Clinical Trial Protocol

Protocol Title:

Molbio Truenat™ TB platform combined with the Truenat TB assays for detection of tuberculosis and rifampicin resistance in adults with presumptive pulmonary tuberculosis at primary-level diagnostic centres in Tanzania and Mozambique: a pragmatic, cluster- randomized controlled trial

Short Title: TB-CAPT CORE Truenat trial

Protocol Number: TB041 -3/1-MOZ

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Signature page (sponsor)

Protocol Title: *Molbio Truenat™ TB platform combined with the Truenat TB assays) for detection of tuberculosis and rifampicin resistance in adults with presumptive pulmonary tuberculosis at primary-level diagnostic centres in Tanzania and Mozambique: a pragmatic, cluster-randomized controlled trial*

Trial Number: *TB041 -3/1*

Herewith we approve the protocol for the TB-CAPT CORE Truenat trial version 4.0, dated 18th January 2023, and confirm that it contains all information necessary to conduct the study according to the ethical principles laid down in the Declaration of Helsinki, Good Clinical Practice and all applicable local regulations.

p.p. DocuSigned by: Dr Adam Penn-Nicholson 9E4305DE17A64FB... Deputy Director TB Programme FIND — 2/14/2023

Sponsor Responsible Person
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Statement of Principal Investigator

Protocol Title: *Molbio Truenat™ platform combined with the Truenat TB assays for detection of tuberculosis and rifampicin resistance in adults with presumptive pulmonary tuberculosis at primary-level diagnostic centres in Tanzania and Mozambique: a pragmatic, cluster-randomized controlled trial*

Trial Number: *TB041 -3/1*

In signing this page, I, the undersigned, agree to conduct the trial according to the protocol and ICH-GCP E6 (R2) guidelines and in compliance with applicable regulations.

I will ensure that the requirements relating to obtaining Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) review and approval are met. I will promptly report to the IRB/IEC any and all changes in the research activities covered by this protocol.

I have sufficient time to properly conduct and complete the trial within the agreed trial period and I have adequate resources (staff and facilities) for the foreseen duration of the trial.

I am responsible for supervising any individual or party to whom I delegate trial related duties and functions conducted at the trial site. Further, I will ensure this individual or party is qualified to perform those trial-related duties and functions.

I certify that key individuals involved with the conduct of this trial, including myself, have completed GCP training and, if applicable, Human Subjects Protection Training.

I understand that all information obtained during the conduct of the trial with regard to the subjects' state of health will be regarded as confidential. No participant's names or personal identifying information may be disclosed. All participant data will be anonymized and identified by assigned numbers on all Case Report Forms, laboratory samples and other trial related information (such as essential documents) forwarded to FIND. Monitoring and auditing by FIND, and inspection by the appropriate regulatory authority(ies), will be permitted.

I will maintain confidentiality of this protocol and all other related investigational materials. Information taken from the trial protocol may not be disseminated or discussed with a third party without the express consent of FIND.

Clinical Trial Site: (Print) Centro de Investigação em Saúde de Manhiça (CISM)

Name of Principal Investigator: (Print) Dinis Bento Nguenha

Signature: _____ Date: 05/May/2023
DD/MMM/YYYY

Protocol History/Amendment Summary*

Version number	Release date	Comments
1.0	26.06.2020	Initial version
2.0	30.11.2020	• Formatting updates • Change in Ifakara PI to replace Frederick Haraka with Jerry Hella • Change in NIRM clinical site coordinator from Elizabeth Ntapara to Elimina Siyame • Rephrase objectives for clarity • Updated timelines • Added secondary objective endpoint • Clarify inclusion/exclusion criteria • Removed reference to Xpert MTB/XDR • Added detail on OpenClinica database system • Updated risk/benefit section
2.1	4.02.2021	• Updated version and dates • Added detail to inclusion criteria • Added detail to patient enrollment • Clarification in patient withdrawal • Added detailed to sampling for socio-economic and cost data • Update schedule of events
2.2	27.03.2021	• Removed “user appraisal” objectives • Changes secondary objective related to costs and productivity (as it will only be measured at 180 days) • Added details on cost and productivity • Rename “Study register” Study TB outcome worksheet as per the new name of the CRF where indicated • Updated schedule of events
3.0 -MZ	16.11.2021	• Administrative clarifications in text • Replaced the investigational device and assay (GeneXpert Omni and Xpert MTB/Rif Ultra) with the Molbio Truenat platform combined with Truenat™ MTB Plus and MTB-RIF Dx assays • Changed follow-up time to 2 months and removed the face-to-face follow-up visit at 6 months • Added objectives of the qualitative sub-study and Ease of Use assessment • Added Mozambique to Section 16: Description of the procedures, inclusion/exclusion criteria, data management and analysis for the

		qualitative sub-study • Changed sponsor responsible person from Samuel Schumacher to Dr. Morten Ruhwald • Change in CISM Site Research Coordinator from Joaquim Cossa to Delio Elisio.
4.0	18.01.2023	• Added primary endpoint: in additional to the proportion, the count was added • Cost and productivity endpoints were changed to include patient and health system costs • The definition of successful treatment outcomes has been changed to ongoing treatment in view of the reduced follow-up time (60 days)

1. Abbreviations / Glossary of Terms

ART	Antiretroviral therapy
AE	Adverse events
CFU	Colony Forming Unit
CISM	Centro de Investigação em Saúde de Manhiça
CRF	Case Report Form
DNA	Deoxyribonucleic acid
DST	Drug Susceptibility Testing
FIND	Foundation for Innovative New Diagnostics
HIV	Human Immunodeficiency Virus
IEC	Independent Ethics Committee
ICH-GCP	International Council for Harmonisation – Good Clinical Practice
IHI	Ifakara Health Institute
INS	Instituto Nacional de Saúde
LMU	Ludwig-Maximilians-Universität
LTFU	Loss to Follow Up
MDR-TB	Multi-drug resistant tuberculosis
MMRC	Mbeya Medical Research Centre
MOP	Manual of Procedures
MOH	Ministry of health
MTB	Mycobacterium tuberculosis
NIMR	National Institute for Medical Research
NTLP	National Tuberculosis and Leprosy Control Programme
Omni	GeneXpert® Omni
POC	Point-of-Care
RIF	Rifampicin
SAE	Severe Adverse Events
SOP	Standard Operating Procedure
SOC	Standard of Care
SSA	Sub-Saharan Africa
TB	Tuberculosis
TPP	Target Product Profiles
Xpert	Xpert® MTB/RIF or Xpert® MTB/RIF Ultra
WHO	World Health Organization

2. Protocol synopsis

Protocol Title	Truenat™ platform combined with the Truenat TB assays for detection of tuberculosis (TB) and rifampicin resistance in adults with presumptive pulmonary tuberculosis at primary-level diagnostic centres in Tanzania and Mozambique: a cluster-randomized controlled trial
Protocol Code	Project number TB041 -3/1
Short Title	TB-CAPT CORE Truenat trial
Sponsor	FIND, The Global Alliance for Diagnostics
Study Centres	Multicentre study in 4 sites in 2 countries (Mozambique and Tanzania)
Name of investigational device	- Primary study: - Referred to collectively in this protocol as: the Truenat platform and Truenat TB assays: - Devices: - Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device - Truelab® Duo Real Time Quantitative micro PCR Analyzer - TB Assay components: - Truelab® micro–PCR Printer (connected by Bluetooth) - Trueprep® AUTO MTB Sample Pretreatment Pack - Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit - TruenatTM MTB Plus kits, consisting of Truenat chips, a Truepet® 6µl Precision Micropipette - TruenatTM MTB-RIF Dx kits, consisting of Truenat chips, a Truepet® 6µl Precision Micropipette
Indication	Investigation of outpatients presenting with symptoms suggestive of pulmonary tuberculosis (TB)
Intervention and Control Groups	Investigational group: TB testing using the Truenat platform/TB assays placed at primary health care clinics combined with rapid communication of results and same day TB treatment initiation Control group (standard of care): Standard of care for TB testing using a combination of smear microscopy and laboratory (off-site) Xpert testing and may vary by clinic depending on availability of transport and stock of Xpert® MTB/RIF or Xpert® MTB/RIF Ultra cartridges (Xpert).
Objectives	Primary Objective: • Evaluate the effect of testing for TB using the Truenat platform/TB assays combined with rapid communication (intervention arm), compared to standard of care (control arm) in primary health care clinics (“clinics” in the further documents) on TB diagnosis and treatment initiation for

	microbiologically confirmed TB at 7 days from enrolment Secondary Objectives: • Evaluate the effect of the Truenat platform/TB assays on time to TB treatment initiation at 60 days from enrolment: - for microbiologically confirmed TB cases - for clinically diagnosed TB cases - all TB cases (clinically diagnosed and microbiologically confirmed) • Estimate the effect of the Truenat platform/TB assays on morbidity, mortality, on treatment loss to follow-up at 7 and 60 days from enrolment • Estimate the effect of Truenat platform/TB assays on total costs (patient costs and health system costs) and productivity Additional Objectives • Evaluate the reliability of Truenat platform/TB assays as measured by rate of non-determined test results and platform failure User preferences • To investigate user perspectives on the Truenat platform/TB assays (including perspectives of end-users such as patients, but also of professional users such as laboratory technicians, clinicians, nurses, and decision-makers) for use as a diagnostic test for outpatients presenting with symptoms suggestive of pulmonary TB tuberculosis (TB) • To investigate and compare experiences and challenges with diagnostic testing for TB in patients using the different diagnostic approaches (Xpert® MTB/RIF, Xpert® MTB/RIF Ultra, microscopy, culture) • To understand feasibility and preferences with regard to diagnosing TB and how Truenat platform/TB assays changes these • To assess the usability and acceptability of Truenat platform/TB assays in the intended users
Study Design	A cluster randomized controlled trial to evaluate the effect of placing Truenat platform/TB assays at primary health care clinics combined with rapid communication of results on time to treatment initiation of microbiologically confirmed TB. In a setup period (survey of study centers) before randomization, the healthcare facilities (=clinics) of 4 sites will be asked to provide information about the number of TB notifications per quarter covering the period 1/2018-6/2020. From this information the foreseeable number of examined patients (“size of a clinic”) will be derived, which will be used as a strata variable in randomization process. Facilities will be randomly allocated to the standard of care (control), or Truenat platform/TB assays. A cluster refers to a

	clinic. The clusters will be assigned to intervention or control arm to one of two diagnostic procedures (SOC [smear microscopy and/or Xpert MTB/RIF Ultra off-site] vs Truenat platform/TB assays on-site) following a restricted randomization strategy, whereby 6 to 8 strata of clusters will be established using the stratification variables site (clinics belong to a site) and size and apply balance criteria for them. The number of strata per site depends on heterogeneity of the sizes of the clinics as established in setup period and may be increased, typically 1 or 2 strata per site will be established. The primary endpoint will be compared in an individual level analysis with strata and intervention as fixed effects and cluster as random effect.
Population	A total of 29 clinics will be randomized at the four participating sites aiming to enrol up to 150 adults (≥ 18 years of age) each (total approximately 4200) with signs and symptoms suggestive of TB. The number of clinics might be extended up to 37 due to the information gained in the setup period. Dependent on the loss to follow-up at 7 days, recruitment may need to be extended.
Inclusion/Exclusion Criteria (Site)	Based on information gained in the setup period sites will be included in the randomization process using the following criteria: <u>Inclusion Criteria:</u> Provision of TB treatment at the clinic Availability of electricity Willingness to participate <u>Exclusion criteria:</u> Patients with TB referred for treatment to other sites Sites with less than 10 patients registered for TB treatment per quarter
Inclusion/Exclusion Criteria (Patients)	<u>Inclusion Criteria:</u> • Patients with presumptive pulmonary TB, as defined by the national TB treatment guidelines in each country: patients with cough more than 1-2 weeks and/or fever, night sweats, blood-stained sputum (haemoptysis) significant weight loss, abnormalities on chest radiograph who are able to produce a sputum sample • Adults 18 years old and above (including pregnant women) who are able and willing to consent <u>Exclusion criteria:</u> • Children and adolescents <18 years of age • Circumstances that raise doubt on free, informed consent (e.g., in a mentally impaired person or a prisoner) • Already diagnosed with TB

	• Currently receiving anti-TB therapy • Patients with symptoms which are only attributable to extra-pulmonary TB • Patients who are seriously ill and need to be admitted to hospital
Inclusion/Exclusion Criteria (User preferences)	Adult aged ≥ 18 years old Either: (1) Routine healthcare worker participating in the CORE trial or (2) Patient tested or to be tested with the Truenat platform /TB assays as part of the study or (3) Decision maker involved with implementation of novel tests at the local, national and/or regional level.

Evaluation Criteria (Endpoints)	<u>Primary endpoint:</u>
	• Absolute number and point estimate (with 95% CIs) of the proportion of enrolled participants who are diagnosed with microbiologically confirmed TB and are starting TB treatment within 7 days of enrolment
	
	<u>Secondary endpoints:</u>
	Diagnosis related endpoints:
	• Estimate of the time to bacteriological confirmation of TB (up to 60 days) from enrolment
	• Point estimate with 95% Cis of the proportion of patients treated for TB who are diagnosed up to 60 days from enrolment
	  ○ microbiologically
	  ○ clinically
	
	Treatment/outcome related endpoints:
	• Point estimate with 95% Cis of the proportion of participants evaluated for pulmonary TB starting TB treatment with microbiological confirmation within 60 days from enrolment
	• Absolute number and point estimate (with 95% CIs) of the proportion of enrolled participants evaluated for pulmonary TB starting TB treatment regardless of microbiological confirmation within 7 and 60 days from enrolment
	• Estimate of time to TB treatment initiation for those with microbiological confirmation and for all participants (censored at 60 days) from enrolment
	• Point estimate of the proportion of patients with ongoing treatment (on treatment) among:
	  ○ Participants diagnosed with TB either clinically or with microbiological confirmation
	  ○ Participants clinically diagnosed with TB
	  ○ Participants with microbiological confirmation of TB

	Morbidity related endpoints: • Prevalence of current cough, limited appetite, weakness at 60 days from enrolment Cost and productivity related endpoints: • Patients' costs related to care <u>Additional endpoints:</u> Operational characteristics: • Truenat platform / TB assay non-determined test results rates • Rate of Truenat platform failure
Statistical Considerations	Randomization: Restricted randomization approach resulting in 6 to 8 strata applying factors site and size of the clusters (=clinics). The strata are constructed using variables site and size of the clinic, whereby appropriate coarsening of the size and balance criteria are applied. The number of strata may be increased in case of heterogeneity of sizes of the clusters (as indicated by information gained in setup period) <u>Description:</u> Baseline characteristics, such as demographic and analytical data will be summarized using descriptive statistical methods. Continuous data will be summarized using the mean, the median, standard deviation, the range (minimum and maximum value). Categorical values will be summarized using frequency counts and percentages. <u>Analysis:</u> Individual level analysis using generalized linear mixed model (GLMM) with logit link function, intervention (y/n) and stratum as fixed effects and cluster as a random effect. Sensitivity analyses based on cluster-level summaries will be applied. Adjustment for other variables (such as HIV status) may be considered in sensitivity analyses and will be described in detail in the statistical analysis plan (SAP). <u>Sample size:</u> For the purpose of sample size estimation, a matched design is regarded which is known to have a similar power in the range of number of clusters per treatment arm similar to this study (~15) since in that range the effects of gain of precision on one hand and the loss in degrees of freedom on the other hand are similar. Applying an alpha-level of 0.05, a power of 80%, 12% prevalence, LTFU-rates of 10% (diagn., control), 10% (pretreatment intervention), 20% (pretreatment control), a cluster size of 150 and a within-pair

	coefficient of variation (default value 0.25 acc. Hayes & Bennett 1999), 13 clusters per study arm result (3900 patients). In order to address uncertainty, 1 additional cluster per treatment arm will be introduced, thus 14 cluster-pairs (28 clinics, 4200 patients) result. If the information collected in the setup phase indicates that some clusters will be unable to enroll 150 patients during the study period the number of cluster may be increased up to 36	
Study Timeline	Period for ethics clearance	Q4 2021- Q2 2022
	Setup period	Q4 2021 - Q2 2022
	Import permits and importation	Q1 2021 -Q3 2022
	Kick off / training meeting	Q3 2022
	Training of sites on study procedures	Q2 2022
	Training of staff on Truenat procedures	Q2 2022
	First participant enrolled	Q3 2022
	Last participant enrolled	Q3 2023
	Complete follow-up	Q4 2023
	Data cleaning:	Q4 2023
	Data analysis:	Q1 2024
	Final report:	Q1 2024
	Total duration of study:	31 months

3. Study schedule of events

Visit	Visit 1 (V1-D1)	Visit 2 (V2-D7)	Visit 3 (V3-D60)
Timeframe	**Day 1**	**Day 7 (Week 1)**	**Day 60 (Month 2)**
Procedure	*<48h*	Day 8 up to day 21	Day 61 up to day 90
Informed consent	**x**		
Eligibility assessment	**x**		
Enrolment (study register)	**x**		
Contact information (locator form)	**x**	**(update)**	
Baseline questionnaire (CRF-V1-D1)	**x**		
Baseline questionnaire with detailed socioeconomic questions (CRF-V1-D1-SE)	**(x)**		
Investigational product testing	**(x)**		
Follow-up questionnaire day 7 (CRF-V2-D7) – administered by telephone (3 attempts) if unsuccessful conduct home visit or call patient-nominated contact person		**x**	
Follow-up questionnaire day 60 (CRF-V3-D60) - administered by telephone (3 attempts) if unsuccessful conduct home visit or call patient-nominated contact person			**x**

Throughout follow-up data from the TB register and health facilities will be collected (study TB outcome worksheet)

4. Summary of the proposed research in lay terms

Tuberculosis (TB) is the infectious disease that causes the highest number of deaths worldwide. In 2017, about 1,6 million persons died of TB globally.

While TB that is sensitive to commonly used medications rifampicin and isoniazid can be treated within 6 months and mostly cured, TB with resistance to these medications requires a different, longer treatment. Besides the success in treating drug sensitive TB, it is believed that 4.3 million persons living with TB are missed every year. The reasons for this are that they cannot access quality diagnostic testing that would detect their disease at a healthcare facility close enough to their home. These are the so-called “missing millions” which may die due to undiagnosed TB or are diagnosed very late resulting in severe and irreversible lung damage. Also individuals with undiagnosed TB may spread the disease in their families and communities.

Xpert MTB/RIF, a molecular diagnostic test developed by Cepheid, detects TB more accurately than microscopy and provides a result within 90 min. The diagnostic test is run on the GeneXpertplatform. Smaller analysers are able to run 2-4 test concurrently while the infinity module can run up to 80 tests. Xpert MTB/RIF correctly diagnoses TB in 90% of individuals who have TB of the lung, this is in contrast to microscopy (still widely used) which only diagnoses about 50% of individuals with TB. Xpert MTB/RIF has the added advantage that it rapidly detects rifampicin (RIF) resistance. Patients diagnosed with rifampicin resistance can thus be referred to appropriate treatment. Xpert MTB/RIF was endorsed by WHO in 2011, and many countries are using the test (or planning to use it) as primary TB diagnostic replacing microscopy. A newer similar version of the test Xpert MTB/RIF Ultra is even more sensitive than the “conventional” Xpert MTB/RIF and detects TB in 95% of individuals who have the disease in their lungs. This Xpert MTB/RIF Ultra was endorsed by WHO in 2017.

Another rapid molecular platform and assay the Molbio Truenat platform (consisting of the Trueprep DNA extraction device and the Truelab micro-PCR machine) and the Truenat MTB Plus assay for detection of *Mycobacterium tuberculosis* complex (MTBC) and Truenat MTB-RIF Dx for detection of RIF resistance was endorsed in 2020 by the WHO for TB diagnosis (collectively referred to as the Truenat TB assays). The system is designed to be operated as a point-of-care diagnostic solution in peripheral laboratories with minimal infrastructure. It takes about 25 minutes to do the DNA extraction and another 35 minutes to diagnose TB. Extracted DNA eluate from samples testing MTBC-positive can be used for RIF-resistance reflex testing. The limit of detection was determined to be 100 CFU/ml sputum sample which is similar to the widely used Xpert MTB/RIF. Despite the successful roll-out of the Xpert MTB/RIF, which is more sensitive than microscopy, across Africa TB notifications have not increased and the impact on mortality remains uncertain. Most GeneXpert machines are placed in central or district laboratories requiring sample transport from primary health care clinics (where patients are usually seen) to the laboratory. Once the sample is tested, the results are sent back to the primary health care facility and the patient will have to be called back to start treatment. There are a number of bottlenecks across the diagnostic TB pathway, which results in substantial attrition during the diagnostic process. Essential steps include the individual accessing care, the health care worker referring the individual for TB investigations, the specimen being sent for TB diagnostics and the results being returned. Initiation of appropriate treatment relies on timely receipt of laboratory results and the patient returning to clinic. The proposed study aims to address some of the bottlenecks by bringing

the diagnostic device closer to the patients in the primary health clinic.
The Truenat MTB Plus assay runs on the Trueprep/Truelab platform and has similar performance characteristics as the Xpert MTB/RIF run on the GeneXpert platform. The Truenat platforms/TB assays have several advantages over the GeneXpert platform making them more suitable for placement at primary health care facilities. The standalone platforms are portable and battery operated, have no need for a computer or laptop, and operate from 2- 40°C ambient temperature. The system uses room temperature stable reagents with long shelf life.

For this study we will randomize 29-37 primary health care facilities to diagnosing persons with signs and symptoms suggestive of TB of the lung using the existing diagnostic algorithm (mostly microscopy or off-site Xpert testing) or to placing Truenat platform at the facility to enable Truenat MTB Plus and Truenat MTB-RIF Dx testing on site.

Whether or not the Truenat platform/TB assays will improve the TB diagnostic pathway will be measured across a number of indicators: time to TB treatment, proportion of patients with laboratory confirmed TB and number of deaths. We will also ask the staff at the health care facilities operating the machine about their experience (ease of use, test failure rate and other problems encountered when operating the machine).

5. BACKGROUND INFORMATION

5.1 Tuberculosis epidemiology

Tuberculosis (TB) remains the leading infectious killer globally with 1.6 million deaths each year of which 300,000 were associated with HIV in 2017[1]; 10 million new cases occur annually disproportionately affecting disadvantaged populations. *TB is recognised to be both preventable and curable, yet 40% of people newly affected remain undiagnosed*[1], and millions do not receive quality care each year.[2,3]

Relative to the population, the number of incident TB cases (per 100 000 population per year) has been falling slowly, as has the number of deaths. Worldwide, TB incidence decreased at about 2% per year.[1] The fastest regional declines from 2013 to 2017 were in the world health organization (WHO) European (5% per year) and African Region (4% per year). In the same 5 years, particularly impressive reductions (4–8% per year) occurred in southern Africa following a peak in the HIV epidemic, and the expansion of TB and HIV prevention and care. However, to achieve the sustainable development goals and End TB Strategy targets set for 2030 the annual decline in the global TB incidence rate would need to be accelerated to an average of 17% per year.

While progress has undoubtedly been made, the *disease burden in Sub-Saharan Africa (SSA), and particularly southern Africa, remains incredibly high*. This is due to the HIV-driven TB epidemic, which resulted in a 500% rise in TB case notifications between 1985 and well into the late 1990s in some SSA countries including Mozambique and Tanzania.[4,5] TB case notifications in these countries still remain increased compared to the pre-HIV era and over 50% of TB cases in SSA are co-infected with HIV. Of the 48 high TB, HIV-TB and MDR (multi-drug resistant)-TB (which is TB resistance to at least rifampicin and isoniazid) burden countries 21 are African. Furthermore 8 of 14 countries featuring on all three high burden lists are located in Africa. Mozambique is one of those countries facing a triple burden of TB, HIV-TB and MDR-TB in the context of a national adult HIV prevalence of 13.2%, while Tanzania is a high TB and HIV-TB burden countries.

5.2 Limited diagnostic capacity in sub-Saharan Africa

Timely and appropriate diagnosis and treatment is the key to reduce TB mortality, morbidity and prevent transmission. However, almost half (4.3 million) of the 10 million TB cases and more than two-thirds (71%) of the MDR-TB cases remain undiagnosed.[1] The main reasons are inaccessibility of diagnostics and attrition during the diagnostic process.[6-10]

The Xpert MTB/RIF (Cepheid, Sunnyvale, CA) a rapid molecular test with 90% sensitivity and 98% specificity to diagnose TB and rifampicin resistance has revolutionised TB diagnostics.[11-13] The test was endorsed by the WHO in 2011 and a newer and more sensitive version, the Xpert® MTB/RIF Ultra (Ultra) has been endorsed more recently.[14,15] South Africa was the first country to rollout the Xpert MTB/RIF nationwide as replacement for smear microscopy. In comparison with South Africa, access to Xpert MTB/RIF testing is far more limited in other SSA countries, and smear microscopy (which has low sensitivity for TB and cannot determine drug susceptibility) remains the main tool for TB diagnosis.[16-18] However, even in SSA countries that have rolled out Xpert MTB/RIF testing remains a centralised service, and limited capacity and financial constraints mean that Xpert MTB/RIF is often restricted to high risk groups.[17-19] Furthermore while Xpert MTB/RIF has increased the proportion of

microbiologically confirmed TB and for the first time enables drug susceptibility testing (DST) for rifampicin (RIF), DST for other first and second line drugs such as isoniazid and moxifloxacin is only available at central level (mainly at the national reference laboratories).

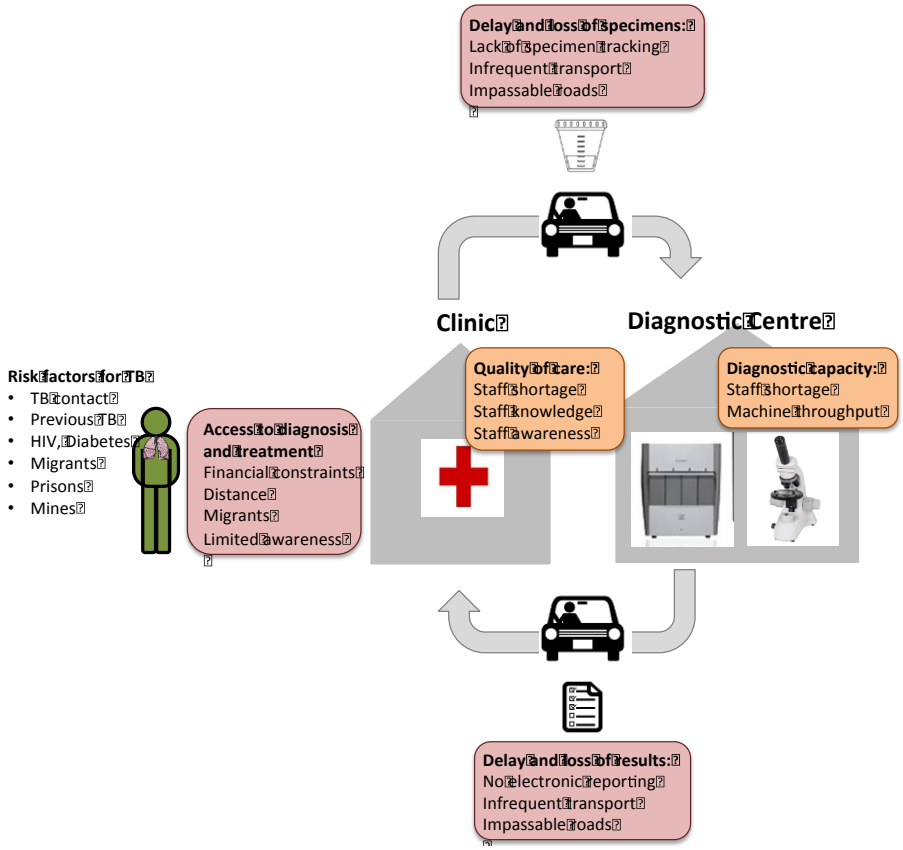

Figure 1: TB diagnostic pathway

As shown in **Figure 1**, there are a number of bottlenecks across the diagnostic pathway for TB, which results in substantial attrition during the diagnostic process. Essential steps include the individual accessing care, the health care worker referring the individual for TB investigations, the specimen being sent for TB diagnostics and the results being returned. Initiation of appropriate treatment relies on timely receipt of laboratory results and the patient returning to clinic. Many SSA countries have reported significant attrition along the diagnostic pathway.[20-24]

In 2014 the WHO convened a stakeholder meeting aimed at establishing high-priority target product profiles (TPPs) for new TB diagnostics.[25] A point-of-care (POC) sputum-based test to replace smear microscopy for detecting pulmonary TB (the smear-replacement test) was identified as one of four high priority TB diagnostics. The Xpert MTB/RIF using the GeneXpert platform falls short of this TPP on a number of WHO ASSURED criteria for evaluating POC devices for resource-limited environments. ASSURED stands for affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users. The current GeneXpert platform and TB assays are sensitive, specific, user-friendly and relatively robust. However, Xpert MTB/RIF and Ultra take 90 and 70 minutes respectively. The platform needs continuous electricity supply (or a powerpack of considerable size), cannot be

operated at high temperatures, is sensitive to dust and the software requires either a laptop or a desktop computer. This limits the use of the GeneXpert platform in primary health care clinics, where these conditions are often not met. Some of these shortcomings are overcome by the new Truenat platform and TB assays.

5.3 GeneXpert implementation in Tanzania

The majority of the more than 1000 TB diagnostic facilities in Tanzania still use smear microscopy as their primary diagnostic tool. Roll out of Xpert MTB/RIF started in 2011 with a total of 67 GeneXpert devices placed across the country by 2014.[17] Operational challenges include logistics of sample transport, unreliable electric power supply, lack of maintenance of the GeneXpert devices, inadequate funds for consumables and servicing contracts and limited expertise regarding supply chain management and stocks.

5.4 GeneXpert implementation in Mozambique

The Xpert MTB/RIF was adopted as the initial diagnostic tool in all presumptive TB cases in 2016. However, smear microscopy continues to be the main diagnostic and treatment monitoring tool for TB. The number of devices has been increasing with 32 in 2014, 85 in 2017 and 109 in 2019. The Ministry of Health (MoH) aims to have at least one device available in every district in the country, the facility with the device will act as the reference center for TB diagnosis in that district.

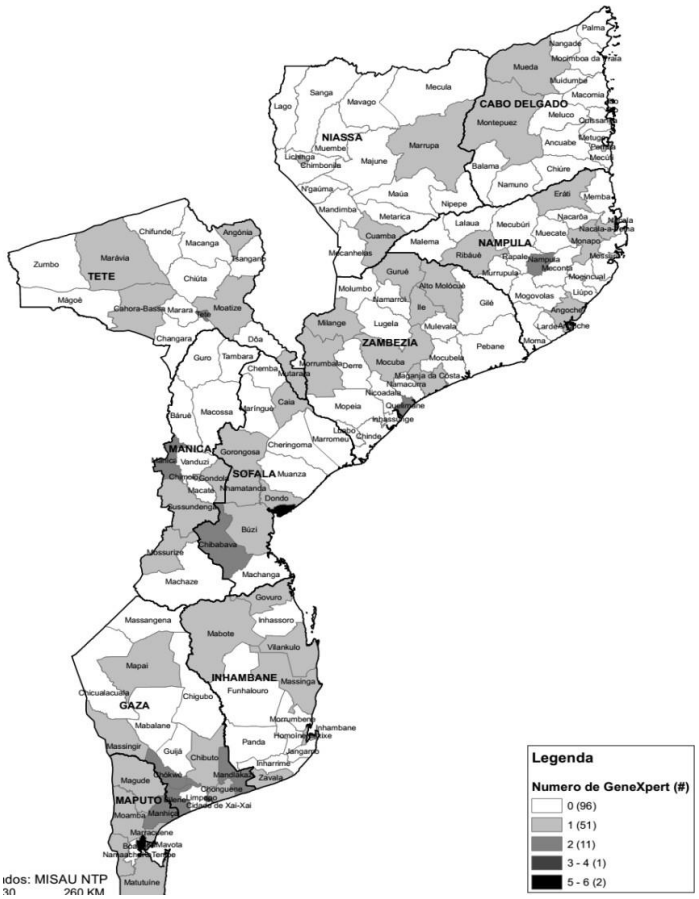

Figure 2: GeneXpert coverage in Mozambique in 2018 (Source: NTP report 2018)

6. INVESTIGATIONAL PRODUCT

All products used for clinical decision-making, including the Truenat platform and the Truenat TB assays for detection of MTBC and RIF-resistance already have CE-IVD mark. The WHO has endorsed the Truenat platform and Truenat MTB Plus and Truenat MTB RIF-Dx assays for diagnosis of TB and detection of RIF resistance in 2020.[26,27]

6.1 Truenat TB Platform/TB Assays definition

The following Molbio Truenat equipment and materials used in this study are referred to collectively in this protocol as: the Truenat TB platform:

- Equipment:
  - Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device
  - Truelab® Duo Real Time Quantitative micro PCR Analyzer
  - Truelab® micro–PCR Printer
- TB Assay components:
- Trueprep® AUTO MTB Sample Pretreatment Pack
- Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit
- TruenatTM MTB Plus kits, containing Truenat chips, a Truepet® 6µl Precision Micropipette
- TruenatTM MTB-RIF Dx kits, containing Truenat chips, a Truepet® 6µl Precision Micropipette

No other Molbio products are to be used in the intervention arm of this protocol.

6.2 Truenat system

The Truenat (Molbio Diagnostics, Goa, India) testing system (Trueprep DNA extraction device and the Truenat micro-PCR machine) uses portable, battery operated devices to rapidly detect MTBC and RIF-resistance. The assay for detecting MTBC DNA is Truenat MTB Plus, while the assay to detect RIF resistance is the Truenat MTB-RIF Dx. The system involves two main devices: the Trueprep AUTO v2 Universal Cartridge based Sample Prep Device for the automated extraction and purification of DNA, and the Truelab Real Time micro PCR Analyzer for performing real-time polymerase chain reaction (PCR), resulting in the semi-quantitative detection of MTBC. The system uses room temperature stable reagents (Trueprep AUTO Sample Pre-treatment and Prep kits) and Truenat micro PCR chips. The system is designed to be operated in peripheral laboratories with minimal infrastructure. In 2020 WHO has reviewed performance characteristics of this molecular point-of-care test for TB and endorsed the test for TB diagnosis. It takes about 25 minutes to do the DNA extraction and another 35 minutes to diagnose TB. Extracted DNA eluate from samples testing MTBC-positive can be used for rifampicin resistance reflex testing. The limit of detection was determined to be 100 CFU/ml sputum sample which is similar to the widely used Xpert MTB/RIF.[28]

In the control arm, standard of care will be conducted according to country specific protocols and guidelines. This will be a combination of smear microscopy at level 1 and/or Xpert MTB/RIF or Xpert MTB/RIF Ultra.

The Truenat testing system has great potential to enable true POC testing for TB and could be placed at the lowest tier of the health care system (i.e. primary health care clinics) potentially reducing

attrition along the diagnostic pathway. This in turn may reduce diagnostic delays and loss to follow-up during the diagnostic process and before initiating treatment resulting in decreased morbidity, mortality and reduced costs for the patient and health care system

6.3 Acquisition

Procurement of the investigational products will be done through FIND, who will coordinate shipments from the manufacturer. It is the responsibility of each trial site to maintain an updated inventory of the trial materials and to inform FIND immediately if additional materials are required. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for the investigational product received and any discrepancies are reported and resolved before its use.

6.4 Storage

Procedures for product storage and disposal will be described in the Trial Manual. The investigational product must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorized site staff.

6.5 Test handling and performance

Testing using the investigational products will be performed according to the manufacturer's instructions outlined within the Trial Manual. Only sputum samples from participants enrolled in the trial will be processed with the investigational product and only authorized site staff will be responsible for processing.

6.6 Accountability

The investigator is responsible for trial intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). Investigational Product Accountability logs filled at each site will ensure the proper follow-up of the used, failed and remaining investigational products. Further guidance and information for the final disposition of unused investigational product are provided in the Trial Manual.

6.7 Export and import permits

It is expected that most countries will require import permits for receiving the investigational materials. Local sites are responsible for making import permit applications in a timely manner.

6.8 Quality control check for incoming shipments

Upon arrival of each new shipment of assays, the sites will conduct and document an incoming quality check following the Trial Manual. New lots may only be used after this quality check is successfully passed.

6.9 Local procurement

Sites are responsible for assessing their needs and procuring any supplies, reagents and kits needed for the trial that are locally available in order to include these costs in the trial budget.

7. RATIONALE FOR THE STUDY

The Truenat platform/TB assays could in principle enable more rapid treatment initiation and thus reduce pre-treatment loss to follow up. However, it is uncertain whether this potential for improvement can and will be realised when implemented as part of routine practice.

This cluster randomized trial aims to assess whether improvements in patient outcomes are truly achieved using a new diagnostic strategy involving the Truenat platform and the Truenat MTB Plus and the Truenat MTB-RIF DX assays implemented in primary health care in Tanzania and Mozambique. In addition, data on operational characteristics of the Truenat platform/TB assays will be determined.

The findings of this trial will inform policy for decisions on use and scale up of the Truenat platform/TB assays for TB diagnosis in resource- limited settings.

8. STUDY OBJECTIVES

8.1 Primary objective

The primary objective is to evaluate the effect of Truenat platform/TB assays (intervention arm) combined with rapid communication of results compared to SOC on TB diagnosis and treatment initiation for microbiologically confirmed TB at 7 days from enrolment.

8.2 Secondary objectives

The secondary objectives are:

- To evaluate the effect of the Truenat platform/TB assay on time to TB treatment initiation at 60 days from enrolment:
  - for microbiologically confirmed TB cases
  - for clinically diagnosed TB cases
  - all TB cases (clinically diagnosed and microbiologically confirmed)
- Estimate the effect of the Truenat platform/TB assays on morbidity, mortality and on treatment loss to follow-up at 7 and 60 days from enrolment
- Estimate the effect of Truenat platform/TB assays on total costs (patient costs and health system costs) and productivity

8.3 Additional objectives

The additional objectives are:

- To evaluate the reliability of Truenat platform/TB assays as measured by rate of non-determined test results and platform failure
- User preferences
  - To investigate user perspectives on the Truenat platform/TB assays (including perspectives of end-users such as patients, but also of professional users such as laboratory technicians, clinicians, nurses, and decision-makers) for use as a diagnostic test for outpatients presenting with symptoms suggestive of pulmonary TB tuberculosis (TB)
  - To investigate and compare experiences and challenges with diagnostic testing for TB in patients using the different diagnostic approaches (Xpert® MTB/RIF, Xpert® MTB/RIF Ultra, microscopy, culture)
  - To understand feasibility and preferences with regard to diagnosing TB and how Truenat platform/TB assays changes these
  - To assess the usability and acceptability of Truenat platform/TB assays in the intended users

9. STUDY DESIGN

9.1 Summary

This is a cluster randomized controlled trial to evaluate the effect of placing Truenat platforms /TB assays at clinics in Mozambique and Tanzania.

In a setup period (survey of study centers) before randomization, the clinics of 4 sites will be asked to provide information about the number of TB notifications per quarter covering the period 1/2018-6/2020. From this information the foreseeable number of examined patients ("size of a clinic") will be derived, which will be used as a strata variable in randomization process with rapid communication of results on time to treatment initiation of microbiologically confirmed TB. Facilities will be randomly allocated to the SOC (control), or Truenat platform/TB assays (intervention). A cluster refers to a clinic. The clinics will be randomly assigned to intervention or control arm following a restricted randomization strategy, whereby 6-8 strata of clusters will be established using the stratification variables site (clinics belong to a site) and size and apply balance criteria for them. The number of strata per site depends on heterogeneity of the sizes of the clinics as established during the setup period and may be increased, typically 1 or 2 strata per site will be established.

Individual level analysis will be performed with strata and intervention as fixed effects and cluster as random effect.

9.2 Intervention

The intervention is the placements of the Truenat platform/TB assays at primary health care clinics combined with rapid communication of results and same day TB treatment initiation.

9.3 Standard of care (control)

All control clinics will have access to off-site Xpert testing. The degree to which patients and/or samples are referred for Xpert testing will vary across clinics and potentially across time dependent on availability of transport.

9.4 Setup period (survey of study centres)

During the setup period, the sites will be asked to collect TB notification numbers per quarter for each of the clinics for the period 1/2018-6/2020.

From this information, the number of adults ("size of a clinic") predicted to be investigated for TB at each of the clinics will be estimated.

9.5 Assignment of intervention

9.5.1 Allocation concealment mechanism

The intervention will be allocated to clinics (i.e., clusters) rather than to individuals. Clinics will be identified and recruited before randomization, i.e. the allocation sequence will be concealed until participating clinics have agreed to participation.

9.5.2 Clinic randomization and sequence generation

Taking into account the strata, a restricted randomisation approach will be applied to achieve balance

between the treatment arms.[29] Among all possible distributions of clusters within strata those which are balanced due to specific stratification variables will be used. This stratification will be by site and size of clinic. In addition, the same or similar number of clusters (max. difference=1) should be allocated to each treatment arm within each stratum. The number of strata per site will depend on heterogeneity of the sizes of the clinics as established during setup period. Typically, 1 or 2 strata per site will be established. A total of 6-8 strata are expected but might be increased dependent on the results of the setup period. All possible allocations will be established, whereby balance criteria covering a prespecified variability of the stratification variables within a stratum will be applied. The size of the clinic (number of TB patients within a 2-month period) should not differ more than 20%. The allocations will be checked for validity, especially whether the clusters are spread independently among the allocations. If this is not the case, the balance criteria will be chosen less restrictive. If the number of allocations excess 10,000, a subsample is drawn. Finally, one of the allocations will be chosen randomly.

9.5.3 Implementation

Clusters will be identified and asked to be part of the study by staff of participating sites. The allocation sequence will be generated by the trial statistician. The allocation to be applied is chosen randomly. All eligible individuals within each cluster will be enumerated and those willing to participate included in the study.

9.5.4 Bias reducing measures

The objective of the trial is to assess the Truenat platform/TB assays in clinical care. The use of Truenat platform/TB assays does not allow blinding of clinicians and participants in this trial. The trial-related procedures will be embedded into the routine practice at the primary-level facility. Data analysts will be blinded to intervention allocation.

Randomisation takes place on a cluster level rather than for the individual patients to avoid contamination between the arms within a health facility. Since clinics will be relatively far apart, contamination is unlikely.

We will contact participants for the 7- and 60-day outcomes by telephone to investigate whether more diagnostics available at the level of the primary health care facility (without the need to refer samples) result in more microbiologically confirmed cases and more rapid treatment initiation. Losses to follow-up pre-diagnosis and during treatment are among the endpoints of the trial. There is a chance that contacting the participants after 7 days may influence their treatment seeking behaviour even in the control arm and reduce loss to follow-up. By keeping contact with trial participants to a minimum (i.e., two time points) we hope to limit the impact on treatment seeking behaviour.

9.6 Study endpoints

Primary and secondary endpoints are summarised in Table 1.

9.6.1 Primary endpoint

The primary endpoint is the estimate of the number and proportion of participants with microbiological confirmation starting TB treatment within 7 days of their first visit among enrolled participants.

9.6.2 Secondary endpoint

Secondary endpoints include diagnosis and treatment related endpoints.

Diagnosis related endpoints:

- Time to bacteriological confirmation of TB (up to 60 days) from enrolment
- Number and proportion of patients treated for TB who are diagnosed within 60 days of enrolment
  - microbiologically
  - clinically

Treatment/outcome related endpoints:

- Number and proportion of participants with signs and symptoms suggestive of pulmonary TB starting TB treatment with microbiological confirmation within 60 days from enrolment
- Number and proportion of participants with signs and symptoms suggestive of pulmonary TB starting TB treatment without microbiological confirmation within 7 and 60 days from enrolment
- Number and proportion of participants with signs and symptoms suggestive of pulmonary TB starting TB treatment regardless of microbiological confirmation within 7 and 60 days from enrolment
- Time to TB treatment initiation for those with microbiological confirmation and for all participants (censored at 60 days) from enrolment
- Number and proportion of patients with ongoing treatment(on treatment) among:
  - Participants diagnosed with TB either clinically or with microbiological confirmation
  - Participants clinically diagnosed with TB
  - Participants with microbiological confirmation of TB

Morbidity related endpoints:

- Prevalence of current cough, limited appetite, weakness at 60 days from enrolment

Cost and productivity related endpoints

- Patient costs related to diagnosis and treatment

9.6.3 Additional endpoints

Operational characteristics

- Truenat TB assays rate of non-determined test results
- Rate of Truenat platform device failure

9.7 Study population and eligibility criteria

The trial population will comprise consecutively recruited adults presenting with symptoms suggestive of pulmonary TB at primary-level facilities (clinics). Presumptive pulmonary TB patients will receive diagnostic procedures according to the random allocation assigned to the clinic. Clinics randomized to the intervention will have Truenat platforms placed in the facility and sputum samples will be investigated with the Truenat MTB Plus and the Truenat MTB-RIF DX assays. In addition, procedures will be put in place to ensure rapid communication of results and same day TB treatment initiation. These procedures will be site specific and informed by the local context. Participants accessing clinics randomized to SOC will be investigated according to the national standard, which in most cases will be smear microscopy and/or Xpert testing.

9.7.1 Inclusion criteria

- Presumptive pulmonary TB patients, as defined by the national TB treatment guidelines in each country: patients with cough more than 1-2 weeks and/or fever, night sweats, blood-stained sputum (haemoptysis) significant weight loss, abnormalities on chest radiograph and who are able to provide a sputum sample
- Adults 18 years old and above (including pregnant women) who are able and willing to consent.

9.7.2 Exclusion criteria

- Children and adolescents <18 years of age
- Circumstances that raise doubt on free, informed consent (e.g. in a mentally impaired person or a prisoner)
- Already diagnosed with TB
- Currently receiving anti-TB therapy
- Patients with symptoms which are only attributable to extra-pulmonary TB
- Patients who are seriously ill and need to be admitted to hospital
- Enrolment into the trial at a previous visit

9.8 Schedule of events

Consent, recruitment, and baseline questionnaire will be done by the primary health care nurses in each of the clinics. Microscopists in the intervention clinics will be trained in using the Truenat platform and will be performing the Truenat MTB Plus and the Truenat MTB-RIF DX assays. Care providers at the intervention clinics will be trained in how to interpret Truenat TB assay results. Referral pathways for patients diagnosed with RIF resistant TB are already in place in both countries. These referral pathways will be re-iterated as part of the training. Each site will develop procedures to ensure rapid communication of Truenat platform/TB assays results to the clinician. Also measures will be taken to ensure same day TB treatment initiation.

For all participants who do not own a phone at the time of enrolment, a close contact number will be registered, otherwise, a home visit will be conducted for the study follow-up visits.

Staff in the control clinics will receive refresher training on the national guidelines on how to diagnose and treat TB. Referral pathways for drug resistant TB will be discussed in these trainings.
Participants will be asked about their contact details (phone number, phone number of a trusted proxy and home address) to enable follow-up. Each participant will get a study-ID.
Contact details will be recorded in a *locator form*. Study-ID, names, age, sex, and date of first presentation will be noted in a *study register*, which will remain in the clinic. The study registers will be checked against the TB register by the research team supervisor every week. If and when a patient has started TB treatment, the TB-register number and the date of TB treatment initiation will be documented in the *study TB outcome worksheet*. A paper-based clinical report form (CRF) will be used to document the baseline (day 1) questionnaire (V1-D1). No names, addresses or contact details will be entered on the CRFs. Participants will be identified by study-ID (see above). The only identifiable information recorded on CRFs will be age and sex. The V1-D1 will be retrieved from each clinic in regular intervals. The V1-D1 will be entered into an electronic database centrally in real time to enable timely follow-up by the research team by phone. The locator forms (including the phone numbers and addresses) will not be entered electronically but kept in locked cupboards. Timely data entry of V1-D1

will allow creating lists of study-IDs for locator forms to be retrieved and follow-up to be coordinated. A proportion of participants (5-10%) will be sampled, by selecting every tenth participant, across intervention and control clinics and across both countries to administered all of the questions of the baseline questionnaire plus some additional questions about costs of care-seeking. The data will be documented on the V1-D1-SE CRF. All clinics will be given a list of study-ID numbers identifying those participants who will be asked to answer the V1-D1-SE CRF before the start of enrolment.

Sites, which have the capacity to perform real-time data entry into electronic tablets, will be able to do so (as an alternative to paper-based CRFs).

Visit 2 (Day 7) and Visit 3 (Day 60) interviews will be conducted between days 8-21 and 61-90 post enrolment, respectively. Three attempts will be made to contact the participant. If the participant cannot be reached, the trusted proxy (documented on the locator form) will be contacted and asked about the participant's whereabouts. The trusted proxy will be asked on how best to contact the participant (i.e., other phone number or home visit). A home visit will be conducted for participants that cannot be reached by phone. A maximum of three home visit attempts will be made. Visit 2 and Visit 3 interviews will be recorded on paper-based CRFs (*V2-D7, V3-D60)* and entered electronically. Face-to-face visits may be considered for the Visit 3 interviews if a participant was sampled for a more extended socio-economic questionnaire (see above and below).

Only the study-ID and not the participant's name will be entered on the Truenat platform when a sample is tested. Pseudonymised Truenat TB assays results will be automatically uploaded to a secure, encrypted server at FIND using a Global SIM card on each Truelab micro PCR device in the intervention clinic on a regular basis using a File Transfer Protocol (FTP) or a similar system. This data maintains participant's confidentiality thereby ensuring subject's anonymity. Laboratory results such as smear microscopy or off-site Xpert MTB/RIF results will be captured by the research team supervisor and recorded in the study TB outcome worksheet.

Assay data (rates of non-determined results) and device data (instrument failures) will be securely uploaded to the FIND server and access by the trial data manager. Maintenance logs will be reviewed at the end of the study.

All sputum samples of participating patients in the intervention clinics will undergo Truenat platform/TB assays testing. The study-ID will be used as an identifier.

9.9 Participating sites

This study will be coordinated by two research institutions (sites) in each country and a total of 29 to 37 primary health care clinics (clinics).

Tanzania

1. Ifakara Health Institute (IHI)
2. National Institute for Medical Research (NIMR/MMRC)

Mozambique

1. Centro de investigação de Saúde de Manhiça
2. Instituto Nacional de Saúde (INS)

If a primary health care clinic has to stop recruitment during the study, another clinic may be selected to replace that clinic. An additional 8 clinics have been selected as backup clinics, 6 by NIMR-MMRC and 2 by INS.

9.10 Clinical laboratories

Health care providers may be asked to send a sputum sample for culture for patients with high suspicion of TB but negative molecular results according to national guidelines. These samples will be processed in the following laboratories:

Tanzania:

TB Laboratory
NIMR Mbeya Medical Research Centre,
Hospital Hill Rd, P.O.Box 2410,
Tel: +255 25 250 3364,
Mbeya, Tanzania

TB Laboratory,
Ifakara Health Institute,
Bagamoyo Research and Training Centre,
P. O. Box 74,
Tel: +255 23 244 0065
Bagamoyo,
Pwani, Tanzania

Mozambique:

Laboratório Nacional de Tuberculose
Instituto Nacional de Saúde
Av. Eduardo Mondlane, No 1653
Maputo, Moçambique
Tel/Fax: (+258) 21 311 038
Carla Madeira BSc, MSc
Email: carlamariamadeira@hotmail.com

Biosafety Level 3 (BSL3) TB laboratory
Centro de Investigação em Saúde de Manhiça
CP 1929 Maputo – Moçambique
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9.11 Data collection and handling

There will be a maximum of three questionnaires administered per participant: at Visit 1 (baseline; V1-D1 or V1-D1-SE), Visit 2 (Day 7; V2-D7) and Visit 3 (Day 60; V3-D60 or V3-D60-SE). All interviews will be documented on paper CRFs entered electronically, either centrally at the four sites, or at the clinics, into the OpenClinica eCRF/data base located at the LMU. Direct electronic data entry will be supported if a research site has the capacity to do that. The paper CRFs (V1-D1) will be retrieved from clinics on a weekly basis and stored at the central site or entered at the clinic. Data entry of V1-D1 will need to be entered within 6 days of enrolment as it is particularly critical to support the conduct of the V2-D7 phone calls.

V2-D7 and V3-D60 questionnaires will be administered mainly by phone by the research team, entered into the database and filed and stored centrally at the sites. For a subset of participants (approximately 50 per site) a more detailed socio-economic questionnaire (V1-D1-SE) will be conducted at baseline and at the last visit (V3-D60-SE). For those participants the Visit 3 questionnaire may be administered face-to-face.

In addition, details (study-ID, name, sex, age, date of enrolment) of each participant will be documented in a study register, which will stored in a secure location by the site until 10 years after the end of the study unless local regulations or institutional policies require a longer retention period. The study register will be compared with the TB registers and clinic records to ascertain diagnostic results (such as smear results in the standard of care clinics), TB registration number and TB initiation dates. This will be documented on the study TB outcome worksheet. The completed worksheet will be retrieved at the end of the study, entered electronically and stored centrally.

Data on number of contact attempts, contact information provided by trusted proxy, date and location of home visits will be documented on paper in free text. This information will not be entered and stored centrally.

All paper forms (including consent form) will be stored in secure and locked cupboards with only authorised person having access to the forms. The electronic database will not include any names, but only study numbers. The OpenClinica Enterprise version 4.0 database will be secure, password protected and regularly backed-up. Only authorised people will have access to the database.

Truenat TB assay results and error logs will be regularly backed-up on secure FIND servers. Interviews with users will be documented on paper-based questionnaires (user appraisal form). These will be entered into an electronic database.

10. STUDY ASSESSMENTS

10.1 Visit 1:

Demographic and background assessments

Following demographic and background variables will be collected according to the schedule of events table:

- Inclusion and exclusion criteria
- Written informed consent
- Demographic data: date of birth, gender
- Symptoms suggestive of TB
- TB treatment history
- HIV status (including antiretroviral therapy), diabetes
- Smoking and mining history
- Household assets
- Number of visits to a health care provider
- Health care provider first visited
- Costs of care
- Loss of income (SE questionnaire only)

TB investigations and treatment

All participants will be asked to submit a sputum sample for testing using Truenat platform/TB assays (intervention clinics) or standard of care testing (control clinics). TB treatment will be initiated by the clinic team not the study staff.

10.2 Visit 2 and 3 assessments

The following efficacy variables will be assessed for evaluation of efficacy outcomes, at the time points indicated in the schedule of events table and documented on V2-D7 and V3-D60 CRFs.

- TB treatment initiation (at 7 and 60 days from enrolment)
- Microbiological confirmed TB up to 60 days from enrolment from Truenat assay result downloads and study registers

11. PATIENT ENROLMENT

A total of 4200 patients will be enrolled at a minimum of 29 and a maximum of 37 clinics, coordinated by 4 sites in 2 countries, Tanzania and Mozambique. In Mozambique the aim is to recruit 2300 participants (800-1000 recruited by CISM and 1300-1500 by INS). The total number of participants recruited in Mozambique may change dependent on recruitment rates in Tanzania. Tanzania aims to recruit 2100-2600 participants.

Patients will be enrolled into the study only if they meet all the inclusion criteria and none of the exclusion criteria. The unit of randomisation is the clinic; hence patients accessing a certain clinic and consenting to the participate will receive investigations according to the arm into which the clinic was randomised.

11.1 Recruitment procedures

All patients presenting with symptoms suggestive of pulmonary TB will be approached by the primary health care nurses and invited to participate in the study. Posters explaining the study will be placed in the clinics. All materials will be used only upon approval of the ethics committees and/or regulatory authorities relevant for this study.

11.2 Patient informed consent

Patients presenting with signs and symptoms suggestive of pulmonary TB (as per the National Guidelines) will be invited to be screened for inclusion in the study. They will be given a patient information sheet and consent form (ICF) about the study and will be explained the anticipated benefits and the potential risks associated with the protocol procedures. The principal investigator or a person designated by the principal investigator will fully inform the patient. The language used will be as non-technical as possible and the patient will not unduly be influenced to participate in the study.

Patients will be told that screening includes asking for symptoms suggestive of pulmonary TB and age. Patients will be told that agreeing to be screened does not mean that they have to join the study, and that participation in the study is voluntary. Patients will be informed that they will be free to withdraw from the study at any time, and a withdrawal will have no negative effects on them receiving standard care afterwards.

Written (or witnessed oral informed consent) must be obtained from every patient prior to any procedures being done specifically for the study.

For illiterate patients, study information is given in the presence of an impartial, literate witness, who will read the information sheet to the patient or will witness the complete reading of the information sheet to the patient. The patient will give consent by thumb printing the ICF and the witness states that free, informed consent has been given by his/her signature on the ICF.

The signed original ICF will be kept in a locked cabinet at each research institute. A second original of the signed ICF will be given to the patient or to the patient's legally acceptable representative.

The ICF template will be provided to the investigator by the sponsor. If any modifications to the form are proposed by the site, the consent form must be submitted to the sponsor for approval prior to

submission to the ethics committee. The ICF will be revised whenever new important information becomes available and will undergo ethics committee review. All active patients must then sign additionally the revised form, after ethics approval was received.

11.3 Incentives and expenses

Participants will not receive any incentives for the participation in the study. For participants who opt to be interviewed and reviewed at the clinics rather than at their homes for the Visit 3, visit transport costs will be reimbursed. The exact amount will be decided by each of the sites.

11.4 Exclusion of particular groups

Children and adolescents aged <18 years will be excluded, because clinical presentation of TB disease differs between children and adults.

11.5 Participant withdrawal

Participant may decide to withdraw from the study at any time and for any reason. The investigator may also withdraw a patient for any of the following reasons:

- Protocol deviation
- If, for any reason, the investigator concludes that continued participation in the study would not be in the participant's best interest

The investigator will also withdraw a patient upon request of the sponsor or if the study is terminated as a whole. Participants who decide to withdraw or who have been withdrawn from the study will not be replaced. After discontinuation of the study participant, the study team will not collect more information for the study but clinical care should continue in the health center. The study will not interfere with treatment; however, it will guarantee adequate referral and assist with screening in the event of loss of follow-up.

12. STUDY PROCEDURES

12.1 Visit 1 Baseline visit

Patients presenting with symptoms suggestive of pulmonary TB to one of the study clinics will be invited to be screened for inclusion in the study. Before any study-specific screening procedures will be performed, all patients will sign and date (or thumbprint) the latest version of the ICF. Illiterate participants will be encouraged to have a witness present.

After informed consent has been obtained, the patient will receive a study number. The ICF will be labelled with the study number. A primary health care nurse or dedicated researcher staff will administer a questionnaire (V1-D1) and ask the patient about their contact details and the contact details of a trusted proxy (locator form). In addition, 5 to 10% participants per site will be invited to answer a socio-economic questionnaire about income, cost of care seeking, productivity losses, diagnostics and treatment (V1-D1-SE). For these participants a face-to-face interview may be conducted.

Depending on the clinic, the patient will receive a diagnostic work-up according to the national algorithm (smear microscopy and/or off-site Xpert testing) or the study intervention (Truenat

platform/TB assays on site). Further diagnostic investigations and TB treatment initiation will be as clinically indicated. All clinical care and further investigations will be provided within the governmental health care service. Patient details (including name, sex, age, and study-ID) will be documented in a study register, which will remain at the clinic. All patients will receive a study card with their details (including name, sex, age and study number and enrolment date) to take home with them. They will be asked to show the card if they present to the clinic again.

12.2 Visit 2: Day 7

All participants will be called by telephone 7 days after enrolment (between day 8 and day 21 after enrolment). Three attempts will be made to contact a participant by phone. If these attempts are unsuccessful the trusted proxy will be contacted to find out about the participant's whereabouts and arrange for a return call by the participants or a home visit. A home visit will be conducted for those participants who cannot be reached by phone.

The questionnaire administered by phone or face-face (V2-D7 CRF) will include questions about symptoms, whether TB was diagnosed (and by what means) and treatment started and if the participant had accessed another health care provider.

12.3 Visit 3: Day 60

All participants will be called by telephone 60 days after enrolment (between day 61 and day 90 after enrolment). Three attempts will be made to contact a participant by phone. If these attempts are unsuccessful the trusted proxy will be contacted to find out about the participant's whereabouts and arrange for a return call or a home visit. A home visit will be conducted for those participants who cannot be reached by phone.

The questionnaire administered by phone or face-face (V3-D60) will include questions about symptoms, whether TB was diagnosed (and by what means) and treatment started and if the participant had accessed another health care provider and how often.

13. STATISTICAL CONSIDERATIONS

13.1 Populations to be analyzed

Intent-to treat (ITT) population: The ITT population will consist of all enrolled patients in the groups to which randomly assigned to intervention and controls. Patients with missing outcome data at days 7 and 60 will be assumed to not have started treatment.

Per-Protocol (PP) population: The PP population will consist of all patients who fulfil the protocol in the terms of eligibility, interventions, and outcome assessment without any major protocol deviation.

The Statistical Analysis Plan (SAP) will define the criteria for the exclusion of patients from any of the above-mentioned analysis populations and will be finalized prior to database lock. The SAP will also provide a detailed summary of the analysis methodology for each of the outcomes of the trial.

13.2 Design considerations

Matched paired design and stratification have been considered as a study design. In fact, matched paired design can be regarded as an extreme form of stratification. Stratification involves the grouping of available clusters into strata that are expected to be similar in outcome. We have decided for stratification for the following reasons[33]:

- Fewer degrees in freedom are lost which is effective considering the relative low number of clusters per arm (10 .. 15).
- Adjustment for covariates via (generalized) linear models is possible
- Single missing clusters can be handled without losing a pair.

13.3 Randomization

See section 9.5.2

13.4 Descriptive statistics

Baseline characteristics, such as demographic and analytical data will be summarized using descriptive statistical methods. Continuous data will be summarized using the mean, the median, standard deviation, the range (minimum and maximum value). Categorical values will be summarized using frequency counts and percentages.

13.5 Analysis of endpoints

Endpoints referring to proportion of patients (primary endpoint, further secondary endpoints acc. Table 1) will be compared across the two arms via generalized linear models using logit link function with intervention and stratum as fixed effect and cluster as random effect. Sensitivity analyses based on cluster-level summaries will be applied. Adjustment for other variables (such as HIV status) may be considered in sensitivity analyses and will be described in detail in the statistical analysis plan (SAP). Endpoints referring to time to event analyses will be described by Kaplan-Meier-curves by treatment and stratum. Depending on the hazard functions the analysis method will be chosen. Details will be

presented in the SAP. Table 1 describes the definition of endpoints and the way to calculation of referring proportions.

Table 1:definition and derivation of endpoints

Endpoint	**Numerator**	**Denominator** (applicable to proportions and rates)	**Data source**
Number and proportion of participants with microbiological confirmation starting TB treatment within 7 days	Participants with microbiological confirmation starting TB treatment within 7 days	All participants	Visit 2 telephone call (V2-D7) Register Study TB outcome worksheet
Time to bacteriological confirmation	Participants with microbiological confirmed TB	Person Time at risk	Visit 2 telephone call (V2-D7) Visit 3 telephone call (V3-D60) Study TB outcome worksheet
Number and proportion of patients treated for TB with microbiological diagnosis	Participants with microbiological confirmed TB and treated	All participants treated for TB	Visit 2 telephone call (V2-D7) Visit 3 telephone call (V3-D60) Study TB outcome worksheet
Number and proportion of patients treated for TB with clinical diagnosis	Participants with clinically diagnosed TB and treated	All participants treated for TB	Visit 2 telephone call (V2-D7) Visit 3 telephone call (V3-D60) Study TB outcome worksheet
Number and proportion of participants with microbiological confirmation starting TB treatment within 60 days	Participants with microbiological confirmation starting TB treatment within 60 days	All participants	Visit 2 telephone call (V2-D7) Visit 3 telephone call (V3-D60) Study TB outcome worksheet
Time to TB treatment initiation for those with microbiological confirmation	Participants with microbiological confirmed TB starting treatment	Person Time at risk	Visit 2 telephone call (V2-D7) Visit 3 telephone call (V3-D60) Study TB outcome worksheet
Time to TB treatment initiation and for all participants	Participants starting TB treatment	Person Time at risk	Visit 2 telephone call (V2-D7) Visit 3 telephone call (V3-D60) Study TB outcome worksheet
Number and proportion of participants with ongoing TB treatment outcome	Ongoing TB treatment outcomes (on TB treatment at 60 days)	Participants diagnosed with TB Participants diagnosed with TB clinically Participants diagnosed with TB microbiologically	Visit 2 telephone call (V2-D7) Visit 3 telephone call (V3-D60) Study TB outcome worksheet TB treatment register
Cost of diagnosis and treatment	Total cost to participants (among participants who respond to the costing survey)	Not applicable	Visit 1 extended questionnaire (V1-D1-SE)

13.6 Other analyses

Other analysis will include

i) comparison of proportions using similar methodology as for proportion-related endpoints:

- initiating TB treatment with microbiologically diagnosed (or all) TB at 60

ii) time to TB treatment initiation (adjusted for strata) across arms

- for microbiologically confirmed TB cases
- for clinical diagnosed TB case
- for all TB cases

Analysis of cost, number of health care visits and symptoms will be done either by comparing proportions or mean (adjusted for strata).

13.7 Interim analysis

For this study no formal interim analysis is planned.

13.8 Sample size determination

For the purpose of sample size estimation, a matched design is regarded which is known to have a similar power in the range of number of clusters per treatment arm similar to this study (~15,[30]). Sample size calculation were based on 972 scenarios using assumptions for

- TB prevalence: 12%, 16%, 20%
- Sensitivity for intervention group: 89%
- Sensitivity for control group: 70%, 75%, 80%
- Diagnostic LTFU intervention arm: 2%
- Diagnostic LTFU control arm: 5%, 10%
- Pre-treatment LTFU[1] intervention arm: 8%, 10%, 12%
- Pre-treatment LTFU control arm: 16%, 20%, 24%[31]
- Cluster size CS 100, 125, 150, 200 (cluster sizes are regarded as average values of a distribution of cluster sizes).
- Within pair coefficient variation CV_m: 0.25 according to recommendation in case of missing information given in
- Alpha-level α = 0.05
- Power: 80% (β = 0.2)

From the prevalence, sensitivity and LTFUs in the diagnostics the proportions p of treated patients is derived ($p = prev \cdot sens \cdot (1 - LTFU_{diag}) \cdot (1 - LTFU_{pre-ther})$ for each of intervention and control arm (→ p_i, p_c).

The number of cluster pairs N_{cp} is calculated according to the following formula [Hayes RJ, Moulton LH 2017]:

$$N_{cp} = A + \left(z_{\alpha/2} + z_{\beta}\right)^2 \frac{p_i(1-p_i)/CS + p_c(1-p_c)/CS + {CV_m}^2({p_i}^2 + {p_c}^2)}{(p_i - p_c)^2}$$

whereby the result is rounded up to the next whole number, with A = 1 [Hayes RJ, Moulton 2017][29]. Note that Software PASS [PASS 2020] uses A=2, [Hayes and Bennett 1999][30] uses A=0.

z = quantile of normal distribution.

One should note that smaller cluster sizes (which would result in more clusters) are advantageous in terms of total number of patients to be recruited. Thus, the scenario has to be selected taking in into account number of patients on the one hand and efforts to recruit and organise sites on the other hand.

Following scenarios have been selected according to feasibility and mid-range choice of the ranges presented above:

Cluster size	Prevalence	Sensitivity control	diagn. LTFU control (diagn. LTFU for intervention: 0.02)	pre-trt. LTFU intervention	pre-trt. LTFU control	success proportion intervention	success proportion control	number of cluster pairs	Number of clusters	Number of patients
100	16%	70%	10%	10%	20%	13%	8%	14	28	2800
100	12%	70%	10%	8%	20%	10%	6%	15	30	3000
100	12%	70%	10%	10%	20%	9%	6%	17	34	3400
100	12%	70%	10%	12%	20%	9%	6%	18	36	3600
150	**12%**	**70%**	**10%**	**10%**	**20%**	**9%**	**6%**	**13**	**26**	**3900**
150	16%	70%	5%	10%	20%	13%	9%	14	28	4200
150	16%	70%	10%	10%	20%	13%	8%	12	24	3600
200	12%	70%	10%	10%	20%	9%	6%	12	24	4800
200	16%	70%	5%	10%	20%	13%	9%	13	26	5200
200	16%	75%	10%	10%	20%	13%	9%	14	28	5600

Table 2: selected scenarios. Colours vary with cluster size and prevalence. Red: final scenario.

Applying an alpha-level of 0.05, a power of 80%, 12% prevalence, diagnostic LTFU-rates of 10% (control), 10% (pre-treatment intervention), 20% (pre-treatment control), a cluster size of 150 and a within-pair coefficient of variation (default value 0.25[29]), the results bring a total of 13 cluster pairs (3900 patients). In order to address uncertainty, 1 additional cluster pair will be added, bringing the total to 14 cluster-pairs (28 clinics, 4200 patients) to be included in the trial. If based on what will be observed in the initial setup period, smaller than average cluster sizes are expected, more sites will need to be included in the trial in order to match the total sample size.

14. DATA MANAGEMENT

14.1 Data management plan

A data management plan will be drafted and agreed upon before the start of enrolment.

14.2 Data collection

The investigator agrees to maintain accurate source data and CRFs. For each patient screened a CRF will be completed, even if the patient drops out at any time point during the study. All information from a telephone interview or a performed visit must be entered in the CRF.

- CRFs should be completed legibly with black ballpoint pen
- CRF information should be complete
- Corrections should be made such that the original information is not obscured, i.e., by striking through the incorrect entry with a single line and the corrected information should be entered next to the deleted item. Corrections should be initialled and dated by the person making the correction
- Each completed CRF must be reviewed, signed, and dated by the investigator or a responsible person the investigator has delegated this task to in a timely manner. The complete CRF will be collected by study monitors as soon as practical after completion.

14.3 Source documents

The investigator agrees to maintain accurate source documents as part of the case histories and permits direct access for domestic and foreign regulatory authorities, sponsors, monitors and auditors.

The following data will be recorded directly into the CRF and will be considered source data:

- Date of enrolment or interview
- Sex
- Age
- Symptoms
- Medical history
- TB diagnostic status
- TB treatment status
- Health seeking
- Symptoms
- Costs
- Vital status

14.4 Data entry

Timely data entry is of utmost importance for this trial for the following reasons:

- To closely monitor patient recruitment and ensure recruitment is on target given the tight timelines
- To ensure Visit 2 follow-up calls (which directly inform the primary outcome) are conducted within 8-21 days post recruitment

The investigator agrees to timely entry of all V1-D1, V2-D7 and V3-M2 within 7 days of data collection.

14.5 Data handling and record keeping

Records and documents, including signed ICFs, pertaining to the conduct of this trial must be retained by the investigator for 10 years after trial completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of FIND. No records may be transferred to another location or party without written notification to FIND.

15. MONITORING AND QUALITY ASSURANCE

15.1 Study monitoring

One or more monitors will be assigned to the study. The monitor, as a representative of the sponsor, has the obligation to follow the study closely. The monitor will visit the site at regular intervals and will be in contact by phone and written communication, as required.

Site investigators and designated study personnel will allow the monitors to inspect study documents, clinic records as well as site facilities, as required. All aspects of the study will be carefully monitored in order to ensure compliance with Good Clinical Practice and all applicable regulatory guidelines. The monitor will be responsible for verification of

- adequacy of study personnel's qualifications as well as facilities
- the accuracy and completeness of the CRF entries, source documents and other study-related records
- appropriate IMP storage, usage, and accountability
- informed consent procedures and patient eligibility
- maintenance of the essential documents
- all other aspects of the study relating to protection of the rights and well-being of patients, accuracy of study data and adherence to the protocol

The study database will only be closed after data has been verified by the monitor and the sponsor, and all queries issued through data cleaning activities have been completed.

15.2 Inspection of record

The investigator will allow the sponsor, the sponsor's representatives, regulatory agencies and ethics committees access to all study records, if requested. The investigator will promptly notify the sponsor of any inspections scheduled by regulatory authorities or ethics committees and promptly forward copies of any inspection reports received to the sponsor.

15.3 Record retention

The sponsor will keep essential study documents (including CRFs) for at least 10 years after completion or discontinuation of the study.

15.4 Confidentiality of personal data

All patient records, lab specimen etc. will be identified in a manner to maintain patient confidentiality and will be kept in a secure storage area with limited access. Each participant will be assigned a pseudonymous study number. No data that could identify the patient other than this identification number (sex and date of birth) will appear on the CRFs.

Data of study patients will only be used as defined in the ICF and in line with applicable data privacy regulations. Accordingly, patient records may be reviewed by inspectors of regulatory authorities or ethics committees, study monitors and auditors, who ensure the quality of the study.

An individual's study data will not be released without the written agreement of the patient (or their legal guardian), except as necessary for monitoring and auditing by the sponsor or its representative, regulatory authorities, or ethics committees, or in case of medical emergencies when written consent cannot be obtained, as deemed in the participant's best interest by the investigator.

16. USER PREFERENCES (Qualitative sub-study assessing usability, feasibility, and acceptability)

Incorporating patients' preferences in the development of a POC strategy for TB diagnosis using the Truenat platform/TB assays may facilitate its implementation and improve health outcomes. Aside from evaluating the test accuracy, it is important to understand experiences and preferences of those who will use the technology. Qualitative methods are ideal for making sense of user experiences with and perspectives on diagnostic tools within "real-world" situations. Furthermore, an assessment of values and preferences, acceptability and ease-of-use is an integral part of the WHO evaluation of novel diagnostic tests. We intend to explore these aspects alongside the prospective study for the Truenat platform/TB assays.

16.1 Objectives

Primary objectives	To investigate user perspectives on the Truenat platform/TB assays (including perspectives of end-users (such as patients), but also of professional users such as laboratory technicians, clinicians, nurses, and decision-makers) for use as a diagnostic test for outpatients presenting with symptoms suggestive of pulmonary TB. To investigate and compare experiences and challenges with diagnostic testing for TB in patients using the different diagnostic approaches (Xpert® MTB/RIF (Ultra), microscopy, culture). To understand feasibility and preferences with regard to diagnosing TB and how the Truenat platform /TB assays change these. To assess the usability and acceptability of the Truenat platform/TB assays in the intended users.
Secondary objectives	To explore potential implications for health equity of this POC test (for instance by cutting diagnostic delay, reducing loss to follow up, cost concerns, increased accessibility, linkage to care).

16.2 General Design and participants

We will use a qualitative approach using semi-structured interviews with patients, professional users (laboratory staff, nurses, clinicians) and decision makers. In addition, to assess the usability of the Truenat platform/TB assays, we will conduct direct observations of the testing procedures and perform Ease-of-use and usability surveys.

Semi-structured interviews with professional users (healthcare and laboratory staff, study staff), decision makers and adult patients seeking diagnosis for TB (involved in the CORE trial):

To gain insights from various professional users, we will include 3-5 healthcare workers from each user group (including nurses, clinicians and laboratory technicians involved in routine care provision as well as study staff involved in the study) at each site, or until saturation is reached. Separately, we will include interviews with 3 TB and HIV programme officers (decision makers), which will allow us to gain insights in the perspective of decision makers in the program at each site. In total, we seek to interview approximately 10-15 health care workers and decision makers per country.

To gain end-user perspectives, we will invite 10-12 participants from each coordinating site (approx. 20-24 per country) for an interview, either when being enrolled in the study or later through the follow-up mechanisms that are part of the study. We will aim to obtain an equal number of patients who tested positive or negative, as well as from the intervention and control clinics.

Observations at study sites and surveys (Ease-of-Use and System Usability Scale):

At the intervention clinics, research staff will conduct a standardized training for the Truenat platform /TB assays. After completing the training, health workers will be asked to perform specimen collection and/or testing under direct observation up to 4 patients enrolled in the study. Namely, research staff will observe them while performing the test and will record the time spent on specimen collection and/or testing, as well as any errors or failures that occur. They will also ask participants to provide general comments on device usability.

Following direct observation, participants will also be asked to complete a survey. The survey will include the System Usability Scale (SUS), a 10-item questionnaire on a 5-point Likert scale, to rate the usability of the test[32,33], and additional questions for health workers to rate the acceptability, and an Ease-of-Use questionnaire developed especially for the Truenat platform /TB assays.

Ten eligible health workers (operators of the test) preferably from the intervention clinics will be invited to participate in the direct observations and surveys per country. Health workers will be purposively sampled to reflect the intended setting of use for the Truenat platform /TB assays.

16.3 Sample size and sampling

Sample size determination in qualitative research rests on maximizing potential for "saturation" (when new interviews do not meaningfully add to codes and themes already represented in the previously collected data). Based on previous experience on assessing user preferences, and literature in sampling for qualitative research[34], we expect to reach saturation for thematic analysis of patients (20-24/country) and professional users, while an exploratory analysis of the decision makers perspectives (overall professionals: 10-15/country, although only 3/country are expected to be decision makers).

Participants will be purposively sampled[35], with a maximum variation approach[36], which seeks to capture the maximum variation for a defined spectrum to identify information-rich cases. A sampling frame, with potential participants matching the eligibility and purposive sampling criteria, will be created based on demographic and trial data. Patients will then be invited to participate by the local study staff.

16.4 Inclusion & Exclusion Criteria

Inclusion criteria for semi-structured **interviews**:

- Adult aged ≥ 18 years old
- Either:
    - (1) Routine healthcare worker participating in the CORE trial, or
    - (2) Patient tested or to be tested for TB as part of the CORE study, or
    - (3) Decision maker involved with implementation of novel tests at the local, national and/or regional level.

Inclusion criteria for **direct observations and surveys (Ease-of-Use and SUS)**:

- Adult aged ≥ 18 years old
- Involved in routine TB testing (collecting specimens for or performing TB tests).

Exclusion criteria (all): unable or unwilling to provide consent
Exclusion criteria (semi-structured interviews): does not agree to allow audio recording of interviews

16.5 Topic guide, data management (protection) and analysis

An interview topic guide to support the qualitative data collection will be developed for the study.

Data will be stored in a secure way accessible only to the researchers involved: password encrypted (digital) and in a locked office in a locked fireproof cabinet (hard copies/notebooks). All the information collected for this study will be kept strictly confidential by identifying the participants with a unique code (or study ID) to which only the study investigators have access. Only de-identified transcripts, survey answers and notes will be kept on a password protected server or a locked cabinet for 10 years following Proposals for Safeguarding Good Scientific Practice (version 2013) of the German Research Foundation (or longer if local regulations require). Audio-files will be destroyed after transcription. Information may only be passed on to other institutions involved in the study if they are pseudonymized for data protection reasons and after signing Data Transfer Agreement between collaborating institutions. The third parties will not have access to the data. Personal information and names will not be revealed at publication and all personal data will be anonymized accordingly to the research purpose.

Thematic analysis will be conducted for the qualitative data using NVivo software. We will use a pre-defined coding framework, which will be updated as the analysis is conducted, to reflect emerging themes. Reporting will follow guidelines for qualitative studies (Consolidated criteria for reporting qualitative research (COREQ), 2007).

Descriptive analysis will be done for the survey data (Ease of Use and SUS).

17. ETHICAL CONSIDERATIONS

17.1 Risks and benefits

As the Truenat platform and TB assays are WHO approved diagnostics for smear replacement, and no additional study specific samples are being collected, there are no specific ethical considerations and risks for this study. Participants accessing the intervention clinic will receive timely diagnosis with a CE-marked WHO endorsed diagnostic (the Truenat platform). Diagnostic testing will be closely monitored and all staff members performing the testing will be adequately trained.

17.2 Safety and incident reporting

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an adverse events (AE) or severe adverse events (SAE) and remain responsible for following up and reporting AEs that are serious, considered related to the trial intervention or trial procedures, or that caused the participant to discontinue the trial.
Given that this is a trial of near patient testing of a WHO endorsed assay (the Truenat platform) the probability of an AE or SAE occurring to a trial participant to be associated with the investigational products is extremely low.

17.3 Basic principles

This study will be performed in accordance with the study protocol, the current ICH- Guideline for GCP E6 (R2) (2016)/WHO Guidelines for GCP/WHO Handbook for Good Clinical Research Practice /Declaration of Helsinki/CIOMS International Ethical Guidelines for Biomedical Research Involving Human Subjects as well as any other applicable national and other regulatory guidelines.

17.4 Involvement of ethics committees and regulatory authorities

The protocol and the informed consent document to be used in this study must be submitted to the responsible investigators' ethics committees and regulatory authorities, and also to the sponsor's EC for approval. Written documentation of approval of the project and all relevant documents must be provided to the sponsor before starting the study.
The investigator will promptly report to the EC deviations from the protocol and all unanticipated risks to human subjects or others, and will not make changes in the defined research without EC approval, except where necessary to eliminate apparent immediate hazards to human subjects.

17.5 Protocol amendment policy

Any substantial change to the protocol will be affected by means of a protocol amendment and has to be submitted to ethics committees and regulatory authorities. No amendment will be implemented until approved and signed by all required parties. Exceptions to this are when the investigator considers that the subject's safety is compromised.

Protocol amendments detailing minor administrative changes should be agreed in advance with the

sponsor and submitted by the investigator to ethics committees and regulatory authorities for notification purposes as appropriate.

17.6 Discontinuation criteria

FIND reserves the right to close the trial site or terminate the trial at any time for any reason at its sole discretion. Investigational sites will be closed upon trial completion. A trial site is considered closed when all required documents and trial supplies have been collected. The investigator may initiate trial-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for early closure of a trial site by FIND are described in the contractual agreement.

17.7 Falsification of data

Any proven evidence of falsification of data will be dealt with in accordance with the policy of the sponsor and appropriate action will be taken.

18. ADMINISTRATIVE CONSIDERATIONS

18.1 Financing

The study sponsor is the Foundation of Innovative Diagnostics. The study is funded by the European and Developing Countries Clinical Trials Partnership (EDCTP).

18.2 Study registration

Before study start, the study will be registered in a WHO recognised study registry (clinicaltrials.gov).

18.3 Patient insurance and compensation

The sponsor certifies that it has obtained or will obtain clinical study insurance in line with the requirements in each country prior to study start and will provide an associated certificate upon request. The insurance does not relieve the investigators of the obligation to maintain their own liability insurance as required by applicable law. The sponsor does not assume any obligation for the medical treatment of other injuries and illnesses.

18.4 Training for staff involved in the study

During the initiation visit the monitor will ensure all involved site personnel is trained on all procedures relevant for their study responsibilities. This will include weekly monitoring visits to each of the clinics to collect CRFs, administration of V1-D1, V2-D7 and V3-M2 CRFs.

Training and supervision of primary health care nurses on study procedures and microscopists will be performed separately and documented in the electronic trial master file. The site personnel will be responsible to monitor the primary health care nurses and microscopists and re-train as necessary.

18.5 Publication policy

A dedicated TB-CAPT publication policy has been developed. In brief, after completion of the study, the data will be considered for presenting at a scientific conference or for publication in a scientific journal. The sponsor will be responsible for these activities and will collaborate with the Scientific Advisory Board to determine how the manuscript is written and edited, the number and order of authors, the journal to which it will be submitted and other related issues.

The results of the study will be published independent of the outcome – positive or negative - of the study.

Under certain circumstances, i.e., when the publication of particular findings (of an epidemiological, sociological or genetics study) may present a risk to the interest of a community or population or a racially or ethnically defined group of people, it may be considered inappropriate to publish findings.

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