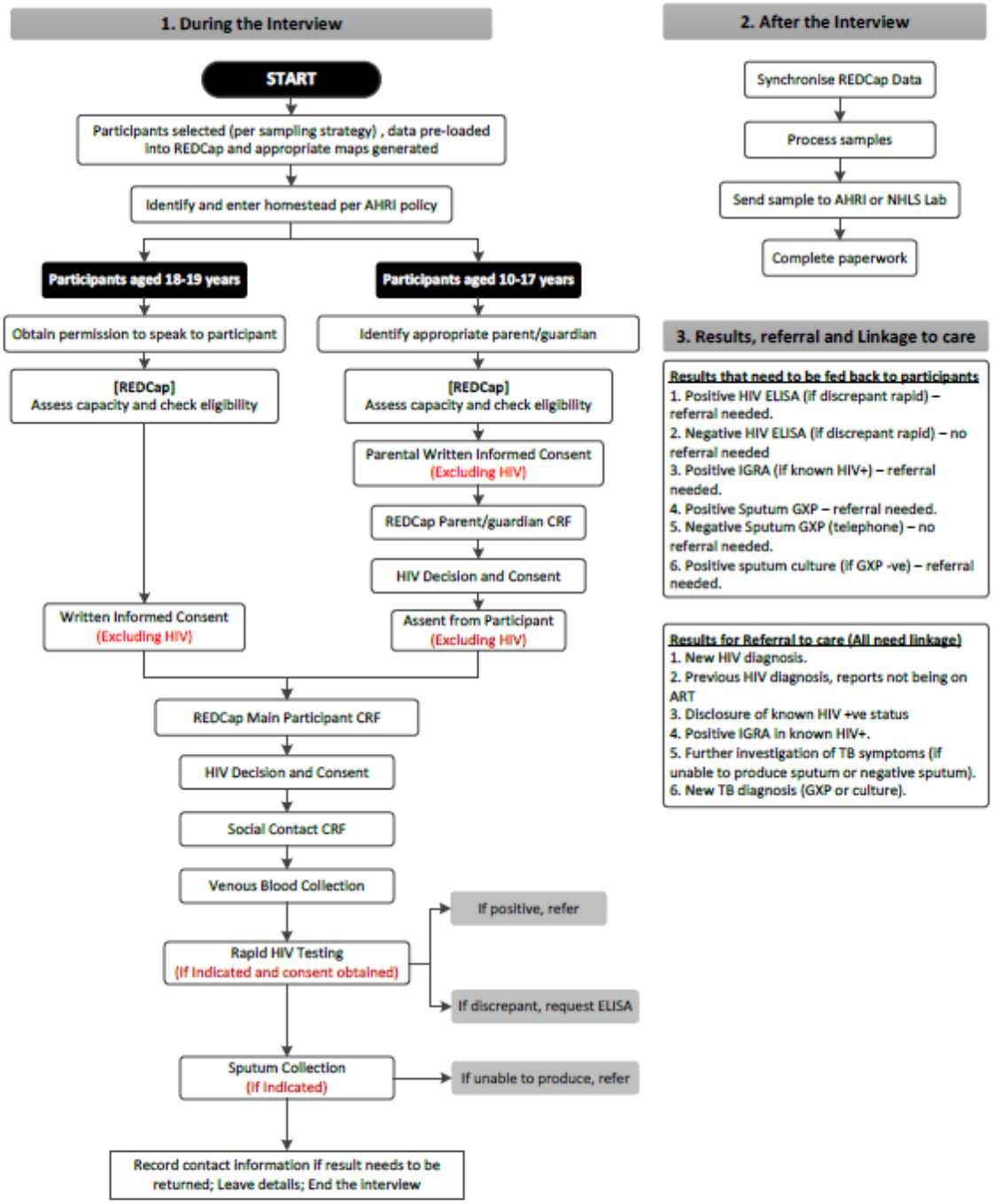

Figure 2. Sample referral form from Research Nurse to AHRI nurse at DoH clinic

HAYA TB Study

REFERRAL TO PRIMARY CARE: GROUP B1

To

DSID: ABCDE-F

The AHRI PIP Nurse,

SOMKHELE Clinic

DATE: 09 APRIL 2018

Dear Colleague,

Re: MR AB SOMEONE DATE OF BIRTH: 15 JUNE 2001

The individual listed above has been referred to your facility by the HAYA 'Incidence of TB infection in Adolescents' study team.

This is a GROUP B1 referral: SYMPTOMS OF TB BUT UNABLE TO PRODUCE SPUTUM

Please log this visit and review appropriately.

If you have any questions or require any further information, please call the research nurse, SR A. NURSE on 0712-345-6789 or the Study Manager on 0698-765-4321

Thank you for your help.

The HAYA TB Study Team

Appendix 9: Study questionnaire for participants (10-19 years)

Contact Attempts

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Contact Attempts - Memory Aid * Introduce AHRI * I am a nurse (AHRI uniform and badges) * Structure and time outline * Find private space to speak with the participant		
	Section taken from PIP *[Up to 6 attempts will be made. At 4th attempt, provide information to study coordinator to evaluate whether further attempts are necessary.]*		

Personal Information

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	<u>Personal Information Memory Aid</u> ** If participant is below 18 years and parent/guardian has already been interviewed, skip to Q10* ** if participant is 18 years and above start with Q1.1*		
1.1 **Pre-loaded**	Participant's (child's) name:	[Text]	**c_parti_name** [Text, 50 characters]
2.1 **Interview**	*Is the participant's pre-loaded name correct?*	**Radio** 0 No 1 Yes *If Yes, Skip to Q3.1* *Do not show if Q1.1 is YES*	**c_partnam_corr** [integer; 1 digit; range 0–1]
2.1.1 **Interview**	If Not correct, please provide the correct participant's name:	[Text] (First Name)	**c_parti_corfname** [Text, 50 characters]
		[Text] (Last Name)	**c_parti_corlname** [Text, 50 characters]
3.1 **Preloaded**	Participant's sex	**Radio** 1 Male 2 Female	**c_sex** [integer; 1 digit; range 1,2]
4.1 **Pre-loaded**	Participant's date of birth	[Date]	**c_dobd** [standard AHRI date variable; range 1999/01/01-2009/12/31]
4.1.1	Age	Number	**c_age**

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
Pre-loaded			[integer; 2 digit; range 10-19]
5.1 **Interview**	*Is the participant's pre-printed date of birth correct?*	**Radio** 0 No 1 Yes *(If Yes, Skip to Q6.1)*	**c_d_prep_dob** [integer; 1 digit; range 0–1]
5.1.1 Interview	If not correct, please provide the correct date of birth If exact date of birth is not known, please request to see a ID or birth certificate and check the date of birth from this document. If document is not available and only the year of birth is known, enter 15 as the day of birth and June as the month of birth.	**[date]**	**c_correct_dob**
	Age Eligibility Memory Aid ** Participants are eligible if date of birth if after 1 May 1999 and before 30 April 2008*		
calculated	*Participant's correct age on day of interview*	Number	**c_age_correct**
5.1.2 **Interview**	*Basing on the corrected date of birth, is the participant still eligible?*	**Radio** 0 No 1 Yes *(If Yes, Go to Q6.1)*	**c_age_eligible** [integer; 1 digit; range 0–1]
	If No, Pop Up Message: ***Participant if not eligible, please go to the End of Survey Instrument and terminate the interview***		
6.1 **Pre-loaded**	BSID	Number	**c_resp_bsid** [integer; 7 digits; range 0000000-9999999]
7.1 **Pre-loaded**	BS Owner	Text	**c_bs_owner** [text; 50 characters]
8.1 **Pre-loaded**	Location/Isigodi	Text	**c_location_isigodi** [text; 50 characters]
9.1 **Pre-loaded**	Household Head	Text	**c_hh_head** [text; 50 characters]
10.1 **Interview**	**Interview Details**		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
10.1.1 **Interview**	Interviewer code	**Drop down** 1 Staffcode1 2 Staffcode2 3 Staffcode3 4 Staffcode4	**c_intv_code** [integer; 1 digit; range 1,2]
10.2 **Interview**	Start time of interview	[Time]	**c_resp_dob** [standard AHRI time variable; 00.00-23:59]
10.3 **Interview**	Date of Interview	[Date]	**c_date_of_interview** [standard AHRI date variable; range 2017/11/01-2019/12/31]

Assessment of Capacity to Consent

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Section taken from PIP		

Eligibility Checklist

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Eligibility Checklist (Previous/current TB treatment) *Before giving detailed information about the study, perform the following eligibility checklist for the study participant*		
1.1 **Interview**	Have you ever received treatment for TB disease? **NB**: *Participant may confuse IPT from TB treatment. Make sure this is clear.* *(If needed, explain that treatment for **TB disease** will have been with at least four drugs [though it may have been in one tablet, e.g., Rifafour] every day for two months, and then two drugs [again, may have been in one tablet, e.g., Rifinah] every day for a further four months [i.e., a total of at least 6 months].* *This is different from **isoniazid preventive therapy** [IPT], which is used to treat **TB infection**, and will have involved taking one drug every day for six months.)*	[Dropdown] 0 No 1 Yes 9 Don't know	**c_tbtx_ever** [integer; 1 digit; range 0–1, 9]
if No or Don't Know, Pop Up Message:			

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Please provide detailed information about the study to the participant and obtain informed consent		
1.1.1 Interview	If yes, when did you start TB treatment? (*If more than once, get details for the most recent episode*)	[Dropdown] 99 = Don't know [Dropdown]	**c_tbtx_dstartm** [integer; 2 digits; range 1–12, 99] **c_tbtx_dstarty** [integer; 4 digits; range 1998–2017]
1.1.2 Interview	Where (at which health facility) did you start TB treatment?	[Dropdown] [List of hospitals and clinics in PIP] 96 = Other (specify) 99 = Don't know	**c_tbtx_clin** [integer; 2 digits; range 01–XX, 96, 99]
1.1.2.1 Interview	Other, specify **[*only appear if 96*]**	*[Text]*	**c_tbtx_clin_o** [string, 30 characters]
	If Q1.1 is Yes, Pop Up Message: ***Participant is not eligible please go to the End of Survey Instrument and terminate the interview***		

Informed Consent Form

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	<u>Informed consent – Memory aid</u> * Check if participant can read and write and can read information sheet on their own. * If the participant is not able to read or write, ask for a witness within the homestead to witness the process of giving information and obtaining consent.		
	Informed Consent Information sheet		
1.1 **Interview**	*Name of interviewer*	*Text*	**p_staff_name** [Text; 50 Characters]
1.2 **Interview**	*Signature*	Picture	**p_staff_signature** [format: .png]
1.3 **Interview**	*Date*	Date and time	**p_staff_date** [standard AHRI date variable; range 2017/11/01-2019/12/31, 00.00-23.59]
1.4 **Interview**	*Did the participant consent to participate in the study?*	[Radio] 0 No 1 Yes	**p_consent** [integer; 1 digit; range 0–1]
	If No, Pop Up Message ***Parent/guardian refused participation, please go to the End of Survey Instrument and conclude the interview.***		
1.5 **Interview**	*Name of respondent*	*Text*	**p_pg_name** [Text; 50 Characters]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
1.6 **Interview**	*Signature*	Picture	**p_pg_signature** [format: .png]
1.7 **Interview**	*Date*	Date and time	**p_pg_date** [standard AHRI date variable; range 2017/11/01-2019/12/31, 00.00-23.59]
1.8 **Interview**	*Is the participant able to sign?*	[Radio] 0 No 1 Yes *(If Yes, skip to section 6)*	**p_sign** [integer; 1 digit; range 0–1]
1.8.1 Interview	*Name of witness*	*Text*	**p_witness_name** [Text; 50 Characters]
1.8.2 Interview	*Signature*	Picture	**p_witness_signature** [format: .png]
1.8.3 Interview	*Date*	Date and time	**p_witness_date** [standard AHRI date variable; range 2017/11/01-2019/12/31, 00.00-23.59]

Adolescent TB Study (Study specific questions)

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
Question 6	**Education**		
6.1 Interview	Have you ever been to school?	[Dropdown] 0 No, never been to school 1 Yes, previously 2 Yes, currently *(If No, skip to Q7)*	**c_edu** [integer; 1 digit; range 0–2]
6.1.1 Interview	What is the highest grade of school that you have reached?	[Number] 96 = Other, specify	**c_edu_grade** [integer; 2 digits; range 01–12, 96]
6.1.1.1 Interview	Other grade if not on the list **[*only appear if 96*]**	[*Text*]	**c_edu_grade_o** [Text, 30 characters]
6.1.2 Interview	Which school do you currently go to? (*Most recent school if not going to school now*)	[Dropdown] [List of schools] 1 2 3 …	**c_edu_school** [integer; 2 digits; range 01–XX, 96]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
		96 Other, specify	
6.1.2.1	Other, specify **[*only appear if 96*]**	[Text]	**c_edu_school_o** [string, 30 characters]
Question 7	**BCG Vaccination**		
7.1 Interview/ [Parent CRF]	Have you ever been vaccinated against TB? (*If the participant is unsure, explain that this would have been an injection in one of the arms (upper side) at birth [before the mother was discharged from hospital] or within the first year of life. The site of the injection may have become very inflamed (a swollen and painful blister), and may have developed into an opening in the skin with pus. This will then have left a scar on the arm.)*	[Radio] 0 No 1 Yes 9 Don't know	**c_bcg_vac** [integer; 1 digit; range 0–1, 9]
7.2 Interview/ [Parent CRF]	Is your road to health card available? (*Ask participant if RTHC card is available and if you can have a look*)	[Radio] 0 No 1 Yes *(if No, skip to Q7.3)*	**c_rthc** [integer; 1 digit; range 0–1]
7.2.1 Interview/ [Parent CRF]	*Is BCG vaccination* documented?	[Radio] 0 No 1 Yes	**c_bcg_doc** [integer; 1 digit; range 0–1]
	***Ask participant if they have a visible scar on the right or left upper arm, obtain permission to examine the scar*:**		
7.3 Interview	May I examine your arm for a TB vaccination scar?	[Radio] 0 No 1 Yes *(If No, skip to Q8)*	**c_bcg_check** [integer; 1 digit; range 0–1]
7.3.1 Interview	*Is BCG scar present?*	[Dropdown] 0 No 1 Yes 2 Doubtful	**c_bcgscar** [integer; 1 digit; range 0–2]
Question 8	**TB Symptoms**		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Tell participant: *I will now ask some questions about symptoms of TB. If I think it might be needed, I might ask you to give me sputum samples, at the end of the interview, which will then be tested for TB at the clinic*.		
8.1 Interview	Do you currently have a cough?	[Radio] 0 No 1 Yes (*If No, skip to Q8.2*)	**c_cough** [integer; 1 digit; range 0–1]
8.1.1 Interview	If yes, how long have had the cough?	[Number] (weeks) *Enter 1 if less than one week* 99 = Don't know	**c_cough_dur** [integer; 2 digits; range 1–52, 99]
8.2 Interview	Do you have fever?	[Radio] 0 No 1 Yes (*If No, skip to Q8.3*)	**c_fever** [integer; 1 digit; range 0–1]
8.2.1 Interview	If yes, how long have you had fever?	[Number] (weeks) *Enter 1 if less than one week* 99 = Don't know	**c_fever_dur** [integer; 2 digits; range 01–52, 99]
8.3 Interview	Do you have drenching night sweats? (*Sweating at night that soaks your clothes or beddings*)	[Radio] 0 No 1 Yes (*If No, skip to Q8.4)*	**c_nsweat** [integer; 1 digit; range 0–1]
8.3.1 Interview	If yes, how long have you had drenching night sweats?	[Number] (weeks) *Enter 1 if less than one week* 99 = Don't know	**c_nsweat_dur** [integer; 2 digits; range 01–52, 99]
8.4 Interview	How much do you weigh?	[Number] (kilograms) 999 = Don't know	**c_wt** [integer; 3 digits; range 015–120, 999]
8.5 Interview	Have you lost weight in the last 6 months? (*Explain to the participant that this is unexplained or unintentional weight loss*)	[Dropdown] 0 No 1 Yes 9 Don't know (*If No or Don't know, skip to Q8.6)*	**c_wtloss_6m** [integer; 1 digit; range 0–1, 9]
8.5.1 Interview	If yes, how much weight have you lost in the last 6 months?	[Number] (kilograms) 99 = Don't know	**c_wtloss_kg** [integer; 2 digits; range 01–52, 99]
8.6 Interview	Do you have any other symptoms apart from those already mentioned?	[Radio] 0 No 1 Yes	**c_tbsx_oth** [integer; 1 digit; range 0–1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	(*Prompt for TB specific symptoms [fatigue, haemoptysis, chest pain, and lymphadenitis]*)	(*If No, skip to Q9)*	
	[Add sub-form for each symptom for questions 8.6.1 to 8.6.1.2]		
8.6.1 Interview	If yes, which symptom	[Dropdown] 1 Fatigue 2 Haemoptysis 3 Chest pain 4 Lymphadenitis 6 Other, specify	**c_tbsx_oth1** [integer; 1 digit; range 1–4, 6]
8.6.1.1 Interview	Other, specify **[*only appear if 6*]**	[Text]	**c_tbsx_oth1_o** [string, 30 characters]
8.6.1.1.1 Interview	Duration of symptom	[Number] (weeks) *Enter 1 if less than one week* 99 = Don't know	**c_tbsx_oth1_dur** [integer; 2 digits; range 01–52, 99]
8.6.1.2 interview	Do you have another symptom?	[Radio] 0 No 1 Yes ***[If Yes, add sub-form for another symptom (Qs 8.6.1–8.6.1.2)]***	**c_tbsx_oth1** [integer; 1 digit; range 0–1]
Question 9	**TB contacts: Household**		
9.1 Interview	Has anyone in your homestead (or residential plot), during your lifetime, ever had TB disease? *(Including those who might not have started TB treatment but were told by a clinician that they had TB disease*)	[Dropdown] 0 No 1 Yes 9 Don't know (*If No, or Don't know skip to Q9.2*)	**c_hh_tbever** [integer; 1 digit; range 0–1, 9]
9.1.2 Interview	Is anybody in your homestead (or residential plot) **currently** on TB treatment?	[Dropdown] 0 No 1 Yes 9 Don't know	**c_hh_tbcur** [integer; 1 digit; range 0–1, 9]
	TB contacts memory aid *Please tell the participant that you would like to ask them a few questions about the people in their homestead who have ever had TB in their lifetime. Tell them that you will ask about each person separately and encourage them to think back and remember how many individuals in their homestead have ever had TB*		
	Details of person(s)		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
9.1.3.1 Interview	What was the age of this person at the time you lived in the same homestead (or residential plot)?	[Number] (years) 999 = Don't know	**c_curhh_tbcontact1age** [integer; 3 digits; range 001–099, 999]
9.1.3.2 Interview	What is the sex of the person?	[Radio] 1 Male 2 Female	**c_curhh_tbcontact1sex** [integer; 1 digit; range 1–2]
9.1.3.3 Interview	In which isigodi did you live with this person?	[Dropdown] [List of isigodis] 96 = Other, specify 99 = Don't know	**c_curhh_tbcontact1isi** [integer; 2 digits; range 01–XX, 96, 99]
9.1.3.3.1 Interview	Other, specify **[*only appear if 96*]**	[Text]	**c_curhh_tbcontact1isi_o** [string, 30 characters]
9.1.3.4 Interview	In which year did you live with this person?	[Dropdown] 9999= don't know	**c_curhh_tbcontact1yr** [standard AHRI year variable; range 1998–2017]
9.1.3.5 Interview	For how long did you live in the same homestead (or residential plot) with this person when they had TB?	[Number] (Months) (*enter 01 if less than one month*) 99 = Don't know	**c_curhh_tbcontact1dur** [integer; 2 digits; range 01–24, 99]
9.1.3.6 Interview	Did you live in the same house as this person when they were ill?	[Radio] 0 No 1 Yes (If No, skip to 9.1.3.7)	**c_curhh_tbcontact1house** [integer; 1 digit; range 0–1]
9.1.3.6.1 Interview	Did you sleep in the same room as this person?	[Radio] 0 No 1 Yes	**c_curhh_tbcontact1rshare** [integer; 1 digit; range 0–1]
9.1.3.7 Interview	Were you involved in the care of this person when they were ill? *(i.e., you came into close contact with this person whilst caring for them)*	[Radio] 0 No 1 Yes	**C_curhh_tbcontact1care** [integer; 1 digit; range 0–1]
9.1.3.8 Interview	Apart from the person(s) described above, has anyone else in your homestead ever had TB disease during your lifetime? *Encourage the participant to think back about whether there have been any others*	[Radio] 0 No 1 Yes If yes, add sub-form (Qs 9.1.3.1 to 9.1.3.8)	**c_curhh_tbcontact_o1** [integer; 1 digit; range 0-1]
	TB Contacts in other households		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
9.2 Interview	Apart from your homestead, have you ever lived (slept overnight) in the same homestead (or residential plot) as someone with TB for a total of 2 or more weeks? *(Including those who might not have started TB treatment but were told by a clinician that they had TB disease*)	[Dropdown] 0 No 1 Yes 9 Don't know (*If No, skip to Q9.4)*	**c_hh_tbcontact** [integer; 1 digit; range 0–1, 9]
	Details of person(s)		
9.2.1.1 Interview	What was the age of this person at the time you lived in the same homestead (or residential plot)?	[Number] (years) 999 = Don't know	**c_hh_tbcontact1age** [integer; 3 digits; range 001–099, 999]
9.2.1.2 Interview	What is the sex of the person?	[Radio] 1 Male 2 Female	**c_hh_tbcontact1sex** [integer; 1 digit; range 1–2]
9.2.1.3 Interview	In which isigodi did this person live?	[Dropdown] [List of isigodis] 96 = Other, specify 99 = Don't know	**c_hh_tbcontact1isi** [integer; 2 digits; range 01–XX, 96, 99]
9.2.1.3.1 Interview	Other, specify **[*only appear if 96*]**	[Text]	**c_hh_tbcontact1isi_o** [string, 30 characters]
9.2.1.4 Interview	In which year did you live with this person?	[Dropdown] 9999= don't know	**c_hh_tbcontact1yr** [standard AHRI year variable; range 1998–2017]
9.2.1.5 Interview	For how long did you live in the same homestead (or residential plot) with this person when they had TB?	[Number] (Months) (*enter 01 if less than one month*) 99 = Don't know	**c_hh_tbcontact1dur** [integer; 2 digits; range 01–24, 99]
9.2.1.6 Interview	Did you live in the same house as this person when they were ill?	[Radio] 0 No 1 Yes (If No, skip to 9.2.1.7)	**c_hh_tbcontact1house** [integer; 1 digit; range 0–1]
9.2.1.6.1 Interview	Did you sleep in the same room as this person?	[Radio] 0 No 1 Yes	**c_hh_tbcontact1rshare** [integer; 1 digit; range 0–1]
9.2.1.7 Interview	Were you involved in the care of this person when they were ill? *(i.e., you came into close contact with this person whilst caring for them)*	[Radio] 0 No 1 Yes	**C_hh_tbcontact1care** [integer; 1 digit; range 0–1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
9.2.1.8	Apart from the person(s) described above, have you ever lived (slept overnight) in the same homestead with anyone else with TB? *Encourage the participant to think back about whether there have been any others*	[Radio] 0 No 1 Yes If yes, add sub-form (Qs 9.2.1.1 to 9.2.1.8)	
	TB Contacts: Care of TB Patients		
9.3 Interview	Apart from people with TB who you lived with in the same homestead (the ones described above), have you ever been involved in the care of another person(s) with TB disease? *(i.e., you came into contact with this person whilst caring for them when they were ill)*	[Radio] 0 No 1 Yes *(if No, skip to Q10)*	**c_tb_care** [integer; 1 digit; range 0–1]
	Details of person(s)		
9.3.1.1 Interview	What was the age of this person at the time you were involved in their care?	[Number] 999 = Don't know	**c_tb_care1age** [integer; 3 digits; range 001–099, 999]
9.3.1.2 Interview	What is the sex of this person?	[Radio] 1 Male 2 Female	**c_tb_care1sex** [integer; 1 digit; range 1,2]
9.3.1.3 Interview	In which isigodi did this person live at the time you were involved in their care?	[Dropdown] [List of isigodis] 96 = Other, specify 99 = Don't know	**c_tb_care1isi** [integer; 2 digits; range 01–XX, 96, 99]
9.3.1.3.1 Interview	Other, specify **[*only appear if 96*]**	[Text]	**c_tb_care1isi_o** [string, 30 characters]
9.3.1.4 Interview	In which year were you involved in the care of this person?	[Dropdown] 9999=don't know	**c_tb_care1yr** [standard AHRI year variable; range 1998–2017]
9.3.1.5	Is there another person with TB who did NOT live in the same homestead, but in whose care you was involved when this person was ill? *Encourage the participant to think back about whether there have been any others*	[Radio] 0 No 1 Yes *[If Yes, add sub-form (Qs 9.3.1.1–9.3.1.5)]*	
Question 10	**Health care utilisation**		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
10.1 Interview	Have you ever been admitted to a hospital? *(Explain, that 'admitted' means sleeping for at least one night in a hospital)*	[Dropdown] 0 No 1 Yes 9 Don't know (*If No, or don't know skip to Q10.2*)	**c_adm_ever** [integer; 1 digit; range 0–1, 9]
10.1.1 Interview	If yes, how many times have you ever been admitted (slept overnight) to hospital?	[Number] 99 - don't know	**c_adm_n** [integer; 1 digit; range 1–9]
10.1.2 Interview	When were you most recently admitted to hospital?	[Dropdown] 99 = Don't know	**c_adm_dadmm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**c_adm_dadmy** [standard AHRI year variable; range 1998–2017]
10.1.3 Interview	Which hospital were you most recently admitted to?	[Dropdown] [List of hospitals] 34 = Private GP 96 = Other, specify 99 = Don't know	**c_adm_hosp** [integer; 2 digits; range 1–XX, 96, 99]
10.1.3.1 Interview	Other, specify **[*only appear if 96*]**	[Text]	**c_adm_hosp_o** [string, 30 characters]
10.1.4 Interview	What was the reason for your most recent admission?	[Dropdown] 01 Respiratory tract infection 02 Diarrhoea 03 Injury 04 Convulsions (fits) 05 Other infection 06 Surgery 96 Other, specify 99 Don't know *(If 96 go to 10.1.4.1, otherwise skip to Q10.2)*	**c_adm_reas** [integer; 1 digit; range 1–4, 6, 9]
10.1.4.1 Interview	Other, specify **[*only appear if 6*]**	[Text]	**c_adm_reas_o** [string, 50 characters]
	Visits to Hospitals		
10.2 **Interview**	Excluding overnight admission, did you visit any hospital in the last 12 months **for any reason** *(including accompanying someone else)*?	[Radio] 0 No 1 Yes	**c_hospvis_12m** [integer; 1 digit; range 0–1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	(*Clarify hospital, as participant may confuse with other health facilities*)	*(If No, skip to Q10.3)*	
10.2.1 Interview	If yes, how many times did you visit any hospital in the last 12 months?	[Number] 99 = Don't know	**c_hospvis_12m_n** [integer; 2 digits; range 01–20, 99]
10.2.2 Interview	When was your most recent visit to any hospital?	[Dropdown] 99 = Don't know	**c_hospvis_dvism** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**c_hospvis_dvisy** [standard AHRI year variable; range 1998–2017]
10.2.3 Interview	Which hospital did you visit most recently?	[Dropdown][List of hospitals] 34 = Private GP 96 = Other, specify	**c_hospvis_hosp** [integer; 2 digits; range 01–XX, 96]
	Other hospital if not on the list **[*only appear if 96*]**	[Text]	**c_hospvis_hosp_o** [text, 30 characters]
10.2.4.1 Interview	Other, specify **[*only appear if 96*]**	[Text]	**c_hospvis_reas_o** [string; 50 characters]
10.2.5 Interview	What was the duration of your most recent visit to hospital?	[Number] (hours) *Enter 1 if less than one hour.* 99 = Don't know	**c_hospvis_dur** [integer; 2 digits; range 1–23, 99]
	Visits to PHC Facilities		
10.3 **Interview**	In the last 12 months, did you visit any primary health care (PHC) facility or clinic **for any reason** *(including accompanying someone else)*?	[Radio] 0 No 1 Yes *(If No, skip to Q11)*	**c_phcvis_12m** [integer; 1 digit; range 0–1]
10.3.1 Interview	If yes, how many times did you visit any primary health care facility (PHC) in the last 12 months?	[Number] 99 = Don't know	**c_phcvis_12m_n** [integer; 2 digits; range 01–20, 99]
10.3.2 Interview	When was your most recent visit to a PHC facility?	[Dropdown] 99 = Don't know	**c_phcvis_dvism** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**c_phcvis_dvisy** [standard AHRI year variable; range 1998–2017]
10.3.3	Which PHC facility did you visit most recently?	[Dropdown] [List of PHC facilities]	**c_phcvis_clin**

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
Interview		96 = Other, specify 99 = Don't' know	[integer; 2 digits; range 01–XX, 96, 99]
10.3.3.1 Interview	Other facility if not on the list **[*only appear if 96*]**	[Text]	**c_phcvis_clin_o** [text, 30 characters]
10.3.5 Interview	What was the duration of your most recent visit to a PHC facility (in hours)?	[Number] *Enter 1 if less than one hour.* 99 = Don't know	**c_phcvis_dur** [integer; 2 digits; range 01–23, 99]
10.3.6	At your most recent visit to a PHC facility, how many people were present at the clinic?	[Number] 999= Don't know	**C_phcvis_natt** [integer; 3 digits; range 01–200, 999]
Question 11	**Chronic illnesses** *(ARV uptake can be captured and noted at this point)*		
Tell participant: *"I will now ask you questions about any chronic/long-term illnesses that you might have and whether you are taking regular medication for that illness"*			
11.1 **Interview**	Do you have asthma?	[Radio] 0 No 1 Yes (*if No, skip to Q11.2*)	**c_asthma** [integer; 1 digit; range 0–1]
11.1.1. Interview	If Yes, are you taking regular medication for asthma?	[Radio] 0 No 1 Yes (*if No, skip to Q11.2*)	**c_asthmamed** [integer; 1 digit; range 0–1]
11.1.1.1 Interview	If Yes, what medication are you taking (*tick all that apply*)?	[Tick box] 01 Short-acting beta-agonist inhaler (e.g., Albuterol, Asthavent, Fenoterol, Salbutamol, Terbutaline, Ventolin, Volmax) 02 Long-acting beta-agonist inhaler (e.g., Formoterol, Foradil, Foratec, Foxair, Oxis, Salmeterol, Serevent) 03 Steroid inhaler (e.g., Aerobec, Beclomethasone, Beclate, Becotide, Beloforte, Budeflam, Budesonide, Ciclesonide, Clenil, Flixotide, Flomist, Fluticasone, Inflammide, Rhinocort, Viarox)	**c_asthmamed** [integer; 2 digits; range 01-06,96,99]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
		04 Combination inhaler (e.g., Budesonide-Formoterol, Seretide, Symbicort 05 Oral steroid (e.g., Prednisone, prednisolone, methylprednisone, methylprednisolone) 06 Oral leukotriene receptor antagonists (e.g., Accolate, Montelukast, Singulair, Zafirlukast) 96 Other, specify 99 Don't know (*if No, skip to Q9.2*)	
11.1.1.1.1 Interview	Other, Specify		
11.1.1.2 Interview	When did you start taking regular medication for asthma?	[Dropdown] 99 = Don't know	**c_asthmamed_dstartm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**c_asthmamed_dstarty** [standard AHRI year variable; range 1998–2017]
11.2 **Interview**	Do you have diabetes?	[Radio] 0 No 1 Yes (*if No, skip to Q11.3*)	**c_diab** [integer; 1 digit; range 0–1]
11.2.1 Interview	If Yes, are you taking regular medication for diabetes?	[Radio] 0 No 1 Yes (*if No, skip to Q11.3*)	**c_diabmed** [integer; 1 digit; range 0–1]
11.2.1.1 Interview	If Yes, what medication are you taking for diabetes? (*Select all that apply*)	[Tick box] 01 **Biguanide** (e.g., Metformin [Bigsens, Diabetmin, Diaformin, Diamin, Diaphage, Forminal, Glucophage, Mengen, Metcheck, Metforal, Metored, Romidab]) 02 **Sulphonylurea**	**c_diabmed_det** [integer; 2 digits; range 01–09, 96, 99]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
		(e.g., Glibenclamide [Daonil, Glycomin]; Gliclazide [Adco-Glucomed, Diaglucide, Diaglumed, Diamicron, Gycron, Glygard]; Glimepiride [Amaryl, Diaglim, Euglim, Glamaryl, Sulphonur]; Glipizide) 03 **PPAR agonist** (e.g., Pioglitazone [Actos]) 04 **SGLT2-inhibitor** (e.g., Canagliflozin, Dapagliflozin, Empagliflozin) 05 **DPP-4 inhibitor** (e.g., Saxagliptin [Onglyza, Kombiglyze], Sitagliptin [Januvia, Janumet, Juvisync], Vildagliptin) 06 **GLP-1 agonist** (e.g., Exenatide [Byetta, Bydureon], Liraglutide [Victoza], Lixisenatide, Semaglutide) 07 **Alpha-glucosidase inhibitor** (e.g., Acarbose) 08 **Short-acting insulin** (e.g., Isophane insulin [Humulin, Insulin-HMGE-Protaphane], Insulin Lispro [Humalog]) 09 **Long-acting insulin** (e.g., Glargine [Basaglar, Lantus, Optisulin]) 96 Other, specify 99 Don't know	
11.2.1.1.1 Interview	Other, Specify		
11.2.1.2 Interview	When did you start taking regular medication for diabetes?	[Dropdown] 99 = Don't know	**c_diabmed_dstartm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown]	**c_diabmed_dstarty** [standard AHRI year variable; range 1998–2017]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
11.3	Do you have any other chronic or long-term illness?	[Radio] 0 No 1 Yes (*if No, skip to Q12*)	**c_cillness** [integer; 1 digit; range 0–1]
	[*add sub-form for each illness*]		
11.3.1 Interview	If yes, what illnesses?	[Dropdown] 1 HIV/AIDS 2 Epilepsy 6 Other (Specify) 9 Don't know (*If HIV/AIDS, skip to Q11.3.4 inform the participant that you will ask the details of ARV uptake later*]	**c_cillness_det** [integer; 1 digit; range 1–3, 6, 9]
11.3.1.1 Interview	Other, specify **[*only appear if 6*]**	[Text]	**c_cillness_det_o** [string, 30 characters]
11.3.2 Interview	Do you take regular medication for this illness?	[Dropdown] 0 No 1 Yes 9 Don't know *(If No, Skip to 11.3.4)* *If yes, and illness=epilepsy – go to 11.3.2.1* *If yes, and illness = other – go to 11.3.2.1.1*	**c_regmed** [integer; 1 digit; range 0–1, 9]
11.3.2.1 Interview	If Yes, what medication?	[Dropdown] [List of medications] 1 Sodium valproate (e.g., Epilim, Epilizine, Epiproate, Eprolep, Navalpro, Valeptic) 2 Phenytoin sodium (e.g., Epanutin, Phlexy) 3 Carbamazepine (e.g., Degranol, Tegretol) 4 Lamotrigine (e.g., Epitec, Girotec, Lamictin, Lamidus, Lamitor) 5 Levetiracetam (e.g., Epikepp, Keppra, Redilev, Torcetam)	**c_regmed_det** [integer; 1 digit; range 1–6,9]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
		6 Other, specify 9 Don't know	
11.3.2.1.1 Interview	Specify medication **[*only appear if 6, or if Q11.3.1=6 (other illness) and Q11.3.2=yes*]**	[Text]	**c_regmed_det_o** [string; 50 characters]
11.3.3 Interview	When did you start taking regular medication for this condition?	[Dropdown] 99 = Don't know	**c_regmed_dstartm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown]	**c_regmed_dstarty** [standard AHRI year variable; range 1998–2017]
11.3.4 Interview	Do you have any other chronic illness that you take regular medication for?	[Radio] 0 No 1 Yes ***[if Yes, add sub-form for questions 11.3.1–11.3.4]***	**c_cillness_o** [integer; 1 digit; range 0–1]
Question 12	**Smoking**		
12.1 Interview	Have you ever smoked tobacco before? (*At least 100 cigarettes in total*)	[Radio] 0 No 1 Yes *(If No, skip to Q13)*	**c_smoke_ever** [integer; 1 digit; range 0–1]
12.1.1 Interview	If yes, when did you start smoking regularly?	[Dropdown] 99 = Don't know	**c_smoke_dstartm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**c_smoke_dstarty** [standard AHRI year variable; range 1998–2017]
12.1.2 Interview	Do you still smoke?	[Radio] 0 No 1 Yes *(If Yes, skip to Q12.2)*	**c_smoke_still** [integer; 1 digit; range 0–1]
12.1.2.1 Interview	If No, when did you stop smoking?	[Dropdown] 99 = Don't know	**c_smoke_dstopm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown]	**c_smoke_dstopy**

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
		9999=don't know	[standard AHRI year variable; range 1998–2017]
12.2 **Interview**	Does anybody else in your homestead smoke (excluding you)?	[Dropdown] 0 No 1 Yes 9 Don't know (If No, Skip to Q13)	**c_smoke_hh** [integer; 1 digit; range 0–1, 9]
12.2.1 Interview	If Yes, do you live in the same house as this person?	[Dropdown] 0 No 1 Yes	**c_smoke_samehh** [integer; 1 digit; range 0–1, 9]
Question 13	**Alcohol consumption**		
13.1 **Interview**	Have you ever taken alcohol?	[Radio] 0 No 1 Yes *(If No, skip to Q14)*	**c_etoh_ever** [integer; 1 digit; range 0–1]
13.1.1 Interview	If yes, do you currently take alcohol?	[Radio] 0 No 1 Yes	**c_etoh_cur** [integer; 1 digit; range 0–1]
13.1.2 Interview	When was the last time you took alcohol?	[Dropdown] 99 = Don't know	**c_etoh_dlastm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**c_etoh_dlasty** [standard AHRI year variable; range 1998–2017]
Question 14	**HIV & ARVs**		

REDCap Memory support - HIV Section

** Take a moment for rapport*
** Begin the HIV section with a general discussion.*
** Initial evaluation of mental state*
** Assess knowledge of HIV / sex / sexual transmission*
** Benefits of treatment / routes of transmission*
** Remind participant that if they already know their status, importance of telling us for appropriate clinical management of the IGRA results*

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
14.3 **[Piped variable from Q11.3.1]**	***What chronic illnesses did the participant mention in section 11?*** *((This question displays the chronic illnesses that the participant mentioned in Q11. This helps you how to proceed if the participant had already told you that they are HIV positive)*	***[Chronic illness1]*** ***[Chronic illness2]*** ***[Chronic Ilness3]***	**c_cillness_piped_2** [integer, 1 digit, range 1-3]
14.1 **Interview**	Have your ever tested for HIV before? *If the participant said they are HIV positive:* You mentioned that you are HIV positive; I assume you have tested for HIV before?	[Dropdown] 0 No 1 Yes 2 Prefer not to say 9 Don't know (*If Yes, go to 14.1.1 otherwise skip to Q14.2*)	**c_hivtest_ever** [integer; 1 digit; range 0–2, 9]
14.1.1 Interview	If yes, what was the result? *If the participant already said they are HIV positive, select Positive and proceed with follow up questions:*	[Dropdown] 0 Negative 1 Positive 2 Prefer not to say 9 Don't know (*if 0, 2 or 9, skip to Q14.1.1.2*)	**c_hivres** [integer; 1 digit; range 0–1, 9]
14.1.1.1 Interview	If Positive, when did you first test HIV positive?	[Dropdown] 99 = Don't know	**c_hivres_pos_dtestm** [standard AHRI month variable; range 01–12, 99]
		[Dropdown] 9999=don't know	**c_hivres_pos_dtesty** [standard AHRI year variable, range 1998-2017]
14.1.1.1.1 Interview	Where did you first test HIV positive?	[Dropdown] 1 DOH Hospital 2 DOH clinic 3 AHRI Home 4 AHRI Mobile Clinic 6 Other, specify 9 Don't know	**c_hivptest_loc** [integer; 1 digit; range 1–4, 6,9]
14.1.1.1.1.1 Interview	Other, specify **[*only appear if Q14.1.1.1.1 = 6*]**	[Text]	**c_hivtest_loc_o** [integer; 1 digit; range 0–1]
14.1.1.2		[Dropdown]	**c_hivres_pos_dtestm**

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
Interview	If negative, prefer not to say, or don't know, when was your most recent HIV test?	99 = Don't know	[standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**c_hivres_pos_dtesty** [integer; 4 digits; range 1998–2017]
14.1.1.2.1 Interview	Where did you test most recently test for HIV?	[Dropdown] 1 DOH Hospital 2 DOH clinic 3 AHRI Home 4 AHRI Mobile Clinic 6 Other, specify 9 Don't know	**c_hivptest_loc** [integer; 1 digit; range 1–4, 6,9]
14.1.1.2.1.1 Interview	Other, specify **[*only appear if Q14.1.1.2.1 = 6*]**	[Text]	**c_hivtest_loc_o** [integer; 1 digit; range 0–1]
14.2 Interview	Have you ever taken Isoniazid Preventive Therapy (IPT)?	[Radio] 0 No 1 Yes 9 Don't know *(If No or Don't know, skip to Q14.3)*	**c_ipt_ever** [integer; 1 digit; range 0–2, 9]
Isoniazid Preventive Therapy :Memory Aid *Explain to the respondent that this is treatment given to prevent development of TB disease unlike TB treatment which is given to treat TB disease.* *[If needed, explain that treatment for **TB disease** will have been with at least four drugs [though it may have been in one tablet, e.g., Rifafour] every day for two months, and then two drugs [again, may have been in one tablet, e.g., Rifinah] every day for a further four months [i.e., a total of at least 6 months].* *This is different from **isoniazid preventive therapy** [IPT], which is given to prevent development of **TB disease**, and will have involved taking one drug every day for six months.]*			
14.2.1 Interview	If Yes, when did you stop taking IPT?	[Dropdown] 97 = Currently on IPT 99 = Don't know	**c_ipt_dstopm** [standard AHRI month variable; range 1–12, 97, 99]
		[Dropdown] 9997 = Currently on IPT 9999=don't know	**c_ipt_dstopy** [standard AHRI year variable; range 1998–2017, 9997]
14.3 **[Piped variable from Q11.3.1]**	***What chronic illnesses did the participant mention in section 11?*** *(This question displays the chronic illnesses that the participant mentioned in Q11. This helps you know if*	***[Chronic illness1]*** ***[Chronic illness2]*** ***[Chronic Ilness3]***	**c_cillness_piped_2** [integer, 1 digit, range 1-3]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	the participant already told you that they are HIV positive)		
14.4 (piped from Q14.1.1)	***Reported HIV status of the participant***		
	ARV Question Memory Aid * Think back about what the participant said regarding their HIV status. Use this information to guide you how to ask the next set of questions about ART uptake.		
14.5 Interview	Have you ever taken ARVs (*Including PEP or PrEP)?* *If the participant is HIV negative (or has never tested for HIV), explain that PrEP (Pre-Exposure Prophylaxis) may be given to people who are at high-risk, to reduce their risk of acquiring HIV and* *PEP (Post Exposure Prophylaxis) is given after someone may have been exposed to HIV (e.g., through a needlestick injury) to prevent them from becoming infected*)	[Dropdown] 0 No 1 Yes 2 Prefer not to say 9 Don't know (*If Yes, go to 14.5.1 otherwise skip to 15*)	**c_arv_ever** [integer; 1 digit; range 0–2, 9]
14.5.1 Interview	*If Yes, why did you take ARVs?* (*if the participant had already mentioned that they are HIV positive and taking ARVs, select 1 for this question and proceed with follow-up questions*)	[Radio] 1 For routine HIV care 2 As post-exposure prophylaxis (PEP) 3 As pre-exposure prophylaxis (PrEP) 6 Other, specify 9 Don't know (*if 6 go to 14.5.1.1 otherwise skip to 14.5.3*)	**c_arv_reas** [integer; 1 digit; range 0–3, 6, 9]
14.5.1.1 Interview	Other, specify	text	**c_arv_o** [text; 30 characters]
14.5.3 Interview	When did you first start taking ARVs?	[Dropdown] 99 = Don't know	**c_arv_dstartm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**c_arv_dstarty** [standard AHRI year variable; range 1998–2017]
14.5.4 Interview	Do you still take ARVs?	[Radio] 0 No 1 Yes *(If No, skip to Part II)*	**c_arv_still** [integer; 1 digit; range 0–1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
14.5.4.1 Interview	If No, when did you stop?	[Dropdown] 99 = Don't know	**c_arv_dstopm** [standard AHRI month variable; range 1–12, 97, 99]
		[Dropdown] 9999=don't know	**c_arv_dstopy** [standard AHRI year variable; range 1998–2017, 9997]
14.5.5 Interview	Which regimen are you taking, or did you take before stopping?	[Dropdown] [List of ARV regimens] 01 ABC + 3TC + LPV/r (e.g., ABC/3TC fixed-dose [Dumiva, Heteruam, Kivexa] + LPV/r [Norvir, Rinavo]) 02 ABC + 3TC + EFV (e.g., ABC/3TC fixed-dose [Dumiva, Heteruam, Kivexa] + EFV [Efamat, Efrin, Erige, Hevaz, Stocrin, Viref]) 03 TDF + FTC + EFV (e.g., Fixed-dose combination [Atenef, Atreslawin, Atripla, Atroiza, Citenvir, Eftenem, Heftenam, Odimune, Rizene, Trenvir, Tribuss, Triolar]) 04 TDF + 3TC + EFV (e.g., Fixed-dose combination [Arion, eflaten, Elteno, Tenarenz, Virlaten]) 05 AZT + 3TC + LPV/r 06 AZT + ABC + LPV/r 96 Other, specify 99 Don't know	**c_arv_still_det** [integer; 2 digits; range 01–06, 96, 99]
14.5.5.1 Interview	Other, specify **[*only appear if 96*]**	[Text]	**c_arv_still_det_o** [string; 30 characters]
14.5.6 Interview	Which clinic (or facility) are you currently taking ARVs from? Or, if no longer taking ARVs, clinic were you using before stopping?	[Dropdown] [List of PIP clinics] 96 = Other (Specify) 99 = Don't know	**c_arv_curclin** [integer; 2 digits; range 01–XX, 96, 99]
14.5.6.1 Interview	Other, specify **[*only appear if 96*]**	[Text]	**c_arv_curclin_o** [string, 30 characters]

HIV DECISION RULES

Do NOT attempt to gain consent for HIV testing if any of the following apply:

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	1. The parent/guardian reported that the participant is HIV-positive AND taking ARVs. **2. The parent/guardian did not give consent for the child to be tested for HIV.** **3. The participant is aged 10-11 years, and parent/guardian did not consent to be present during the test** **4. The participant reported themselves as HIV-positive AND taking ARVs.** **5. The date of the last HIV test in PIP was within 3 months.**		
	HIV Testing Memory Aid ** Participants reporting themselves to be HIV positive but not on ARVs should be offered a rapid test and referred to care.*		
	Memory Aid - Date for last HIV Test in PIP ** The question below gives date for the participant's last test in PIP. Use the date in deciding whether to offer a rapid HIV test.* ** If the date is not available, this means that the participant has never been tested by AHRI.* ** If the date is within 3 months, do not offer a rapid test* ** If the date is more than 3 months and the participant is not taking ARVs, offer rapid HIV test.*		
15.1 Preloaded	*Date of last HIV test in PIP:*	*date*	**c_piplhivdate** [Standard Date Variable]
Question 15.2 Interview	*Basing on the HIV Decision rules above, should the participant be offered a rapid HIV test?*	[Radio] 0 No 1 Yes (*If No, skip to 16.1.3*)	**c_hiv_dec** [integer; 1 digit; range 0–1]
Question 16	**Consent for rapid HIV testing (if participant should be offered an HIV test)**		
	REDCap Memory support - Offering rapid HIV testing *** Initial evaluation of mental state** *** Confirm: has child capacity to assent?** *** Duty of confidentiality/with limits** *** Right to withhold assent** *** If age 15+, explain that test result can remain confidential, explore who to tell** *** If aged 12-14 years, explain that they must have their own phone so that you will be able to check on them if needed, or they must be willing to share the results with their parent** *** If age 12-14 years with own phone, encourage to share with parent/explore who to tell** *** Deeper assessment of reaction to positive** *** Consider referral to clinic for testing** *** Reaffirm assent of child, then conduct test.** *** Confirm: proceed with test?** *** Choices re presence of parent** *** Would you like your (parent) present now?**		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	*** If age 12+, explain that it is their choice whether parent is present (if aged 10-11 years, must be tested with parent present)** *** Ask: have they wondered if they could have HIV?** *** Ask if they have fear of a key adult** *** Check age of partner (if appropriate)**		
16.1 **Interview**	Would you like to have an HIV test today? *If participant is not willing to have a rapid test, and the parent gave permission for HIV testing of venous blood, obtain permission to test HIV using the venous blood collected - Q16.1.3)*	[Radio] 0 No 1 Yes *(If Yes, and aged <15, go to 16.1.1)* *(If Yes, and aged 15+, go to 16.1.4 then skip to next instrument [Social contacts])* *(If No, go to 16.1.3])* *(If No and HIV positive not on ART, go to Q16.1.3 then Q16.2)*	**c_hiv_rapidconsent** [integer; 1 digit; range 0–1]
16.1.1 Interview	**If age <15 years**: Would you prefer to have your parent/guardian present during the process of HIV testing? *If aged 12-14 years, explain that this is their preference. If aged 10-11 years, both parent/guardian and participant must agree for parent/guardian to be present, in order for test to be offered.*	[Radio] 0 No 1 Yes *(If yes, and age 10-11, skip to 16.1.4, then go to next instrument [Social contacts])* *(If no, and age 10-11, show pop-up message, skip to 16.1.3, then go to next instrument [Social contacts])* *(If age 12-14, go to 16.1.2)*	**c_parpresent** [integer; 1 digit; range 0–1]
	If age 10-11 and NO, Pop up Message: **Please explain to the participant that you cannot offer them an HIV test without parent present**		
16.1.2 Interview	**If aged 12-14 years**: Do you have your own telephone?	[Radio] 0 No 1 Yes *(If No, go to 16.1.2.2)* *(If Yes, go to 16.1.4 then skip to next instrument [Social contacts])*	**c_ownphone** [integer; 1 digit; range 0–1]
16.1.2.2	**If No**: Are you willing to share your test result with your parent/guardian?	[Radio] 0 No 1 Yes	**c_shareres** [integer; 1 digit; range 0–1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Explain that you may need to use their parent's phone number if you need to contact them *If not willing to share the result with parent, explain that you will not be able to offer them an HIV test*	(*If No, show pop up message, go to 16.1.3 then skip to next instrument [Social contacts])* *(If Yes, go to 16.1.4 then skip to next instrument)*	
	Pop up Message: **Please explain to the participant that you cannot offer them an HIV test**		
16.1.3 Interview	If **participant does not want an HIV test, or cannot be offered a test based on the decision rules**: can we test HIV using the venous blood that we have collected for research purposes? *Explain to the participant that the HIV test will be for research purposes only and that the result will not be available. This is to help us understand better the link between HIV and TB. Should they want to know their status at a later stage, advise them that they should go to nearest clinic to be tested.* *If the participant has reported that they are HIV positive and not on ART, go to the referral section.*	[Radio] 0 No 1 Yes	**c_hiv_venoustest** [integer; 1 digit; range 0–1]
	If YES, Pop up Message: **Please make sure HIV ELISA is ticked on the Lab Requisition Form**		
16.1.4 Interview	(*If Yes, ask participant to sign consent for HIV testing*) I agree that my blood can be tested for HIV as part of this study	Signature	**c_hivcons_sign**
16.1.5 Interview	If HIV test was not offered or refused, please comment	Text	**c_hiv_noconsent** [text 200 characters]
16.2 **Interview**	**REFERRAL TO CARE FOR HIV CARE (PREVIOUS HIV+ NOT LINKED TO CARE)**		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Referral To care Memory Aid: * If the participant self-reports that they are HIV positive and not on ART, they should be referred to clinic in this section. * Inform the participant that you need to refer them to clinic in PIP where there is an AHRI nurse. * Ask the participant to choose a clinic within PIP. * If age <15, tell the participant that an AHRI paediatric HIV nurse the AHRI paediatric HIV nurse can see them at the one of following clinics in PIP: KwaMsane (Mondays); Mtubatuba (Tuesdays); Somkhele (Thursdays). If the participant is not able to attend one of those clinics, ask them to choose another clinic in PIP.		
16.2.1 Interview	Which clinic would you like to be referred to? The AHRI Paediatric nurse can see participants in the following clinics: KwaMsane (Mondays); Mtubatuba (Tuesdays); Somkhele (Thursdays)	[drop down] 1 Clinic1 2 Clinic2 3 …. 99 Other If 99, skip to 16.2.1.2	**c_prevhiv_prefclin** **[**integer; 2 digits; range 0–99**]**
16.2.1.2	Specify other clinic		
16.2.2 Interview	What is the agreed target date for attending clinic? *Agree with the participant a target date to attend clinic (this should be within 2 weeks).*	[date]	**c_prevhiv_tdat** [standard AHRI date variable, range: today () + 14]
16.2.3 Interview	What is your primary contact number? *Explain that you need the phone number to be able to contact them should they fail to make it on the agreed date.*	[number]	**c_prevhiv_contnum**
16.2.4 Interview	Do you share this phone with other people?	[Radio] 0 No 1 Yes If aged <15, skip to 16.2.6 If aged 15+, go to 16.2.5	**c_prevhiv_contnum_share** [integer; 1 digit; range 0–1]
16.2.5 Interview	**If aged 15+:** Are you happy to receive text messages on this number?	[Radio] 0 No	**c_prevhiv_sms_cons** [integer; 1 digit; range 0–1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
		1 Yes *(if No, Skip to Q16.2.6)*	
16.2.5.1 Interview	*If Yes, what SMS reminder wording did the participant choose?* *Please choose from standard SMS reminders or use own words.*	[Radio] 1 Please don't forget your appointment 2 We hope you will visit us soon 3 Don't forget to get that present 6 Choose your own words *(If 1-3, Skip to Q16.2.6)*	**c_prevhiv_sms_opt** [integer; 1 digit; range 1-3, 6]
16.2.5.1.1 Interview	Provide your own words for SMS reminder message	[Text]	**c_prevhiv_sms_opt_o** [Text,50 characters]
16.2.6 Interview	*Has the phone number been verified during the visit?*	[Radio] 0 No 1 Yes	**c_prevhiv_contnum_verif** [integer; 1 digit; range 0–1]
	If No, Pop Up Message ***Please verify the number to check if it is working***		

Blood Sample Collection

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Memory Aid – Scanning Sample Barcode * Before scanning the barcode for each sample, make sure the sample has been obtained first.		
Question 1	**Venepuncture: EDTA**		
1.0 **Interview**	Laboratory PT ID number	Barcode	**c_participant_id** [barcode]
1.1 **Interview**	EDTA Specimen number	Barcode	**c_edta_specno** [barcode]
1.2 **Interview**	Was an EDTA sample obtained?	[Radio] 0 No 1 Yes	**c_edta** [integer; 1 digit; range 0–1]
1.2.1 Interview	If no, please give a reason	[Text] *(Skip to Q2)*	**c_edta_noreas** [string; 50 characters]
1.2.2 Interview	If yes, time of collection	[Number]	**c_edta_tcollhh** [integer; 2 digits; range 00–23]
		[Number]	**c_edta_tcollmm** [integer; 2 digits; range 00–59]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
1.2 **Interview**	Has the specimen been successfully QC-ed and can it be sent to the Lab?	[Radio] 0 No 1 Yes	**c_edta_specim_qced** [integer; 1 digit; range 0–1]
Question 2	**Venepuncture: Lithium Heparin**		
2.1 **Interview**	Lithium heparin specimen number	Barcode	**c_lihep_specno** [barcode]
2.2 **Interview**	Was a lithium heparin sample obtained?	[Radio] 0 No 1 Yes	**c_lihep** [integer; 1 digit; range 0–1]
2.2.1 Interview	If no, please give a reason	[Text] *(Skip to Q2)*	**c_lihep_noreas** [string; 50 characters]
2.2.2 Interview	If yes, time of collection	[Number]	**c_lihep_tcollhh** [integer; 2 digits; range 00–23]
		[Number]	**c_lihep_tcollmm** [integer; 2 digits; range 00–59]
2.2 **Interview**	Has the specimen been successfully QC-ed and can it be sent to the Lab?	[Radio] 0 No 1 Yes	**c_edta_specim_qced** [integer; 1 digit; range 0–1]

Rapid HIV Testing

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	REDCap Memory support - Pre-rapid HIV test *** Younger child (age 10-11 years): parent present now?**		
	REDCap Memory support - Pre-test information: *** Importance of knowing status (whether positive or negative)** *** Explain confidentiality/shared confidentiality; how it will be maintained.** **o Give the participant information with regards HIV** *** Check that the participant has understood the following items:** **o The benefits of knowing status (whether positive or negative)** **+ Lifesaving benefits of treatment that is available immediately**		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	+ Window period. **+ Add the following information:** **- The meaning of HIV and AIDS** **- Transmission of HIV** *** It is helpful if you tell me if you are taking ARVs already** *** If you are already on ARVs, the test may react as negative, even though you have HIV.** *** Meaning of both positive and negative results and other results** *** Prevention of HIV infection** *** Right to refuse to test** *** If the participant is not ready DO NOT TEST but emphasize the importance of testing in and knowing their status.**		
	<u>REDCap Memory support - Test Procedure: Items to prepare</u> **[*Review which samples are to be collected for this participant*]** *** Bar code stickers/pen etc.** *** Linen saver** *** 2x cotton wool balls** *** Lancet** *** Alcohol swabs** *** One Step HIV testing Kit: Cassette, buffer/diluent and pipette** *** Biotracer HIV testing Kit: Cassette, buffer/diluent and pipette** *** Gloves** *** Sharps container** *** Red bag for non-sharp waste** *** Timer**		
	<u>REDCap Memory support - Test Procedure: Collecting samples</u> *** Put gloves on** *** Finger chosen (middle and ring finger) in less dominant hand** *** Milk the chosen finger properly to get enough blood** *** Clean finger with swab** *** Prick lateral aspect of finger with lancet** *** Review if a fresh sample needed / avoid excess squeezing** *** Using Pipette from One Step kit draw up one drop of blood** *** Using Pipette from Biotracer kit draw up blood until the second line on the pipette**		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	REDCap Memory support - Test Procedure: Rapid testing kits *** Squeeze 1 drop blood into S- well of One Step cassette** *** Squeeze 1 entire drop of blood (drawn up to 2nd line on pipette) into S-sample well of Biotracer cassette** *** Put 1drops of One Step buffer into the (S)- well of One Step cassette** *** Put 3 drops of Biotracer buffer into (S) well of Biotracer cassette** *** Set timer and wait for tests to process for full 20 minutes.** *** Move the cassette away from participants eyes.** *** Whilst waiting for the results, check if the client still remembers how to read the results and * Labelling**		
	REDCap Memory support - Test Procedure: Results *** Clearly evaluates if One Step test is valid, and gives correct interpretation** *** Clearly evaluates if Biotracer test is valid, and gives correct interpretation** *** Outlines correct response/action to combined (2 to 3) kit results**		
Question 1	**Test Kit 1: Attempt 1**		
1.1 **Interview**	*What was the LOT number for the test kit?*	[Text]	**c_advancekit_lotnum** [Text; 20 digits]
1.2. Interview	*What is the Rapid HIV test result?*	[Radio] 0 Valid Negative 1 Valid Positive 2 Invalid	**c_advance_rapid** [integer; 1 digit; range 0–2]
	Invalid results		
	REDCap Memory support - Post-test information giving		
	*Check if the client would like to consent for another rapid test *Explain the possibility of a second prick. *Repeat the test with same kit (Kit 1)		
	Test Kit 1: Attempt 2		
1.2.1. Interview	*What was the LOT number for the test kit?*	Number	**c_advancekit_lotnum_2** [Text; 20 digits]
1.2.2. Interview	*What is the Rapid HIV test result?*	[Radio] 0 Valid Negative 1 Valid Positive	**c_advance_rapid_2** [integer; 1 digit; range 0–2]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
		2 Invalid	
Question 2	**Kit 2: Attempt 1**		
2.1 Interview	*What was the LOT number for the test kit?*	Number	**c_abonkit_lotnum** [Text; 20 digits]
2.2 Interview	*What is the Rapid HIV test result?*	[Radio] 0 Valid Negative 1 Valid Positive 2 Invalid *(If invalid, second attempt)*	**c_abon_rapid** [integer; 1 digit; range 0–2]
	Invalid results		
	REDCap Memory support - Post-test information giving		
	*Check if the client would like to consent for another rapid test *Explain the possibility of a second prick. *Repeat the test with same kit (Kit 2)		
	Kit 2: Attempt 2		
2.2.1 Interview	*What was the LOT number for the test kit?*	Number	**c_abonkit_lotnum_2** [Text; 20 digits]
2.2.2 Interview	*What is the Rapid HIV test result?*	[Radio] 0 Valid Negative 1 Valid Positive 2 Invalid	**c_abon_rapid_2** [integer; 1 digit; range 0–2]
	Please ensure that you have entered the kit results correctly		
Question 3	**Result Summary**		
3.1 **Interview**	*Confirm the Summary HIV test result*?	[Radio] 0 HIV Negative 1 HIV Positive 2 Discordant 3 Invalid *(If 0, skip to the next instrument)* *(If 1, Skip to Q4.1)* *(If 2-3, go to Q3.2)*	**c_hivrapid_result** [integer; 1 digit; range 0–2]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Please ensure that you have entered the kit results correctly		
	HIV Positive results		
	REDCap Memory support - Post-test information giving		
	* Show the participant both cassettes and let the participant read the results. * Agree on the positive results. * Allow time for the client to absorb the results. * Assess if participant understands meaning of results * Use open ended questions to ensure that the participant believes the result. * Deal with the immediate emotions reactions. * Help participant identify the main concerns at this stage. * Remind about lifesaving benefits of treatment. * Give information of how to access ARV and linkage to care (participant to visit clinic within 10 days). * If aged <15 years, AHRI paediatric HIV nurse will help them, and will meet them at their first appointment * Encourage disclosure, especially to parent/guardian if aged <15 years. * Assess / caution re inappropriate disclosure. * If alone: assess when parent to be brought in, & what to say. * Discuss the risk of STIs and opportunistic infections l * Check availability of immediate support at home. * Discuss positive healthy living with HIV. * Including prevention of transmission * Discuss the implications of not taking ARV. * Ask if there are questions, listen to participant's concerns. * Complete the referral letter and refer participant to the fixed clinic. * If aged 15+, add referral target date and inform re SMS reminder by using REDCap.		
	Negative Results		
	REDCap Memory support - Post-test information giving		
	* Show the participant both cassettes and let the participant read the results * Agree on the negative results * Allow time for the participant to absorb the results * Discuss window period and future re-testing * Check that the participant understands that someone on long term ARV may test negative while being infected. * Encourage healthy lifestyle i.e. stay negative, circumcision for males		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	* Encourage participant to know partner's status (if age-appropriate) * Ask if there are questions, listen to participant's concerns		
	Discordant/Invalid results		
	REDCap Memory support - Post-test information giving		
	* Ensure that the participant understands the discordant results. * Explain that the result can be confirmed in the lab using the venous blood that has already been collected. * Request HIV ELISA on LRF. * Explain that you will feedback this result in person.		
3.2 **Interview**	If final rapid HIV test result is invalid or discordant, was HIV ELISA requested on the Lab Requisition Form?	[Radio] 0 No 1 Yes	**c_elisa_discrapid** [integer; 1 digit; range 0-1]
4.1 **Interview**	**REFERRAL TO CARE FOR HIV CARE (NEW HIV DIAGNOSIS)**		
	Referral To care Memory Aid: * Inform the participant that you need to refer them to clinic in PIP where there is an AHRI nurse. * Ask the participant to choose a clinic within PIP. * If age <15, tell the participant that an AHRI paediatric HIV nurse the AHRI paediatric HIV nurse can see them at the one of following clinics in PIP: KwaMsane (Mondays); Mtubatuba (Tuesdays); Somkhele (Thursdays). If the participant is not able to attend one of those clinics, ask them to choose another clinic in PIP.		
4.1.1 Interview	Which clinic would you like to be referred to? The AHRI Paediatric nurse can see parents in the following clinics: KwaMsane (Mondays); Mtubatuba (Tuesdays); Somkhele (Thursdays)	[drop down] 1 Clinic1 2 Clinic2 3 ….	**c_rapidhiv_prefclin** **[**integer; 2 digits; range 0–99**]**
4.1.2 Interview	What is the agreed target date for attending clinic? *Agree with the participant/parent a target date to attend clinic (this should be within 2 weeks).*	[date]	**c_rapidhiv_tdat** [standard AHRI date variable, range: today () + 14]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
4.1.3 Interview	What is your primary contact number? *Explain that you need the phone number to be able to contact them should they fail to make it on the agreed date.*	[number]	**c_rapidhiv_contnum**
4.1.4 Interview	Do you share this phone with other people?	[Radio] 0 No 1 Yes If aged <15, skip to 4.1.6 If aged 15+, go to 4.1.5	**c_rapidhiv_contnum_share** [integer; 1 digit; range 0–1]
4.1.5 Interview	**If aged 15+:** Are you happy to receive text messages on this number?	[Radio] 0 No 1 Yes *(if No, Skip to Q4.1.6)*	**c_rapidhiv_sms_cons** [integer; 1 digit; range 0–1]
4.1.5.1 Interview	*If Yes, what SMS reminder wording did the participant choose?* *Please choose from standard SMS reminders or use own words.*	[Radio] 1 Please don't forget your appointment 2 We hope you will visit us soon 3 Don't forget to get that present 6 Choose your own words *(If 1-3, Skip to Q4.1.6)*	**c_rapidhiv_sms_opt** [integer; 1 digit; range 1-3, 6]
4.1.5.1.1 Interview	Provide your own words for SMS reminder message	[Text]	**c_rapidhiv_sms_opt_o** [Text,50 characters]
4.1.6 Interview	*Has the phone number been verified during the visit?*	[Radio] 0 No 1 Yes	**c_rapidhiv_contnum_verif** [integer; 1 digit; range 0–1]
	If No, Pop Up Message ***Please verify the number to check if it is working***		

Sputum Sample Collection

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	Sputum Decision Tree		
1.1 **Interview**	*Should sputum be collected from this participant?*	[Dropdown] 1 Yes 2 No, not required *(If No skip to next section-end of survey)*	**c_sputum** [integer; 1 digit; range 1-4]
1.1.1 **Interview**	*Sputum sample 1 barcode* ***Use DOH sputum specimen container and barcode***	Barcode	**c_sput_specno1** [Barcode]
1.1.1.1 **Interview**	*Time of collection*	[Number]	**c_sput1_tcollhh** [integer; 2 digits; range 00–23]
		[Number]	**c_sput1_tcollmm** [integer; 2 digits; range 00–59]
1.1.2 **Interview**	*Sputum sample 2 specimen number* ***Use AHRI sputum specimen container and barcode***	Barcode	**c_sput_specno2** [Barcode]
1.1.2.1 **Interview**	*Time of collection*	[Number]	**c_sput2_tcollhh** [integer; 2 digits; range 00–23]
		[Number]	**c_sput2_tcollmm** [integer; 2 digits; range 00–59]
1.1.2.1.1	Total number of sputum samples collected	[Number]	Integer; 1 digit, range 0-2]
1.2 **Interview**	If sputum should be collected, but fewer than 2 samples are collected, please comment	[Text]	**c_sput_n1reas** [string; 200 characters]
	If participant is not able to produce sputum, initiate referral to clinic		
1.2.1	Does the participant need to be referred?	[Radio] 0 No 1 Yes If yes, go to 1.3	**c_nsput_refer**
1.3 **Interview**	**REFERRAL TO CARE (TB SYMPTOMS, UNABLE TO PRODUCE SPUTUM)**		
	<u>Referral To care Memory Aid:</u> * Explain to the participant that because they have symptoms suggestive of TB, they need to be reassessed at a clinic.		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	* Provide reassurance and reiterate that they are free to contact you whenever necessary * Tell the participant that you need to refer them to clinic in PIP where there is an AHRI nurse. * Ask the participant to choose a clinic within PIP. * If you have already referred the participant during the visit for another reason, ask if can be contacted on the same number for this referral.		
1.3.1 Interview	Which clinic would you like to be referred to?	[drop down] 1 Clinic1 2 Clinic2 3 ….	**c_nsput_prefclin** **[**integer; 2 digits; range 0–99**]**
1.3.2 Interview	*What is the agreed target date for attending clinic?* *Agree with the participant on a target date to attend clinic (this should be within 2 weeks).*	[date]	**c_nsput_tdat** [standard AHRI date variable, range: today () + 14]
1.3.3 Interview	What is your primary contact number? *Explain that you need the phone number to be able to contact them should they fail to make it on the agreed date.*	[number]	**c_nsput_contnum**
1.3.4 Interview	Do you share this phone with other people?	[Radio] 0 No 1 Yes	**c_nsput_contnum_share** [integer; 1 digit; range 0–1]
1.3.6 Interview	*Has the phone number been verified during the visit?*	[Radio] 0 No 1 Yes	**c_nsput_contnum_verif** [integer; 1 digit; range 0–1]
	If No, Pop Up Message ***Please verify the number to check if it is working***		

End of Survey Instrument

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
1.1 Interview	**Summary of results to feedback:** ** Inform the participant and/or the parent/guardian that you may feedback the following results:* ** Give estimated timelines for feeding back the result.*		**c_res_feedback** [lebel]
1.1.1 Calculated	*HIV ELISA.*	*if rapid HIV test is discrepant or invalid*	**c_hivelisa_fdback**
1.1.2 Calculated	*GeneXpert (Sputum)*	*if sputum1 was collected*	**c_xpert_fdback**
1.1.3 Calculated	*Culture (Sputum)*	*If Sputum2 is collected. Results only fed back if Sputum 2 is positive and Sputum 1 is negative*	**c_sptcult_fdback**
1.1.4 Calculated	*IGRA result* *Tell the participant you will feed back if IGRA is positive*	*if the participant also knows their HIV positive status, and the IGRA result is positive*	**c_igra_fdback**
1.2 Interview	**Contact Details for Feeding Back Result**		
	Memory Aid – Feeding Back Results ** Tell Participant: Results will fed back in person (except geneXpert negative), but you will call first to arrange and appointment*		
1.2.1 Interview	Which number can I contact you on to feedback laboratory results?	*[Text]*	**c_res_cntnum** [text; 10 characters]
1.2.2 Interview	Do you share this number with other people?	[Radio] 0 No 1 Yes	**c_res_contnum_share** [integer; 1 digit; range 0–1]
1.2.3 Interview	Are you happy to receive phone calls about your results on this number?	[Radio] 0 No 1 Yes	**c_res_sms_cons** [integer; 1 digit; range 0–1]
1.2.4 Interview	*Has the number been verified during the visit?*	[Radio] 0 No 1 Yes	**c_res_contnum_verif** [integer; 1 digit; range 0–1]
	If No, Pop Up Message ***Please verify the number to check if it is working***		
2.1	**Reimbursement**		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
Interview			
2.1.1 **Interview**	How would you like to be reimbursed?	[Radio] 1 Airtime 2 Food Refreshment	**c_reimburse_opt** [integer; 1 digit; range 1-2]
	If airtime, please confirm number to be reimbursed on		
2.1.1.1 **Preloaded/Interview**	Please provide your primary contact number (mobile)	Number	**c_prim_cntnum** [text; 10 characters]
2.1.1.2 **Preloaded/Interview**	Please indicate network	**Drop down** 1 MTN 2 Vodacom 3 CellC 4 Telkom 6 Other	**c_prim_cntnum_network** [integer; 1 digit; range 0–4,6]
2.1.1.2.1 **Interview**	Specify network	Text	**c_prim_cntnum_network_o** [text; 50 characters]
2.1.2.1 **Preloaded/Interview**	Alternative contact number (mobile)	Number	**c_alt_prim_cntnum** [text; 10 characters]
2.1.2.2 **Preloaded/Interview**	Please indicate network	**Drop down** 1 MTN 2 Vodacom 3 CellC 4 Telkom 6 Other	**c_alt_prim_cntnum_network** [integer; 1 digit; range 0–4,6]
2.1.2.2.1 **Interview**	Specify network	Text	**c_alt_prim_cntnum_network_o** [text; 50 characters]
3.1 **interview**	**Interview Details**		
3.1.1 **Interview**	*Has the participant answered all the questions?*	[Radio] 0 No 1 Yes *(if Yes, Skip to Q15.2)*	**c_surv_comp** [integer; 1 digit; range; 0-1]
3.1.1.1 **Interview**	If No, why were some questions not answered? (give comments why some questions were not completed)	[Text]	**c_nocomp_reas** [Text, 200 characters]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
3.1.2	Please add any other comments or notes that you feel are needed	[text]	**c_survey_comments** [Text, 300 characters]
3.1.3 **Interview**	Interviewer Code	Dropdown 1 Staffcode 1 2 Staffcode 2 3 Staffcode 3 4 Staffcode 4	**c_intid** [integer; 1 digit; range; 1-4]
3.1.4 **Interview**	Time interview completed	[Number]	**c_tintstophh** [integer; 2 digits; range 00–23]
		[Number]	**c_tintstopmm** [integer; 2 digits; range 00–59]

Appendix 10: Social Contacts questionnaire for participants (10-19 years)

HAYA TB Study – Social Contacts Case Report Form

Tell the participant:

Tuberculosis is an air borne infection. This means that it is transmitted by inhaling TB bugs that have been released into the air by people with disease through: coughing, sneezing, singing, or speaking. In this part of the interview I will ask you about the places that you visit, the numbers of people that were present the last time you were at these places, and the numbers of people that you had contact with. This information will help us understand how people mix in their households and in the community. It will also help us understand the link between different social mixing patterns and TB infection.

Describe the following definition of a social contact to the participant and proceed with the interview

Definition of a social contact:

Explain to the participant that for the purposes of this study a social contact is defined as a person with whom the study participant either:

1) Had a conversation in the physical presence of the other person, within arm's reach; OR

2) Physical skin to skin contact, like a handshake, hug, or kiss; OR

3) Had sat next to each other in an enclosed environment, like a classroom, church, hospital or public transport.

Non-physical contacts made by phone call, WhatsApp, Facebook or other social media do not qualify as social contacts for the purposes of this study.

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
Question 1:	**Visit to other households**		
1.1 Interview	Do you sleep in the same room as another person?	[Radio] 0 No 1 Yes *(If No, skip to Q1.3)*	**c_hh_roomshare** [integer; 1 digit; range 0–1]
1.2. Interview	If yes, how many other people sleep in the same room as you?	[Number]	**c_hh_roomshare_n** [integer; 2 digits; range 01–19]
1.2.1 Interview	***Can you provide me details of each person that you sleep in the same room with?***	**[*Enter sub-form for each person*]**	**c_hh_roomshare_det**
1.2.1.1 Interview	Age of person	[Number] (years) 999 = Don't know	**c_hh_roomshareage** [integer; 3 digits; range 001–099, 999]
1.2.1.2 Interview	Sex of person	[Radio] 1 Male	**c_hh_roomsharesex** [integer; 1 digit; range 1–2]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
		2 Female	
1.3 **Interview**	In the last 12 months, did you spend nights in homesteads other than your own for a total of 2 weeks or more? *(explain that this does not have to be the same homestead)*	Radio 0 No 1 Yes (*If No, skip to Q1.4*)	**c_hh_vis12m** [integer; 1 digit; range 0–1]
1.3.1 Interview	If Yes, how many homesteads?	[Number] 99 = Don't know	**c_hh_vis12m_n** [integer; 2 digits; range 01–10, 99]
1.4 **Interview**	In the last four weeks, did you visit homesteads other than your own for 30 minutes or longer (where you did not spend the night)?	Radio 0 No 1 Yes (*If No, skip to Q2*)	**c_hh_vis1m** [integer; 1 digit; range 0–1]
1.4.1 Interview	If Yes, how many homesteads?	[Number] 99 = Don't know	**c_hh_vis1m_n** [integer; 2 digits; range 01–10, 99]
Question 2:	**Attendance to church or prayer meetings**		
2.1 **Interview**	In the last 12 months, did you attend church or any place of worship?	Radio 0 No 1 Yes (*If No, skip to Q3*)	**c_church_att12m** [integer; 1 digit; range 0–1)
2.1 Interview	If Yes, how many times did you attend church in the last 4 weeks?	[Number] 99 = Don' know	**c_church_natt12m** [integer; 2 digits; range 01–31]
2.2 **Interview**	Which church/religion do you belong to?	Dropdown [List of religions] 1 African Evangelical Church 2 Anglican (Church of South Africa) 3 Assemblies of God 4 Baptists …. 96 Other, specify 97 None 98 Prefer not to say *(If None or Prefer not to say, skip to Q2.3)*	**c_church** [integer; 2 digits, range 01–XX, 96–98]
2.2.1 Interview	Other religion if not on the list:	[Text]	**c_church_o** [string; 30 characters]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
2.2.2 Interview	Which branch (location of place of worship) of your church or place of worship do you go to?	[Text]	**c_church_loc** [string; 30 characters]
2.3 Interview	When was the last time that you attended church or any prayer meeting?	[Number] (day) 99 = Don't know	**c_churchvis_dvisd** [standard AHRI day variable; range 01–31, 99]
		[Number] (month) 99 = Don't know	**c_churchvis_dvism** [standard AHRI month variable; range 01–12, 99]
		[Number] (year)	**c_churchvis_dvisy** [standard AHRI year variable; range 1998–2017]
2.3.1 Interview	Was church service or prayer meeting conducted indoors or outdoors?	Drop down 1 Entirely indoors 2 Mostly indoors 3 Mostly outdoors 4 Entirely outdoors	**c_church_indoor** [integer; 1 digit; range 1–4]
	Memory Aid – Contacts at last day of attending school * Questions 2.3.2 to 2.3.2 ask about the time spent in a classroom and the number of students present. * If the participant is not able to recall the time spent in a classroom or the number of students present, assist them to come up with a realistic estimate as much as possible. * if you have assisted the participant to come up with this number indicate this by ticking on the tick box next to the question.		
2.3.2 Interview	How much time did you spend the last time you attended church or player meeting?	[Number] (hours) 99 = Don't know	**c_church_dur** [integer; 2 digits; range 01–15, 99]
		Tick box	**c_church_dur_est** [integer; 1 digit; range 1]
2.3.3 Interview	How many people were present the last time you attended church or player meeting?	[Number] 999 = Don't know	**c_church_natt** [integer; 3 digits; range 001–500, 999]
		Tick box	**c_church_natt_est** [integer; 1 digit; range 1]
2.3.3.1 Interview	How many of these people were aged 11 years or below?	[Number] 999 = Don't know	**c_church_nchild** [integer; 3 digits; range 001–500, 999]
		Tick box	**c_church_nchild_est** [integer; 1 digit; range 1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
2.3.3.2 Interview	How many of these people were males aged 12 years and above?	[Number] 999 = Don't know	**c_church_nadultm** [integer; 3 digits; range 001–500, 999]
		Tick box	**c_church_nadult_est** [integer; 1 digit; range 1]
Question 3:	**School attendance**		
3.1 **Piped from participant CRF**	***Do you currently go to school?*** *(This question shows what the participant said on whether they attend school, it guides you how to proceed with Q3.2)*	[Ribbon] 1 No, never been to school 1 Yes, previously 1 Yes, currently	**c_school** [integer; 1 digit; range 1]
3.2. **Interview**	In the last 12 months, did you attend school? *(if participant mentioned that they are currently attending school, you do not need to ask this question again. Select 'Yes' and proceed with the follow-up questions)*	Radio 0 No 1 Yes (*If No, skip to Q3.3*)	**c_school12m** [integer; 1 digit; range 0–1]
3.2.1 Interview	If you are not going to school, why?	Dropdown 1 Illness 2 Finished Matric 3 Taking care of relative 6 Other specify (*Skip to Q4*)	**c_school12m_no_reas** [integer; 1 digit; range 0–X, 6]
3.2.1.1 Interview	Other reason if not on the list	[Text]	**c_school12m_no_reas_o** [text, 30 characters]
3.3. Interview	In the last week, how many days did you go to school? (*if on holiday, ask in the last week attended school*)	[Number]	**c_school_nvis1wk** [integer; 1 digit; range 1–7]
3.4. Interview	When was the last time you attended school?	[Number] (day) 99 = Don't know	**c_school_dattd** [standard AHRI month variable; range 01–31, 99]
		[Number] (month) 99 = Don't know	**c_school_dattm** [standard AHRI month variable; range 01–12, 99]
		[Number] (year)	**c_school_datty** [standard AHRI year variable; range 1998–2018]
	Memory Aid – Attendance to church or Prayer meetings:		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	* Questions 3.4.1 to 3.4.3 ask about the duration of prayer meetings and the number of people present. * If the participant is not able to recall the duration of the prayer meeting or the number of people present, assist them to come up with a realistic estimate as much as possible. * if you have assisted the participant to come up with this number indicate this by ticking on the tick box next to the question.		
3.4.1 Interview	How much time in total did you spend in a classroom the last day that you attended school?	[Number] (hours) 99 = Don't know	**c_school_class_dur** [integer; 2 digits; range 01–12, 99]
		Tick box	**c_school_classdur_est** [integer; 1 digit; range 1]
3.4.2 Interview	What was the highest number of students in your classroom among the classes that you attended?	[Number] 999 = Don't know	**c_school_class_nstud** [integer; 2 digits; range 01–70, 999]
		Tick box	**c_school_classdnstud_est** [integer; 1 digit; range 1]
3.4.2.1 Interview	How many of these students were aged 11 years or below?	[Number] 999 = Don't know	**c_school_class_nchild** [integer; 2 digits; range 01–70, 999]
		Tick box	**c_school_classdnchild_est** [integer; 1 digit; range 1]
3.4.2.2 Interview	How many of these students were males aged 12 years and above?	[Number] 999 = Don't know	**c_school_class_nadultm** [integer; 2 digits; range 01–70, 999]
		Tick box	**c_school_classd_nadultm_est** [integer; 1 digit; range 1]
3.4.3 Interview	On the last day that you attended school, how many people did you contact at school, including your teachers and other students not in your class? (*repeat the above definition of a contact to the participant*)	[Number] 999 = Don't know	**c_school_ncontact** [integer; 2 digits; range 01–70, 999]
		Tick box	**c_school_ncontact_est** [integer; 1 digit; range 1]
Question 4	**Travel on Public Transport**		
4.1 **Piped from participant CRF**	***Do you currently go to school?***	[List] 1 No, never been to school 1 Yes, previously	**c_school_2** [integer; 1 digit; range 1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	(This question shows what the participant said on whether they attend school, it guides you how to proceed with Q4.2)	1 Yes, currently	
4.2 **Interview**	*If currently in school or not been in school in the last 12 months*: In the last 7 days, did you make road trips in a closed vehicle like a minibus, taxi, bakkie/pickup, or private car? *If currently in school but on holiday; ask in the last week you attended school.*	Radio 0 No 1 Yes (*If No, skip to Q4.2*)	**c_rdtrip** [integer; 1 digit; range 0–1]
4.2.1 Interview	If yes, how many road trips in a vehicle did you make in the last 7 days? *If on holiday, ask in the last week you attended school.*	[Number] 99 = Don't know	**c_rdtrip_n1wk** [integer; 2 digits; range 01–20, 99]
4.3 Interview	When was the last time you made a road trip in a vehicle?	[Number] (day) 99 = Don't know	**c_rdtrip_dlastd** [standard AHRI month variable; range 01–31, 97, 99]
		[Number] (month) 99 = Don't know	**c_rdtrip_dlastm** [standard AHRI month variable; range 01–12, 97, 99]
		[Number] (year)	**c_rdtrip_dlasty** [standard AHRI year variable; range 1998–2017]
	Memory Aid – Travel on public transport: * Questions 4.3.1 to 4.3.2.2 ask about the duration of road trips and the number of other passenger. * If the participant is not able to recall the duration of the road trip or the number of passengers assist them to come up with a realistic estimate as much as possible. * if you have assisted the participant to come up with this number indicate this by ticking on the tick box next to the question.		
4.3.1 Interview	On the last day that you travelled, how much time were you in a vehicle in total?	[Number] (hours) 99 = Don't know	**c_rdtrip_dur** [integer; 2 digits; range 01–20, 99]
		Tick box	**c_rdtripdur_est** [integer; 1 digits; range: 1]
4.3.2 Interview	On the last day that you travelled, how many people were in the vehicle with you?	[Number] 99 = Don't know	**c_rdtrip_npasseng** [integer; 2 digits; range 01–70, 99]
		Tick box	**c_rdtrip_npasseng_est**

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
			[integer; 1 digits; range: 1]
4.3.2.1 Interview	How many of these people were aged 11 years or below?	[Number] 99 = Don't know	**c_rdtrip_nchild** [integer; 2 digits; range 01–70, 99]
		Tick box	**c_rdtrip_nchild_est** [integer; 1 digits; range: 1]
4.3.2.2 Interview	How many of these people were males aged 12 years and above?	[Number] 99 = Don't know	**c_rdtrip_nadultm** [integer; 2 digits; range 01–70, 99]
		Tick box	**c_rdtrip_nadultm_est** [integer; 1 digits; range: 1]
Question 5:	**Visit to bars, nightclubs, or shebeens**		
5.1 **Interview**	In the last 12 months, did you go to a bar, nightclub, or shebeen?	Radio 0 No 1 Yes (*If No, skip to 6)*	**c_barvis** [integer; 1 digits; range 0–1]
5.1.1 Interview	In the last four weeks, how many times did you go to bars, nightclubs, or shebeens?	[Number] 99 = Don't know	**c_barvis_n1m** [integer; 2 digits; range 01–20, 99]
5.2 Interview	When was the last time that you went to a bar, nightclub, or shebeen?	[Number] (month) 97 = Never 99 = Don't know	**c_barvis_dvism** [standard AHRI month variable; range 01–12, 97, 99]
		[Number] (year) 9997 = Never	**c_barvis_dvisy** [standard AHRI year variable; range 1998–2017, 9997]
	Memory Aid – Visits to bars and night clubs: * Questions 5.2.1 to 5.3.2.1 ask about the duration of visits to night clubs and the number of people present during the visit. * If the participant is not able to recall the duration of the visit or the number of people present, assist them to come up with a realistic estimate as much as possible. * if you have assisted the participant to come up with this number indicate this by ticking on the tick box next to the question.		
5.2.1 Interview	How much time did spend at the bar, nightclub, or shebeen at your last visit?	[Number] (hours) 99 = Don't know	**c_barvis_dur** [integer; 2 digits; range 01–20, 99]
		Tick box	**c_barvis_dur_est** [integer; 1 digits; range1]

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
5.2.2 Interview	The last time you were at bar was it indoors or outdoors?	Drop down 1 Entirely indoors 2 Mostly indoors 3 Mostly outdoors 4 Entirely outdoors	**c_barvis_indoor** [integer; 1 digit; range 1–4]
5.3.2 Interview	How many people were present at your last visit to a bar, nightclub, or shebeen?	[Number] 999 = Don't know	**c_barvis_natt** [integer; 3 digits; range 001–200, 999]
		Tick box	**c_barvis_natt_est** [integer; 1 digits; range1]
5.3.2.1 Interview	How many of these people were males aged 12 years and above?	[Number] 999 = Don't know	**c_barvis_nadultm** [integer; 3 digits; range 001–200, 999]
		Tick box	**c_barvis_nadultm_est** [integer; 1 digits; range1]
Question 6:	**Visits to other places**		
6.1 **Interview**	In the last 3 months, did you visit any other gathering places apart from school, church, hospital, clinic, bar and public transport?	Radio 0 No 1 Yes (*If No, skip to the next instrument*)	**c_othvis** [integer; 1 digit; range 0–1]
6.1.1 Interview	If yes, can you tell me more about the gathering places that you visited in the last 3 months?		**c_othvis_det**
6.1.1.1 Interview	If yes, what is the name of this this gathering place?	[Text]	**c_othvis1_loc** [string; 30 characters]
6.1.1.2 Interview	When was the last time that you went to this place?	[Number] (day) 99 = Don't know	**c_othvis1_dvisd** [standard AHRI month variable; range 01–31, 99]
		[Number] (month) 99 = Don't know	**c_othvis1_dvism** [standard AHRI month variable; range 01–12, 99]
		[Number] (year)	**c_othvis1_dvisy** [standard AHRI year variable; range 1998–2017]
	Memory Aid – Visits [*Other Places*]:		

Q number; source	Question	Var type; dropdown options; skip logic	Var name; field type; size; limits
	* Questions 6.1.1.3 to 6.1.1.5.2 ask about the duration of visits to [other places] and the number of people present during the visit. * If the participant is not able to recall the duration of the visit or the number of people present, assist them to come up with a realistic estimate as much as possible. * if you have assisted the participant to come up with this number indicate this by ticking on the tick box next to the question.		
6.1.1.3 Interview	The last time you went to this place, how much time did you spend?	[Number] (hours)	**c_othvis1_dur** [integer; 2 digits; range 01–20]
		Tick box	**c_othvis1_dur_est** [integer; 1 digits; range 1]
6.1.1.4 Interview	Was this gathering place indoors or outdoors?	Drop down 1 Entirely indoors 2 Mostly indoors 3 Mostly outdoors 4 Entirely outdoors	**c_othvis1_indoor** [integer; 1 digit; range 1–4]
6.1.1.5 Interview	How many people were present at your last visit?	[Number] 999 = Don't know	**c_othvis1_natt** [integer; 3 digits; range 001–500, 999]
		Tick box	**c_othvis1_natt_est** [integer; 1 digits; range 1]
6.1.1.5.1 Interview	How many of the people present were aged 11 years or below?	[Number] 999 = Don't know	**c_othvis1_nchild** [integer; 3 digits; range 001–500, 999]
		Tick box	**c_othvis1_nchild_est** [integer; 1 digits; range 1]
6.1.1.5.2 Interview	How many of the people present were males aged 12 years and above?	[Number] 999 = Don't know	**c_othvis1_nadultm** [integer; 3 digits; range 001–500, 999]
		Tick box	**c_othvis1_nadultm_est** [integer; 1 digits; range 1]
6.1.1.6 Interview	Is there another gathering place that you visited in the last 3 months that you have not already told me about?	Radio 0 No 1 Yes (*If No, continue to Q7;* *If Yes, add sub-form [Q6.1.1–6.1.7]*)	**c_othvis1_another** [integer; 1 digit; range 0–1)
			Sub-form variables: second visit [c_othvis2_...]; third visit [c_othvis3_...]; etc.

Appendix 11: Study questionnaire for parent/guardian

Contact Attempts

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	Memory Aid * Introduce AHRI * I am a nurse (AHRI uniform and badges) * Structure and time outline * Find private space to speak with parent		
	Section taken from PIP *[Up to 6 attempts will be made. At 4th attempt, provide information to study coordinator to evaluate whether further attempts are necessary.]*		

Personal Information

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
1 Pre-loaded	Participant's (child's) name:	[Text]	**p_parti_name** [Text, 50 characters]
2 Interview	*Is the participant's (child's) pre-loaded name correct?*	**Ribbon** 0 No 1 Yes If Yes, Skip to Q3	**p_partnam_corr** [integer; 1 digit; range 0–1]
2.1 Interview	If Not correct, please provide the correct participant's name:	[Text] (First Name)	**p_parti_corfname** [Text, 50 characters]
		[Text] (Last Name)	**p_parti_corlname** [Text, 50 characters]
3 Pre-loaded	Participant's sex	**Ribbon** 1 Male 2 Female	**p_sex** [integer; 1 digit; range 1,2]
4 Pre-loaded	Participant's (child's) date of birth	[Date]	**p_dobd** [standard AHRI date variable; range 1999/01/01-2009/12/31]
5 Pre-loaded	Age	Number	**p_age** [integer; 2 digits; range 10-19]
6 Interview	*Is the participant's (child's) pre-printed date of birth correct?*	**Ribbon** 0 No	**p_d_prep_dob** [integer; 1 digit; range 0–1]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
		1 Yes If Yes, Skip to Q7	
6.1 Interview	If not correct, please provide the correct date of birth If exact date of birth is not known, please request to see a ID or birth certificate and check the date of birth from this document. If document is not available and only the year of birth is known, enter 15 as the day of birth and June as the month of birth.	**[Date]**	**p_correct_dob** [integer; 2 digits; range 01-31]
	Age Eligibility Memory Aid ** Participants are eligible if date of birth is after 1 May 1999 and before 30 April 2008*		
6.2 Interview	*Basing on the corrected date of birth, is the participant still eligible?*	**Ribbon** 0 No 1 Yes If Yes, Go to Q7	**p_age_eligible** [integer; 1 digit; range 0–1]
6.2.1 calculated	Participant's correct age	Number	**p_age_correct**
If No, Pop Up Message: Participant if not eligible, please terminate the interview			
7 Pre-loaded	BSID	Number	**p_resp_bsid** [integer; 7 digits; range 0000000-9999999]
8 Pre-loaded	BS Owner	Text	**p_bs_owner** [text; 50 characters]
9 Pre-loaded	Location/Isigodi	Text	**p_location_isigodi** [text; 50 characters]
10 Pre-loaded	Household Head	Text	**p_hh_head** [text; 50 characters]
11 Interview	*Is the respondent the head of the household?*	**Ribbon** 0 No 1 Yes	**p_resp_hhead** [integer; 1 digit; range 0–1]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
		If Yes, Skip to Q12	
11.1 Interview	Respondent's first name	Text	**p_resp_fname** [text; 50 characters]
11.2 Interview	Respondent's last name	Text	**p_resp_lname** [text; 50 characters]
11.3 Interview	Respondent's sex	**Ribbon** 1 Male 2 Female	**p_resp_sex** [integer; 1 digit; range 1,2]
11.4 Interview	Respondent's date of birth	[Date]	**p_resp_dob** [standard AHRI date variable; range 1900/01/01-2009/12/31]
11.5 Interview	Respondents relationship to participant:	**Drop down** 1 Biological Parent (Father/Mother) 2 Legal guardian 3 Foster parent caregiver 4 Nominated trusted adult (*If 3 or 4 go to Q11.5.1)*	**p_resp_rel** [integer; 2 digit; range 1-6, 96]
11.5.1 Interview	Specify relationship	**Text**	**p_resp_rel_o** [Text, 50 characters]
12 Interview	Interviewer code	**Drop down** 1 Staffcode1 2 Staffcode2 3 Staffcode3 4 Staffcode4	**p_intv_code** [integer; 1 digit; range 1,2]
13 Interview	Start time of interview	[Time]	**p_resp_dob** [standard AHRI time variable; 00.00-23:59]
14 Interview	Date of Interview	[Date]	**p_date_of_interview** [standard AHRI date variable; range 2017/11/01-2019/12/31]

Assessment of Capacity to Consent

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	Section taken from PIP		

Eligibility Checklist

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	Eligibility Checklist (Previous/current TB treatment) *Before giving detailed information about the study, perform the following eligibility checklist for the study participant*		
1.1 **Interview**	Has your child ever received treatment for TB disease? *(If needed, explain that treatment for **TB disease** will have been with at least four drugs [though it may have been in one tablet, e.g., Rifafour] every day for two months, and then two drugs [again, may have been in one tablet, e.g., Rifinah] every day for a further four months [i.e., a total of at least 6 months].* *This is different from **isoniazid preventive therapy** [IPT], which is used to treat **TB infection**, and will have involved taking one drug every day for six months.)*	[Radio] 0 No 1 Yes 9 Don't know	**p_tbtx_ever** [integer; 1 digit; range 0–1, 9]
	if No or Don't Know, Pop Up Message: ***Please provide detailed information about the study to the parent/guardian and obtain informed consent***		
1.1.1 Interview	If yes, when did your child start TB treatment? (*If more than once, get details for the most recent episode*)	[Dropdown] 99 = Don't know	**p_tbtx_dstartm** [standard AHRI month variable; range 01-12, 99]
		[Dropdown] 9999=don't know	**p_tbtx_dstarty** [standard AHRI year variable; range 1998–2017]
1.1.2 Interview	Where (which facility) did your child start TB treatment?	[Dropdown] [List of clinics in PIP] 96 = Other (specify) 99 = Don't know	**p_tbtx_clin** [integer; 2 digits; range 1–XX, 96, 99]
1.1.2.1 Interview	Other, specify	[Text]	**p_tbtx_clin_o** [string; 30 characters]
	If Q1.1 is Yes, Pop Up Message ***Participant is not eligible please go to the End of Survey Instrument and conclude the interview***		

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	Informed consent – Memory aid ** If the parent is not able to read or write, ask for a witness within the home to witness the process of giving information and obtaining consent*		
	Insert Informed Consent Information sheet		
1.1 Interview	*Name of interviewer*	*Text*	**p_staff_name** [Text; 50 Characters]
1.2 Interview	*Signature*	Picture	**p_staff_signature** [format: .png]
1.3 Interview	*Date*	Date and time	**p_staff_date** [standard AHRI date variable; range 2017/11/01-2019/12/31, 00.00-23.59]
1.4 Interview	*Did parent/guardian consent to participation in the study?*	[Radio] 0 No 1 Yes (*If No, Show IC1*)	**p_consent** [integer; 1 digit; range 0–1]
	If No, Pop Up Message ***Parent/guardian refused participation, please go to the End of Survey Instrument and conclude the interview.***		
1.5 Interview	*Name of respondent*	*Text*	**p_pg_name** [Text; 50 Characters]
1.6 Interview	*Signature*	Picture	**p_pg_signature** [format: .png]
1.7 Interview	*Date*	Date and time	**p_pg_date** [standard AHRI date variable; range 2017/11/01-2019/12/31, 00.00-23.59]
1.8 Interview	*Is the parent/guardian able to sign*	[Radio] 0 No 1 Yes *(If Yes, No go to Q1.8.1)*	**p_sign** [integer; 1 digit; range 0–1]
1.8.1 Interview	*Name of witness*	*Text*	**p_witness_name** [Text; 50 Characters]
1.8.2 Interview	*Signature*	Picture	**p_witness_signature** [format: .png]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
1.8.3 **Interview**	*Date*	Date and time	**p_witness_date** [standard AHRI date variable; range 2017/11/01-2019/12/31, 00.00-23.59]

Adolescent TB Study (Study specific questions)

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
Question 5	**BCG Vaccination**		
5.1 **Interview**	Has your child been vaccinated against TB? (*If the parent is unsure, explain that this would have been an injection in one of the child's arms at birth [before the mother was discharged from hospital] or within the first year of the child's life. The site of the injection may have become very inflamed (a swollen and painful blister), and may have developed into an opening in the skin with pus. This will then have left a scar in the child's arm.)*	[Radio] 0 No 1 Yes 9 Don't know	**p_bcg** [integer; 1 digit; range 0–1, 9]
5.2 **Interview**	Is your child's road to health card available? (*Ask parent/guardian if RTH card is available and if you can have a look*)	[Radio] 0 No 1 Yes *(If No, skip to Q6)*	**p_rthc** [integer; 1 digit; range: 0–1]
5.2.1 Interview	*Is BCG vaccination documented?*	[Radio] 0 No 1 Yes	**p_bcg_doc** [integer; 1 digit; range: 0–1]
Question 6	**Health care utilisation**		
6.1 **Interview**	Has your child ever been admitted to hospital? *(Explain, if needed, that 'admitted' means sleeping for at least one night in a hospital)*	[Dropdown] 0 No 1 Yes 9 Don't know (*if No, skip to Q7*)	**p_adm_ever** [integer; 1 digit; range 0–1, 9]
6.1.1 Interview	If yes, how many times has your child ever been admitted to hospital?	[Number] 99 = Don't know	**p_adm_num** [integer; 2 digits; range 01–20, 99]
6.1.2 Interview	When was your child most recently admitted to hospital?	[Dropdown] 99 = Don't know	**p_dadmm**

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
			[standard AHRI month variable; range 01–12, 99]
		[Dropdown] 9999=don't know	**p_dadmy** [standard AHRI year variable; range 1998–2017]
6.1.3 Interview	Where (to which hospital) was your child most recently admitted?	[Dropdown] [List of facilities in PIP] 96 = Other (specify) 99 = Don't know	**p_adm_hosp** [integer; 2 digits; range 01–XX, 96]
6.1.3.1 Interview	Other, specify	[Text]	**p_adm_hosp_o** [string; 50 characters]
6.1.4 Interview	What was the reason for your child's most recent admission to hospital?	[Dropdown] 01 Respiratory tract infection 02 Diarrhoea 03 Injury 04 Convulsions (fits) 05 Other infection 06 Surgery 96 Other, specify 99 Don't know *(If 1, 2, 3, 4, 5, or 9, skip to Q7)*	**p_adm_reas** [integer; 1 digit; range 01–06,96,99]
6.1.4.1 Interview	Other, specify	[Text]	**p_adm_reas_o** [string; 50 characters]
Question 7	**TB contacts: Household**		
7.1 **Interview**	Has anyone in your homestead (or residential plot) ever had TB disease during your child's lifetime? *(Including those who might not have started TB treatment but were told by a clinician that they had TB disease*)	[Dropdown] 0 No 1 Yes 9 Don't know (*if No or don't know, skip to Q7.2*)	**p_hh_tbever** [integer; 1 digit; range 0–1, 9]
7.1.1 Interview	Is anybody in your household **currently** on TB treatment?	[Dropdown] 0 No 1 Yes 9 Don't know	**p_hh_tbcur** [integer; 1 digit; range 0–1, 9]
		TB contacts memory aid	

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	Please tell the parent that you would like to ask them a few questions about these people. Tell them that you will ask about each person separately and encourage them to think back and remember how many individuals in their homestead had TB in their child's lifetime		
	Details of person(s)		
7.1.2 Interview	What was the age of this person at the time they lived in the same homestead as your child?	[Number] 999 = Don't know	**p_curhh_tbcontact** [integer; 3 digits; range 001–099, 999]
7.1.3 Interview	What is the sex of this person?	[Radio] 1 Male 2 Female	**p_curhh_tbcontactsex** [integer; 1 digit; range: 1–2]
7.1.4 Interview	In which year did your child live in the same homestead as this person?	[Dropdown] 9999 = Don't know	**p_curhh_tbcontactyr** [Integer; 4 digits; range 1998–2018, 9999]
7.1.5 Interview	In which isigodi was the person staying at the time?	[Dropdown] [List of isigodis] 96 = Other, specify 99 = Don't know *If 96 go to 7.1.4.1, otherwise skip to 7.1.5*	**p_curhh_tbcontactisi** [integer; 2 digits; range 01–XX, 96, 99]
7.1.5.1 Interview	Other, specify	[Text]	**p_curhh_tbcontactisi_o** [string; 30 characters]
7.1.6 Interview	Did your child sleep in the same **house** as this person when the person was ill?	[Radio] 0 No 1 Yes 9 Don't know *(if No or Don't know, skip to Q7.1.6)*	**p_curhh_contacthouse** [integer; 1 digit; range 0–1, 9]
7.1.6.1 Interview	Did your child sleep in the same **room** as this person when the person was ill?	[Dropdown] 0 No 1 Yes 9 Don't know	**p_curhh_tbcontactshare** [integer; 1 digit; range 0–1, 9]
7.1.7 Interview	Was your child involved in the care of this person when the person was ill? *(i.e., your child came into close contact with this person whilst caring for them)*	[Dropdown] 0 No 1 Yes 9 Don't know	**p_curhh_tbcontactcare** [integer; 1 digit; range 0–1, 9]
7.1.8 Interview	Apart from the person(s) described above, has anyone else in your homestead ever had TB disease during your child's lifetime?	[Radio] 0 No 1 Yes *[If Yes, add new sub-form (Qs 7.1.1–7.1.7)]*	**p_curhh_tbcontactotherp** [integer; 1 digit; range 0–1]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	Encourage the parent to think back about whether there have been any others		
	TB Contacts in other households		
7.2 Interview	Apart from your homestead (or residential plot), has your child ever lived (slept overnight) in the same homestead (or residential plot) as someone with TB for a total of 2 weeks or more? *(Including those who might not have started TB treatment but were told by a clinician that they had TB disease*)	[Dropdown] 0 No 1 Yes 9 Don't know *(If No or Don't know, skip to Q8)*	**p_hh_tbcontact** [integer; 1 digits; range 0–1,9]
	Details of person(s)		
7.2.1.1 Interview	What was the age of this person at the time they lived in the same homestead as your child?	[Number] 999 = Don't know	**p_hh_tbcontact** [integer; 3 digits; range 001–099, 999]
7.2.1.2 Interview	What is the sex of this person?	[Radio] 1 Male 2 Female	**p_hh_tbcontactsex** [integer; 1 digit; range: 1–2]
7.2.1.3 Interview	In which year did your child live in the same homestead as this person?	[Dropdown] 9999 = Don't know	**p_hh_tbcontactyr** [Integer; 4 digits; range 1998–2018, 9999]
7.2.1.4 Interview	In which isigodi was the person staying at the time?	[Dropdown] [List of isigodis] 96 = Other, specify 99 = Don't know	**p_hh_tbcontactisi** [integer; 2 digits; range 01–XX, 96, 99]
7.2.1.4.1 Interview	Other, specify	[Text]	**p_hh_tbcontactisi_o** [string; 30 characters]
7.2.1.5 Interview	Did your child sleep in the same **house** as this person when the person was ill?	[Radio] 0 No 1 Yes 9 Don't know *(if No or Don't know, skip to 7.2.1.7)*	**p_hh_contacthouse** [integer; 1 digit; range 0–1, 9]
7.2.1.6 Interview	Did your child sleep in the same **room** as this person when the person was ill?	[Dropdown] 0 No 1 Yes 9 Don't know	**p_hh_tbcontactshare** [integer; 1 digit; range 0–1, 9]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
7.2.1.7 Interview	Was your child involved in the care of this person when the person was ill? *(i.e., your child came into close contact with this person whilst caring for them)*	[Dropdown] 0 No 1 Yes 9 Don't know	**p_hh_tbcontactcare** [integer; 1 digit; range 0–1, 9]
7.2.1.8 Interview	Apart from the person(s) described above, has your child ever lived (slept overnight) in the same homestead as another person with TB? *Encourage the parent to think back about whether there have been any others*	[Radio] 0 No 1 Yes *[If Yes, add sub-form (Qs 7.2.1.1–7.2.1.8)]*	**p_hh_tbcontactotherp** [integer; 1 digit; range 0–1]
Question 8	**TB contacts: Carer**		
8.1 **Interview**	Apart from people with TB who lived in the same homestead as your child (the ones mentioned in the sections above), has your child ever been involved in the care of another person with TB?	[Dropdown] 0 No 1 Yes 9 Don't know *(If No or Don't know, skip to Q9)*	**p_tb_care** [integer; 1 digit; range 0–1, 9]
	Details of person(s)		
8.1.1 Interview	What was the age of this person at the time your child was involved in their care?	[Number] 999 = Don't know	**p_tb_careage** [integer; 3 digits; range 001–099, 999]
8.1.2 Interview	What is the sex of this person?	[Radio] 1 Male 2 Female	**p_tb_caresex** [integer; 1 digit; range 1–2]
8.1.3 Interview	In which isigodi was this person staying at the time?	[Dropdown] [List of isigodis] 96 = Other, specify 99 = Don't know	**p_tb_careisi** [integer; 2 digits; range 01–XX, 96, 99]
8.1.3.1 Interview	Other, specify	[Text]	**p_tb_careisi_o** [string; 30 characters]
8.1.4 Interview	In which year did your child care for this person?	[Dropdown] 9999 = Don't know	**p_tb_careyr** [integer; 4 digits; range 1998–2018, 9999]
8.1.5 Interview	Is there another person with TB who did NOT live in the same house as your child, but in whose care your child was involved when this person was ill?	[Radio] 0 No 1 Yes *[If Yes, add sub-form (Qs 8.1.1–8.1.5)]*	**p_tb_careotherp** [integer; 1 digit; range 0–1]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	Encourage the parent to think back about whether there have been any others		
Question 9	**Chronic conditions** *(ARV uptake **can** be captured and noted at this point. If the parent indicates that the child is taking ARVs, record details later, in the HIV section)*		
9.1 Interview	Does your child have asthma?	[Radio] 0 No 1 Yes (*if No, skip to Q9.2*)	**p_asthma** [integer; 1 digit; range 0–1]
9.1.1. Interview	If Yes, does your child take regular medication for asthma?	[Radio] 0 No 1 Yes (*if No, skip to Q9.2*)	**p_asthmamed_take** [integer; 1 digit; range 0–1]
9.1.1.1 Interview	If Yes, what medication? (*Select all that apply*)	[Radio] 01 Short-acting beta-agonist inhaler (e.g., Albuterol, Asthavent, Fenoterol, Salbutamol, Terbutaline, Ventolin, Volmax) 02 Long-acting beta-agonist inhaler (e.g., Formoterol, Foradil, Foratec, Foxair, Oxis, Salmeterol, Serevent) 03 Steroid inhaler (e.g., Aerobec, Beclomethasone, Beclate, Becotide, Beloforte, Budeflam, Budesonide, Ciclesonide, Clenil, Flixotide, Flomist, Fluticasone, Inflammide, Rhinocort, Viarox) 04 Combination inhaler (e.g., Budesonide-Formoterol, Seretide, Symbicort 05 Oral steroid (e.g., Prednisone, prednisolone, methylprednisone, methylprednisolone) 06 Oral leukotriene receptor antagonists	**p_asthmamed** [integer; 2 digits; range 01-06,96,99]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
		(e.g., Accolate, Montelukast, Singulair, Zafirlukast) 96 Other, specify 99 Don't know (*if No, skip to Q9.2*)	
9.1.1.1.1 Interview	Other, specify	[Text]	**p_asthmamed_o** [text; 30 characters]
9.1.1.2 Interview	When did your child start taking regular medication for asthma?	[Dropdown] 99 = Don't know	**p_asthmamed_dstartm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**p_asthmamed_dstarty** [standard AHRI year variable; range 1998–2017]
9.2 **Interview**	Is your child diabetic (or has diabetes)?	[Radio] 0 No 1 Yes (*if No, skip to Q9.3*)	**p_diab** [integer; 1 digit; range 0–1]
9.2.1 Interview	If Yes, is your child taking regular medication for diabetes?	[Radio] 0 No 1 Yes (*if No, skip to Q9.3*)	**p_diabmed** [integer; 1 digit; range 0–1]
9.2.1.1 Interview	If Yes, what medication is your child taking? (*Select all that apply*)	[Tick box] 01 **Biguanide** (e.g., Metformin [Bigsens, Diabetmin, Diaformin, Diamin, Diaphage, Forminal, Glucophage, Mengen, Metcheck, Metforal, Metored, Romidab]) 02 **Sulphonylurea** (e.g., Glibenclamide [Daonil, Glycomin]; Gliclazide [Adco-Glucomed, Diaglucide, Diaglumed, Diamicron, Gycron, Glygard]; Glimepiride [Amaryl, Diaglim, Euglim, Glamaryl, Sulphonur]; Glipizide) 03 **PPAR agonist** (e.g., Pioglitazone [Actos])	**p_diabmed_o** [integer; 1 digit; range 0–1]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
		04 **SGLT2-inhibitor** (e.g., Canagliflozin, Dapagliflozin, Empagliflozin) 05 **DPP-4 inhibitor** (e.g., Saxagliptin [Onglyza, Kombiglyze], Sitagliptin [Januvia, Janumet, Juvisync], Vildagliptin) 06 **GLP-1 agonist** (e.g., Exenatide [Byetta, Bydureon], Liraglutide [Victoza], Lixisenatide, Semaglutide) 07 **Alpha-glucosidase inhibitor** (e.g., Acarbose) 08 **Short-acting insulin** (e.g., Isophane insulin [Humulin, Insulin-HMGE-Protaphane], Insulin Lispro [Humalog]) 09 **Long-acting insulin** (e.g., Glargine [Basaglar, Lantus, Optisulin]) 96 Other, specify 99 Don't know	
9.2.1.1.1 Interview	Other, Specify		**p_diabmed_o** [integer; 1 digit; range 0–1]
9.2.1.2 Interview	When did your child start taking regular medication for diabetes?	[Dropdown] 99 = Don't know	**p_regmed_dstartm** [standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**p_regmed_dstarty** [standard AHRI year variable; range 1998–2017]
9.3 Interview	Does your child have any other chronic or long-term illness?	[Radio] 0 No 1 Yes (*if No, skip to Q10*)	**p_cillness** [integer; 1 digit; range 0–1]
	[*add sub-form for each illness*]		
9.3.1.1	If yes, what illnesses?	[Dropdown]	**p_cillness_det**

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
Interview		1 HIV/AIDS 2 Epilepsy 6 Other (Specify) 9 Don't know (*If [1] HIV/AIDS, skip to Q9.3.1.3, inform the parent that you will ask them for more details later*]	[integer; 1 digit; range 1–2, 6, 9]
9.3.1.1.1 Interview	Other, specify **[*only appear if 6*]**	[Text]	**p_cillness_det_o** [string, 30 characters]
9.3.1.2. Interview	Does your child take regular medication for this illness?	[Dropdown] 0 No 1 Yes 9 Don't know *(If No, Skip to 9.3.1.3)* *(If yes, and illness=epilepsy – go to 9.3.1.2.1* *If yes, and illness = other – go to 9.3.1.2.1.1*	**p_regmed** [integer; 1 digit; range 0–1, 9]
9.3.1.2.1 Interview	If Yes, what medication?	[Dropdown] [List of medications] 1 Sodium valproate (e.g., Epilim, Epilizine, Epiproate, Eprolep, Navalpro, Valeptic) 2 Phenytoin sodium (e.g., Epanutin, Phlexy) 3 Carbamazepine (e.g., Degranol, Tegretol) 4 Lamotrigine (e.g., Epitec, Girotec, Lamictin, Lamidus, Lamitor) 5 Levetiracetam (e.g., Epikepp, Keppra, Redilev, Torcetam) 6 Other, specify 9 Don't know	**p_regmed_det** [integer; 2 digits; range 01–XX, 96, 99]
9.3.1.2.1.1 Interview	Specify medication **[*only appear if 6 – or if 9.3.1.1 = other illness & 9.3.1.2=yes*]**	[Text]	**p_regmed_det_o** [string; 50 characters]
9.3.1.2.2		[Dropdown]	**p_regmed_dstartm**

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
Interview	When did your child start taking regular medication for this condition?	99 = Don't know	[standard AHRI month variable; range 1–12, 99]
		[Dropdown] 9999=don't know	**p_regmed_dstarty** [standard AHRI year variable; range 1998–2017]
9.3.1.3 Interview	Does your child have any other chronic illness?	[Radio] 0 No 1 Yes ***[if Yes, add sub-form for questions 9.3.1-9.3.3]***	**p_cillness_o** [integer; 1 digit; range 0–1]
Question 10	**HIV Section** **HIV Section Memory Aid** ** Assess parental knowledge of HIV* ** Give general HIV information* ** Benefits of treatment / routes of transmission* ** Need to know re ARVs due to impact on test*		
10.1 **Piped from Q9.3.1.1**	***What chronic illnesses did the parent mention in Q 9*** *(This question displays the chronic illnesses that the parent/guardian mentioned in Q9. This helps you how to proceed if the parent had already told you that the child is HIV positive)*	Ribbon 1, [p_cillness] 2, [p_cillness_2] 3, [p_cillness_3]	p_cillness_piped [integer, 1 digit, range 1]
10.2 **Interview**	Has your child ever tested for HIV before? ***If the parent said the child is HIV positive:*** You mentioned that your child is HIV positive; I assume your child has tested for HIV before?	[Dropdown] 0 No 1 Yes 2 Prefer not to say 9 Don't know (*If Yes, go to 10.2.1;* *If 0,2 or 9, skip to Q11*)	**p_hivtest_ever** [integer; 1 digit; range 0–2, 9]
10.2.1 **Interview**	If yes, what was the result? ***if parent said child is HIV positive in section 9;*** *Select Positive, and proceed with follow up questions:*	[Dropdown] 0 Negative 1 Positive 9 Don't know (*if Negative or Don't know, skip to Q*10.2.1.2)	**p_hivres** [integer; 1 digit; range 0–1, 9]
10.2.1.1	If positive, when did your child first test positive?	[Dropdown]	**p_hivres_pos_dtestm**

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
Interview		99 = Don't know	[standard AHRI month variable; range 01–12, 99]
		[Dropdown] 9999=don't know	**p_hivres_pos_dtesty** [standard AHRI year variable, range 1998-2017]
10.2.1.1.1 Interview	Where did your child first test positive?	[Dropdown] 1 DOH Hospital 2 DOH clinic 3 AHRI Home 4 AHRI Mobile Clinic 6 Other, specify 9 Don't know	**p_hivptest_loc** [integer; 1 digit; range 1–4, 6, 9]
10.2.1.1.1.1 Interview	Other, specify	[Text]	**p_hivptest_loc_o** [string; 30 characters]
10.2.1.2 Interview	If Negative, when was your child's most recent test?	[Dropdown] 99 = Don't know	**p_hivtest_neg_drecm** [standard AHRI month variable; range 01–12, 99]
		[Dropdown] 9999=don't know	**p_hivtest_neg_drecy** [standard AHRI year variable; range 1998–2017]
10.2.1.2.1 Interview	Where did your child most recently test for HIV	[Dropdown] 1 DOH Hospital 2 DOH clinic 3 AHRI Home 4 AHRI Mobile Clinic 6 Other, specify 9 Don't know	**p_hivntest_loc** [integer; 1 digit; range 1–4, 6, 9]
10.2.1.2.1.1 Interview	Other, specify	[Text]	**p_hivntest_loc_o** [string; 30 characters]
Question 11	**IPT and ART**		
11.1 **Interview**	Has your child ever taken isoniazid preventive therapy (IPT)?	[Radio] 0 No 1 Yes 9 Don't know	**p_ipt_ever** [integer; 1 digit; range 0–1, 9]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	(*explain to the respondent that this is treatment given to prevent development of TB disease unlike TB treatment which is given to treat TB disease*) ***Pop-up information:*** *[If needed, explain that treatment for **TB disease** will have been with at least four drugs [though it may have been in one tablet, e.g., Rifafour] every day for two months, and then two drugs [again, may have been in one tablet, e.g., Rifinah] every day for a further four months [i.e., a total of at least 6 months].* *This is different from **isoniazid preventive therapy** [IPT], which is given to prevent development of **TB disease**, and will have involved taking one drug every day for six months.]*	*(If No or Don't know skip to Q11.3)*	
11.1.1 Interview	If Yes, when did your child stop taking IPT?	[Dropdown] 97 = Currently on IPT 99 = Don't know	**p_ipt_dstopm** [standard AHRI month variable; range 01–12, 97, 99]
		[Dropdown] 9997 = Currently on IPT 9999=don't know	**p_ipt_dstopy** [standard AHRI year variable, range 1998–2017, 9997]
11.2 **Piped variable from Q9.3**	***What chronic illnesses did the parent mention in section 9*** *(This question displays the chronic illnesses that the parent/guardian mentioned in Q9. This helps you know if the parent already told you that the participant (child) is HIV positive.)*	[Ribbon] 1,[p_cillness] 2,[p_cillness_2] 3,[p_cillness_3]	**p_cillness_piped_2** [integer, 1 digit, range 1-3]
	Memory aid – HIV Status * Think back about what the parent/guardian said regarding the participant's HIV status. They may have already told you that the child is on ART. Use the information to guide you how to ask the next set of questions about ART uptake.		
11.3 **Interview**	**Has your child ever taken ARVs (including PEP or PrEP)?**	[Dropdown] 0 No 1 Yes 2 Prefer not to say	**p_arv_ever** [integer; 1 digit; range 0–2, 9]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	(*if the parent had already mentioned that the child is HIV positive and taking ARVs, select YES for this question and proceed with follow-up questions*) *If needed, explain to the parent/guardian that ARVs may be given to HIV negative individuals in the form of PrEP or PEP.* ** PrEP (Pre-Exposure Prophylaxis) is given to people who are at high-risk, to reduce their risk of acquiring HIV and* ** PEP (Post Exposure Prophylaxis) is given after someone may have been exposed to HIV (e.g., through a needlestick injury) to prevent them from becoming infected.*	9 Don't know (*if No, Prefer not to say, or Don't know **AND** the child was reported HIV-positive and less than 15 years, skip to **Q11.4***) (*if No, Prefer not to say, or Don't know **AND** the child was reported HIV-positive, skip to **Q12***) *(If No, Prefer not to say, or Don't know **AND** the child was reported HIV-negative or with unknown HIV status, skip to **Q13**)*	
11.3.1 Interview	If Yes, why did your child take ARVs/PEP/PrEP? (*if the parent had already mentioned that the child is HIV positive and taking ARVs select "For Routine care" for this question and proceed with follow-up questions*	[Radio] 1 For routine HIV care 2 As **post**-exposure prophylaxis (PEP) 3 As **pre**-exposure prophylaxis (PrEP) 6 Other, specify 9 Don't know (*if 6 go to 11.3.1.1 otherwise skip to 11. 3.2*) (if 1, and parent has not mentioned child is HIV positive, please show pop up message)	**p_arv_reas** [integer; 1 digit; range 0–3, 6, 9]
	Pop Up Message ***Parent has not previously mentioned that child is HIV positive, please check***		
11.3.1.1 Interview	Other reason if not on list	[Text]	**p_arv_reas_o** [text; 30 characters]
11.3.2 Interview	If Yes, when did your child first start taking ARVs/PrEP/PEP?	[Dropdown] 99 = Don't know	**p_arv_dstartm** [standard AHRI month variable; range 01–12, 99]
		[Dropdown] 9999=don't know	**p_arv_dstarty** [standard AHRI year variable, range 1998–2017]

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
11.3.3 Interview	Is your child still taking ARVs (for routine care, as PEP, or as PrEP]?	[Radio] 0 No 1 Yes 9 Don't know *(If Yes, skip to 11.3.3.2)*	**p_arv_still** [integer; 1 digit; range 0–1, 9]
11.3.3.1 Interview	If No, when did your child stop taking ARVs (for routine care, as PEP, or as PrEP]?	[Dropdown] 99 = Don't know	**p_arv_dstopm** [standard AHRI month variable; range 01–12, 99]
		[Dropdown] 9999=don't know	**p_arv_dstopy** [standard AHRI year variable, range 1998–2017]
11.3.3.2 Interview	Which regimen is your child taking, or took before stopping?	[Dropdown] [List of ARV regimens] 01 ABC + 3TC + LPV/r (e.g., ABC/3TC fixed-dose [Dumiva, Heteruam, Kivexa] + LPV/r [Norvir, Rinavo]) 02 ABC + 3TC + EFV (e.g., ABC/3TC fixed-dose [Dumiva, Heteruam, Kivexa] + EFV [Efamat, Efrin, Erige, Hevaz, Stocrin, Viref]) 03 TDF + FTC + EFV (e.g., Fixed-dose combination [Atenef, Atreslawin, Atripla, Atroiza, Citenvir, Eftenem, Heftenam, Odimune, Rizene, Trenvir, Tribuss, Triolar]) 04 TDF + 3TC + EFV (e.g., Fixed-dose combination [Arion, eflaten, Elteno, Tenarenz, Virlaten]) 05 AZT + 3TC + LPV/r 06 AZT + ABC + LPV/r 96 Other, specify 99 Don't know	**p_arv_regimen** [integer; 2 digits; range 01–XX, 96, 99]
11.3.3.3 Interview	At which clinic is your child taking ARVs now? (*or last clinic attended if not taking ARVs now*)	[Dropdown] 96 = Other, specify 99 = Don't know	**p_arvclin** [integer; 2 digits; range 01–XX, 96, 99]
11.3.3.3.1	Other, specify	[Text]	**p_arvclin_o**

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
Interview			[string; 30 characters]
Question 12	**Child HIV status Disclosure and linkage to care**		
	This question should ONLY be asked if the parent/guardian reports that the child is HIV-positive or is taking ARVs. ***If the child's reported HIV status is negative or unknown, skip to Q13.***		
12.1 **Interview**	Does your child know his/her HIV status?	[Radio] 0 No 1 Yes 9 Don't know (*If Yes and on ARVs, Skip to Q14)* *(If Yes but not on ARVs, skip to Q13*)	**p_hivknown** [integer; 1 digit; range 0–1, 9]
12.1.1 Interview	If No or Don't know, would you like assistance in disclosing the HIV status to your child? *(If the parent/guardian needs help in disclosing HIV status to child, complete referral section)*	[Radio] 0 No 1 Yes *(If No and on ARVs, Skip to Q14)* *(If No but not on ARVs, skip to Q13)*	**p_hivdisclos** [integer; 1 digit; range 0–1]
12.2	**REFERRAL TO CARE FOR CHILD HIV DISCLOSURE**		
	Referral To care Memory Aid: **Explain that the AHRI paediatric HIV nurse can help them with disclosure* **Check if the participant (child) is currently attending a clinic for ARVs. Explain that ideally disclosure should take place at this clinic.* ** If the child is not on ARVs, inform the parent that the AHRI paediatric HIV nurse can see them at the one of following clinics in PIP: KwaMsane (Mondays); Mtubatuba (Tuesdays); Somkhele (Thursdays)* *If the parent is not able to attend one of those clinics, ask them to choose another clinic in PIP, where an AHRI nurse will help them..*		
12.2.1	*Clinic child currently attending*	**1, [p_arvclin]** **2, [p_arvclin_o]**	**p_curclin** **[**integer; 1 digit; range 0–1,7**]**

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
Piped from Q11.3.3, Q11.3.3.3 and Q11.3.3.3.1			
12.2.2 Interview	Which clinic would you like to be referred to? The AHRI Paediatric nurse can see parents in the following clinics: KwaMsane (Mondays); Mtubatuba (Tuesdays); Somkhele (Thursdays)	[Dropdown] [List of PIP clinics) 96 = Other, specify	**p_prefclin** **[**integer; 2 digits; range 0–99**]**
12.2.3 Interview	Other, specify	[Text]	**p_prefclin_0** **[**Text, 50 characters**]**
12.2.4	What is the agreed target date for attending the clinic	[Date]	**p_hivdis_tdate** [standard AHRI date variable]
12.2.5 Interview	What is your primary contact number?	[number]	**p_hivdis_contnum** **[**Number, 10 digits**]**
12.2.5.1 Interview	Do you share this phone with other people?	[Radio] 0 No 1 Yes	**p_contnum_personal** [integer; 1 digit; range 0–1]
12.2.5.2 Interview	*Has the phone number been verified during the visit?*	[Radio] 0 No 1 Yes	**p_contnum_verif** [integer; 1 digit; range 0–1]
	If No, Pop Up Message ***Please verify the number to check if it is working***		

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
Question 13	**Consent for Rapid HIV test**		
	<u>Rapid HIV Test Memory Aid</u> *Rapid HIV test should not be offered if:* ** Date of last HIV test in PIP was within 3 months (check date below)* ** Participant is HIV+ and taking ARVs*		
	<u>Rapid HIV Testing: Parent Consent Memory Aid</u> ** Confirm: has the parent capacity to consent?* ** Parental preference re child seen alone* ** Explain right of child to be tested alone (if aged 12+)* ** Explain that if child is aged 10-11 years, he/she can only be tested with parent present* ** Consider referral to clinic for testing*		

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
	** Confirm: proceed with HIV testing?* ** Reaffirm parental consent* * Duty of confidentiality to parent (and child)		
	<u>Date for last HIV Test in PIP</u> ** The question below gives date for the participant's last test in PIP. Use the date in deciding whether to offer a rapid HIV test.* ** If the date is not available, this means that the participant has never been tested by AHRI.* ** If the date is within 3 months, do not offer a rapid test* ** If the date is more than 3 months and the participant is not taking ARVs, offer rapid HIV test.*		
13.1 **Preloaded**	*Date of last HIV test in PIP:*	[date]	**p_lhivdate** [standard AHRI date]
13.2 **Interview**	*Basing on the HIV decision rules, should consent for rapid HIV testing for the child be obtained?*	[Dropdown] 0 No 1 Yes *If No, skip to Q13.4* *If No and HIV+ not taking ARVs skip to Q13.5* If No and HIV+ taking ARVs skip to Q14	**p_hivrdec** [integer; 1 digit; range 0–1]
13.3 **Interview**	Are you happy for your child to be offered an HIV test today?	[Dropdown] 0 No 1 Yes *If Yes, skip to Q13.4.1*	**p_hivrap_cons** [integer; 1 digit; range 0–1]
13.4 Interview	If **No, or if your child does not want a rapid test**, can we offer to test your child for HIV using venous blood for research purposes? *(If needed, explain to the parent/guardian that with their permission we can test for HIV in the lab using the venous blood collected for IGRA testing only for research purposes. Make it clear that this result will remain anonymous – neither you (the researcher) or the parent or child will be able to link the result of this test to the child.)*	[Radio] 0 No 1 Yes 7 Not Applicable *(If No, Skip to Q13.5)*	**p_hivven_cons** [integer; 1 digit; range 0–1]
13.4.1 Interview	If Yes, ask parent to sign consent for HIV testing	[Signature]	**p_hivcons_sign**

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
13.4.2 Interview	Would you prefer to be present for the process of HIV testing? *Explain that this is only a preference and depends also on the wishes of the child (if the child is aged 12 years or above). If the child is aged 10-11 years, the parent must agree to be present. 'Process' in this case refers to consent/assent, conducting the procedure, and giving the results.*	[Radio] 0 No 1 Yes *If No, go to Q13.5* *If Yes, skip to Q14*	**p_hivrap_pres** [integer; 1 digit; range 0–1]
	Parent has refused being present during the test. Do not offer HIV Testing to participant		
13.5 Interview	If HIV rapid test is not offered or refused, please comment.	Text	**c_hiv_noconsent** [text 200 characters]

Question 14	Adolescent consent		
14.1 **Interview**	Do you wish to be present when we obtain assent from your child for their participation in the study? *(Explain that this is only the process of obtaining assent with the child. The interview with the child should take place without the parent/guardian present)*	[Radio] 0 No 1 Yes *(If no, proceed to obtain consent/assent WITHOUT the parent/guardian.)* *(If yes, proceed to obtain consent assent WITH the parent/guardian.)*	**p_childcons_pres** [integer; 1 digit; range 0–1]

End of Survey Instrument

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
1.1 **Interview**	**Contact Details:**		
	Memory Aid: Contact Details for Feeding Back Results ** Inform the parent/guardian that you may need to feed back some of the participant's lab test results to them and that you will need their contact details to be able to do this.*		
1.1.1 Interview	Which number should we use to contact you?	[Text]	**p_res_contnum** **[**text, 10 characters**]**

Q number; source	Question	Field type; dropdown options	Variable name, type, size, limits
1.1.2 Interview	Do you share this phone with other people?	[Radio] 0 No 1 Yes (if Yes?)	**p_res_contnum_personal** [integer; 1 digit; range 0–1]
1.1.4 Interview	*Has the phone number been verified during the visit?*	[Radio] 0 No 1 Yes	**p_res_contnum_verif** [integer; 1 digit; range 0–1]
	If No, Pop Up Message ***Please verify the number to check if it is working***		
1.2 Interview	**Details of interview**		
1.2.1 Interview	*Has the parent/guardian answered all the questions?*	[Radio] 0 No 1 Yes *(if Yes, Skip to Q15.2)*	**p_surv_comp** [integer; 1 digit; range; 0-1]
1.2.1.1 Interview	If No, why were some questions not answered?	[Text]	**p_nocomp_reas** [Text, 200 characters]
1.2.1.2 Interview	Please add any other comments or notes that you feel are needed	[text]	**p_gencomment** **[**text, 300 characters**]**
1.2.2 Interview	Interviewer Name	[Text]	**p_intid** [Text, 50 characters]
1.2.3 Interview	Time interview ended	[Number]	**p_tintstophh** [integer; 2 digits; range 00–23]
		[Number]	**p_tintstopmm** [integer; 2 digits; range 00–59]

Appendix 12: Risk factors for *M. tuberculosis* infection

Appendix Table 1: Risk factors for *Mycobacterium tuberculosis* infection showing odds ratios obtained from the crude, partial and fully adjusted models at each level of hierarchical approach (not taking account for clustering within households).

Variable	QFT Positive n/N (%)	Crude OR (95% CI)	*p*-value	Adjusted OR[1] (95% CI)	*p*-value	Adjusted OR[2] (95% CI)	*p*-value
Community Level factors							
Community HIV prevalence (%)							
<25%	12/85 (14.1)	1	0.05				
25-34.9%	133/618 (21.5)	1.67 (0.88 -3.16)					
35-44.9%	61/261 (23.4)	1.86 (0.95 -3.64)					
≥45%	26/82 (31.7)	2.82 (1.31 -6.08)					
Location							
Rural	156/715 (21.8)	1	0.31	1[a]	0.81		
Urban	93/379 (24.5)	1.17 (0.87-1.56)		0.95 (0.63-1.43)			
Household Level factors							
Distance to nearest clinic (km) (quartiles)							
<1.85	84/301 (27.9)	1	0.08	1[a]	0.15	1[b]	0.24
1.85-3.41	80/403 (19.9)	0.64 (0.45-0.91)		0.67 (0.46-0.97)		0.73 (0.50-1.06)	
3.42-5.36	55/259 (21.2)	0.70 (0.47-1.03)		0.77 (0.50 -1.20)		0.82 (0.52-1.29)	
>5.36	30/131 (22.9)	0.77 (0.48-1.24)		0.96 (0.58 -1.60)		1.10 (0.65-1.85)	
Household social economic index score (tertiles)							
Low	74/305 (24.3)	1	0.62	1[a]	0.71	1[b]	0.72
Middle	79/350 (22.6)	0.91 (0.63 -1.31)		0.87 (0.59 -1.26)		0.87 (0.59-1.26)	
High	83/393 (21.1)	0.84 (0.59 -1.19)		0.88 (0.61 -1.27)		0.89 (0.61-1.28)	
Number of residents							
<6	87/333 (26.1)	1	0.20	1[a]	0.30	1[b]	0.38
6-7	58/252 (23.0)	0.85 (0.58-1.24)		0.85 (0.57 -1.26)		0.90 (0.60-1.36)	
8-10	50/248 (20.2)	0.71 (0.48-1.06)		0.72 (0.48 -1.08)		0.73 (0.48-1.12)	
>10	46/237 (19.4)	0.68 (0.45-1.02)		0.71 (0.47 -1.08)		0.74 (0.48-1.13)	
Reported smoker in household							
No	197/880 (22.4)	1	0.92	1[a]	0.66	1[b]	0.62
Yes	47/207 (22.7)	1.02 (0.71 -1.46)		0.92 (0.63 -1.34)		0.91 (0.62-1.33)	
Individual Level factors							

Variable	QFT Positive n/N (%)	Crude OR (95% CI)	*p*-value	Adjusted OR[1] (95% CI)	*p*-value	Adjusted OR[2] (95% CI)	*p*-value
Sex							
Female	123/548 (22.4)	1	0.80	1[c]	0.98	1[d]	0.81
Male	126/546 (23.1)	1.04 (0.78 -1.38)		1.00 (0.74 -1.35)		0.96 (0.70-1.32)	
Age (years)							
10-11	49/237 (20.7)	1	<0.01	1[c]	<0.01	1[d]	<0.01
12-14	62/349 (17.8)	0.83 (0.55 -1.26)		0.86 (0.56 -1.35)		0.92 (0.58-1.44)	
15-17	71/297 (23.9)	1.21 (0.80 -1.82)		1.13 (0.72 -1.77)		1.22 (0.77-1.92)	
≥18	67/211 (31.8)	1.79 (1.16 -2.74)		1.83 (1.16 -2.89)		1.96 (1.23-3.15)	
Lifetime household TB contact							
No	168/823 (20.4)	1	<0.01	1[c]	0.01	1[d]	<0.01
Yes	78/266 (29.3)	1.62 (1.18 -2.21)		1.57 (1.12 -2.20)		1.73 (1.22-2.45)	
HIV Status							
Negative	193/855 (22.6)	1	0.88	1[c]	0.34	1[d]	0.33
Positive	9/43 (20.9)	0.91 (0.43 -1.93)		0.72 (0.31 -1.68)		0.72 (0.31-1.71)	
Unknown	47/196 (24.0)	1.08 (0.75 -1.56)		1.27 (0.85 -1.91)		1.30 (0.86-1.97)	
BCG Vaccination							
Vaccinated	216/984 (22.0)	1	0.20	1[c]	0.94	1[d]	0.67
Not vaccinated	28/101 (27.7)	1.36 (0.86 -2.16)		1.02 (0.60 -1.72)		1.13 (0.66 -1.92)	
Smoking							
No	240/1070 (22.4)	1	0.29	1[c]	0.86	1[d]	0.61
Yes	6/18 (33.3)	1.73 (0.64 -4.66)		0.90 (0.31 -2.70)		0.74 (0.24-2.26)	
Alcohol intake							
No	226/1021 (22.1)	1	0.21	1[c]	0.68	1[d]	0.65
Yes	16/54 (29.6)	1.48 (0.81 -2.71)		1.15 (0.60 -2.21)		1.16 (0.59-2.28))	
Education level							
Primary	130/631 (20.6)	1	0.05	1[c]	0.21	1[d]	0.33
Secondary or above	118/459 (25.7)	1.33 (1.00 -1.77)		0.72 (0.42-1.21)		0.77 (0.4 -1.33)	
Admission to hospital							
No	221/967 (22.9)	1	0.58	1[c]	0.28	1[d]	0.18
Yes	25/121 (20.7)	0.88 (0.55 -1.40)		0.76 (0.47-1.25)		0.70 (0.4 -1.18)	
Social contact factors							
Contact hours with adult men							
<100	63/274 (23.0)	1	0.97	1[c]	0.72	1[d]	0.63
100-1047	63/275 (22.9)	1.00 (0.67 -1.48)		1.22 (0.80-1.88)		1.34 (0.85-2.10)	
1048-2400	59/272 (21.7)	0.93 (0.62 -1.39)		1.01 (0.65-1.57)		1.14 (0.72-1.81)	
>2400	64/273 (23.4)	1.03 (0.69 -1.53)		1.17 (0.76-1.80)		1.17 (0.75-1.82)	
Contact hour with adult females							

Variable	QFT Positive n/N (%)	Crude OR (95% CI)	*p*-value	Adjusted OR[1] (95% CI)	*p*-value	Adjusted OR[2] (95% CI)	*p*-value
<160	68/277 (24.5)	1	0.42	1[c]	0.82	1[d]	0.85
160-1216	53/274 (19.3)	0.74 (0.49 -1.11)		0.84 (0.54-1.29)		0.92 (0.59-1.44)	
1216-2880	66/270 (24.4)	0.99 (0.67 -1.47)		1.01 (0.66-1.54)		1.11 (0.71-1.72)	
>2880	62/273 (22.7)	0.90 (0.61 -1.34)		0.99 (0.64-1.51)		1.07 (0.69-1.66)	
Church attendance in previous month							
None	165/664 (24.8)	1	0.06	1[c]	0.11	1[d]	0.03
1-2 times	37/176 (21.0)	0.81 (0.54 -1.20)		0.77 (0.50-1.18)		0.70 (0.45-1.08)	
≥3 times	41/233 (17.6)	0.65 (0.44 -0.94)		0.68 (0.46-1.01)		0.60 (0.40-0.90)	
Health facility attendance (12 months)							
No	142/667 (21.3)	1	0.20	1[c]	0.70	1[d]	0.65
Yes	104/422 (24.6)	1.21 (0.91 -1.61)		1.06 (0.78 -1.45)		1.08 (0.78-1.49)	
Visiting other houses during the day							
None	169/720 (23.5)	1	<0.01	1[c]	0.01	1[d]	0.01
1-2 houses	59/227 (26.0)	1.15 (0.81 -1.61)		1.12 (0.78 -1.61)		1.03 (0.71-1.49)	
≥3 houses	17/136 (12.5)	0.47 (0.27 -0.80)		0.42 (0.24 -0.75)		0.39 (0.22 -0.70)	
Sharing sleeping room with other people							
None	84/363 (23.1)	1	0.84	1[c]	0.55	1[d]	0.44
1 person	78/336 (23.2)	1.00 (0.71 -1.43)		1.23 (0.84-1.80)		1.29 (0.87-1.90)	
≥2 persons	84/389 (21.6)	0.91 (0.65 -1.29)		1.06 (0.73-1.55)		1.09 (0.74-1.62)	

BCG: Bacillus Calmette-Guérin; CI: confidence interval; HIV: Human immunodeficiency virus; OR: odds ratios; QFT: QuantiFERON TB-Gold plus; TB: tuberculosis

[1] Partially adjusted by *a priori* confounders and variables remaining significant (p<0.2) at higher levels in the hierarchy

[2] Fully adjusted by *a priori* confounders, variables remaining significant (p<0.2) at higher levels in the hierarchy and variables remaining significant (p<0.2) that levels in the hierarchy.

[a] Adjusted by community HIV prevalence

[b] Adjusted by community HIV prevalence, distance to clinic and socioeconomic status (*a priori* confounder)

[c] Adjusted by community HIV prevalence, socioeconomic status and age (*a priori* confounder)

[d] Adjusted by community HIV prevalence, socioeconomic status, age, lifetime household TB contact, attendance to church and visiting other houses during the day